# 8TH INTERNATIONAL ANATOLIAN AGRICULTURE FOOD, ENVIRONMENT AND BIOLOGY CONGRESS

**11-12 Haziran 2026**

**Sinop - TÜRKİYE**

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## PREFACE

Dear Colleagues...

The " 8th International Anatolian Agriculture, Food, Environment and Biology Congress (TARGID 2026) " has been held in Sinop/Türkiye on 11-12 June 2026, with the participation of scientists working in agriculture and related sciences, with the aim of sharing scientific developments, proposing scientific solutions to international problems, increasing cooperation and creating scientific outputs that will benefit humanity by using international resources economically.

A total of 296 papers from 37 countries were presented to our congress. Of these papers, 158 came from foreign countries and 138 came from Türkiye. The countries of the participants who submitted papers to the congress and the number of incoming papers are as follows: Algeria (5), Azerbaijan (5), Bangladesh (15), Benin (4), Bosnia and Herzegovina (1), Bulgaria (1), Chad (1), China (4), Côte d'Ivoire (1), Ethiopia (3), Ghana (4), Hungary (3), India (0), Iraq (1), Iran (11), Italy (5), Kazakhstan (5), Kenya (1), Malaysia (4), Moldova (1), Morocco (1), Nepal (5), Niger (3), Nigeria (25), Pakistan (18), Philippines (1), Poland (4), Somalia (2), Spain (1), Sri Lanka (7), Sudan (4), Tanzania (1), Tunisia (5), Uganda (2), Ukraine (1), United States (2), Zimbabwe (1), and Türkiye (138). The rate of international papers was 53.4%, while papers from Türkiye accounted for 46.6% of the total.

At the congress; participants discussed such topics as animal production, crop production, aquaculture, food science and technologies, agricultural technologies, soil science, sustainable agricultural systems, agricultural economics, genetics, biology, agricultural ecology, landscape architecture and agricultural environment issues related to original, advanced technology on agriculture and natural life.

During the organization and congress phases of TARGID 2026, Assoc. Prof. Dr. Burak Şen, Prof. Dr. Cem Baltacıoğlu, Assoc. Prof. Dr. Hande Baltacıoğlu, Assist. Prof. Dr. Mustafa Duman, Assist. Prof. Dr. Mutlu Bulut, Assist. Prof. Dr. Ali Kaan Yetik, Assist. Prof. Dr. Mehmet Yetişen, Ahmet Akyol, Furkan Baş, Muhammet Talha İnce, and Ulrich Sosthène Hodonou took on important responsibilities in the congress secretariat.

We would like to express our gratitude to all our stakeholders who supported the successful realization of our congress and all the scientists who participated and supported us with their papers.

Kind regards

# ÖNSÖZ

Değerli Meslektaşlarımız,

Tarım ve ilgili bilim alanlarında çalışan bilim insanlarının katılımıyla gerçekleştirilen "8. Uluslararası Anadolu Tarım, Gıda, Çevre ve Biyoloji Kongresi (TARGID 2026)", bilimsel gelişmelerin paylaşılması, uluslararası sorunlara bilimsel çözümler sunulması, iş birliğinin artırılması ve uluslararası kaynakların ekonomik ve etkin bir şekilde kullanılması yoluyla insanlığa fayda sağlayacak bilimsel çıktılar oluşturulması amacıyla 11–12 Haziran 2026 tarihlerinde Sinop/Türkiye'de gerçekleştirilmiştir.

Kongremize 37 ülkeden toplam 296 bildiri sunulmuştur. Bu bildirilerin 158'i yabancı ülkelerden, 138'i ise Türkiye'den gelmiştir. Kongremize bildiri gönderen katılımcıların ülkeleri ve gönderilen bildiri sayıları şu şekildedir: Cezayir (5), Azerbaycan (5), Bangladeş (15), Benin (4), Bosna-Hersek (1), Bulgaristan (1), Çad (1), Çin (4), Fildişi Sahili (1), Etiyopya (3), Gana (4), Macaristan (3), Hindistan (0), Irak (1), İran (11), İtalya (5), Kazakistan (5), Kenya (1), Malezya (4), Moldova (1), Fas (1), Nepal (5), Nijer (3), Nijerya (25), Pakistan (18), Filipinler (1), Polonya (4), Somali (2), İspanya (1), Sri Lanka (7), Sudan (4), Tanzanya (1), Tunus (5), Uganda (2), Ukrayna (1), Amerika Birleşik Devletleri (2), Zimbabve (1) ve Türkiye (138). Uluslararası bildirilerin toplam bildiriler içerisindeki oranı %53,4, Türkiye'den sunulan bildirilerin oranı ise %46,6 olarak gerçekleşmiştir.

Kongrede; hayvansal üretim, bitkisel üretim, su ürünleri yetiştiriciliği, gıda bilimi ve teknolojileri, tarımsal teknolojiler, toprak bilimi, sürdürülebilir tarım sistemleri, tarım ekonomisi, genetik, biyoloji, tarımsal ekoloji, peyzaj mimarlığı ve tarımsal çevre konularının yanı sıra tarım ve doğal yaşamla ilişkili özgün ve ileri teknoloji uygulamaları ele alınmış ve tartışılmıştır.

TARGID 2026'nın organizasyon ve kongre süreçlerinde; Doç. Dr. Burak Şen, Prof. Dr. Cem Baltacıoğlu, Doç. Dr. Hande Baltacıoğlu, Dr. Öğr. Üyesi Mustafa Duman, Dr. Öğr. Üyesi Mutlu Bulut, Dr. Öğr. Üyesi Ali Kaan Yetik, Dr. Öğr. Üyesi Mehmet Yetişen, Ahmet Akyol, Furkan Baş, Muhammet Talha İnce ve Ulrich Sosthène Hodonou kongre sekretaryasında önemli görevler üstlenmişlerdir.

Kongremizin başarıyla gerçekleştirilmesine katkı sağlayan tüm paydaşlarımıza ve bilimsel çalışmalarıyla kongremize katılım göstererek bizleri destekleyen tüm bilim insanlarına teşekkürlerimizi sunarız.

Saygılarımızla

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## Yayın Ve Düzenlemede Görevlendirilmiş Akademisyen Temsilci

11–12 Haziran 2026 tarihinde Sinop/Türkiye'de "8. Uluslararası Tarım, Gıda, Çevre ve Biyoloji Kongresi (TARGID 2026)" gerçekleştirilmesinde, Niğde Ömer Halisdemir Üniversitesi Rektörlüğü'nün 04/02/2026-777021 sayısı olurları ile aşağıda isimleri verilen akademisyenlere ***yayın ve düzenlemede akademik temsilci*** olarak yer almalarına izin verilmiştir.

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Arş. Gör. Ahmet AKYOL
Arş. Gör. Furkan BAŞ
Arş. Gör. Muhammet Talha İNCE

## ULUSLAR ARASI KONGRE ÖZELLİKLERİ

### 1. Ülkelere göre bildiri sayıları

| No. | Ülke | Bildiri sayısı |
|---|---|---|
| 1 | Algeria | 5 |
| 2 | Azerbaijan | 5 |
| 3 | Bangladesh | 15 |
| 4 | Benin | 4 |
| 5 | Bosnia and Herzegovina | 1 |
| 6 | Bulgaria | 1 |
| 7 | Chad | 1 |
| 8 | China | 4 |
| 9 | Côte d'Ivoire | 1 |
| 10 | Ethiopia | 3 |
| 11 | Ghana | 4 |
| 12 | Hungary | 3 |
| 13 | Iran | 11 |
| 14 | Iraq | 1 |
| 15 | Italy | 5 |
| 16 | Kazakhstan | 5 |
| 17 | Kenya | 1 |
| 18 | Malaysia | 4 |
| 19 | Moldova | 1 |
| 20 | Morocco | 1 |
| 21 | Nepal | 5 |
| 22 | Niger | 3 |
| 23 | Nigeria | 25 |
| 24 | Pakistan | 18 |
| 25 | Philippines | 1 |
| 26 | Poland | 4 |
| 27 | Sri Lanka | 7 |
| 28 | Somalia | 2 |
| 29 | Spain | 1 |
| 30 | Sudan | 4 |
| 31 | Tanzania | 1 |
| 32 | Tunisia | 5 |
| 33 | Türkiye | **138** |
| 34 | Uganda | 2 |
| 35 | Ukraine | 1 |
| 36 | USA | 2 |
| 37 | Zimbabwe | 1 |

2. **Bilim Kurulunda Yer Alan Yabancı Akademisyenler**
(Toplam: 11 yabancı akademisyen, 8 farklı ülke)

| Sıra | Unvan | Ad Soyad | Kurum | Ülke |
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**3. Yurt dışı katılım oranı**

| Group | Number of countries | Participation | Percentage |
|---|---|---|---|
| Türkiye | 1 | 138 | 46.62% |
| Yurt dışı | 36 | 158 | **53.38%** |
| Total | 37 | 296 | 100.00% |

Yukarıda verilen bilgiler çerçevesinde, 11-12 Haziran 2026 tarihinde Sinop/Türkiye'de " 8. Uluslararası Tarım, Gıda, Çevre ve Biyoloji Kongresi (TARGID 2026) ", uluslararası kongre niteliği taşımakta, akademik teşvik kriterleri ve UAK Doçentlik başvuru koşullarını **sağlamıştır.**

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# Performance Analysis of the Turkish Egg Industry Over the Last Ten Years

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## Abstract

In this study, the performance of the Turkish egg industry between 2016 and 2025 was evaluated in terms of production, consumption, exports, and economic indicators. Data obtained from the Turkish Statistical Institute (TURKSTAT), Central Union of Egg Producers of Türkiye (YUM-BİR), FAO statistics, and sectoral reports were used in the research. Egg production volume, laying hen population, per capita consumption, export performance, and changes in feed costs were analyzed. According to the findings, Türkiye has shown significant growth in egg production over the last decade and has become one of the leading countries in global egg production. Continuous increases were observed particularly in production and exports during the 2016–2023 period. However, rising feed costs, avian influenza risks, energy expenses, and export restrictions negatively affected the sector during 2024–2025. As a result, it was concluded that increasing domestic feed raw material production, strengthening biosecurity measures, and maintaining stable export policies are essential for the sustainable growth of the Turkish egg industry.

***Keywords:*** *Türkiye, egg industry, production performance, export, sustainability*

**Türkiye Yumurta Endüstrisinin Son 10 Yıllık Performans Analizi**

## Özet

Bu çalışmada Türkiye yumurta endüstrisinin 2016–2025 yılları arasındaki performansı üretim, tüketim, ihracat ve ekonomik göstergeler açısından değerlendirilmiştir. Araştırmada Türkiye İstatistik Kurumu (TÜİK), Yumurta Üreticileri Merkez Birliği (YUM-BİR), FAO verileri ve sektörel raporlardan yararlanılmıştır. Çalışmada yumurta üretim miktarı, yumurtacı tavuk varlığı, kişi başı tüketim, ihracat performansı ve yem maliyetlerindeki değişimler incelenmiştir. Bulgulara göre Türkiye, son on yılda yumurta üretiminde önemli bir büyüme göstermiş ve dünya yumurta üretiminde önde gelen ülkeler arasında yer almıştır. Özellikle 2016–2023 döneminde üretim ve ihracatta sürekli artış gözlenmiştir. Ancak 2024–2025 yıllarında yem maliyetlerindeki yükseliş, kuş gribi riskleri, enerji giderleri ve ihracat kısıtlamaları sektör üzerinde olumsuz etkiler oluşturmuştur. Sonuç olarak Türkiye yumurta sektörünün sürdürülebilir büyümesi için yerli yem hammaddesi üretiminin artırılması, biyogüvenlik uygulamalarının güçlendirilmesi ve ihracat politikalarının istikrarlı şekilde yürütülmesi gerektiği belirlenmiştir.

***Anahtar Kelimeler:*** *Türkiye, yumurta endüstrisi, üretim performansı, ihracat, sürdürülebilirlik*

## Giriş

Yumurta, içerdiği yüksek kaliteli protein, vitamin ve mineral maddeler nedeniyle insan beslenmesinde önemli bir hayvansal protein kaynağıdır. Ekonomik olması, kolay ulaşılabilirliği ve yüksek biyolojik değeri sayesinde dünya genelinde yaygın olarak tüketilmektedir. Kanatlı hayvancılık sektörü içerisinde yumurta üretimi, hem ekonomik katkısı hem de gıda güvenliği açısından stratejik bir öneme sahiptir.

Türkiye yumurta sektörü son yıllarda önemli bir gelişim göstermiş ve modern üretim sistemleri sayesinde dünya yumurta üretiminde önde gelen ülkeler arasında yer almıştır. Özellikle entegre üretim tesislerinin yaygınlaşması, teknolojik yatırımların artması ve ihracat kapasitesinin gelişmesi sektörün büyümesine katkı sağlamıştır. Türkiye, yıllık yaklaşık 20 milyar adedin üzerindeki üretim kapasitesiyle dünya yumurta üretiminde ilk sıralarda yer almakta ve önemli ihracatçı ülkeler arasında bulunmaktadır.

2016–2025 döneminde sektörde üretim miktarı, yumurtacı tavuk varlığı ve ihracat hacminde genel olarak artış gözlenmiştir. Bununla birlikte yem hammaddesi maliyetlerindeki yükseliş, döviz kuru dalgalanmaları, enerji giderleri, kuş gribi vakaları ve ihracat düzenlemeleri sektörün sürdürülebilirliği üzerinde baskı oluşturmuştur. Özellikle yem giderlerinin toplam üretim maliyetlerinin büyük bölümünü oluşturması, sektörün ekonomik kırılganlığını artırmıştır.

Dünya genelinde artan nüfus ve protein ihtiyacı, yumurta sektörünün gelecekte daha da önem kazanacağını göstermektedir. Bu nedenle Türkiye yumurta endüstrisinin mevcut durumunun değerlendirilmesi ve son yıllardaki performans değişimlerinin ortaya konulması önem taşımaktadır.

Bu çalışmanın amacı, Türkiye yumurta endüstrisinin 2016–2025 yılları arasındaki performansını üretim, tüketim, ihracat ve ekonomik göstergeler açısından incelemek; sektörün gelişim sürecini değerlendirmek ve sürdürülebilir büyümeye yönelik öneriler sunmaktır.

## Materyal ve Yöntem

### *Veri Kaynakları*

Bu çalışmada Türkiye yumurta endüstrisinin 2016–2025 yılları arasındaki performansının değerlendirilmesi amacıyla çeşitli resmi kurum ve sektörel kaynaklardan elde edilen ikincil veriler kullanılmıştır. Araştırmanın temel veri kaynaklarını Türkiye İstatistik Kurumu (TÜİK) verileri, Yumurta Üreticileri Merkez Birliği (YUM-BİR) raporları, T.C. Tarım ve Orman Bakanlığı istatistikleri ve çeşitli sektörel değerlendirme raporları oluşturmuştur. Ayrıca konu ile ilgili ulusal ve uluslararası akademik çalışmalar incelenerek literatür desteği sağlanmıştır.

TÜİK verilerinden yumurta üretim miktarı, tavuk sayısı ve dış ticaret istatistikleri elde edilirken, YUM-BİR raporlarından sektörün üretim kapasitesi, ihracat performansı ve sektörel gelişimine ilişkin bilgiler alınmıştır. Tarım ve Orman Bakanlığı kaynaklarından ise kanatlı hayvancılık sektörüne ilişkin güncel değerlendirmeler ve destek politikaları incelenmiştir. Bunun yanında sektörel raporlar ve akademik yayınlardan yararlanılarak ekonomik gelişmeler, yem maliyetleri ve sürdürülebilirlik uygulamaları değerlendirilmiştir.

### *İncelenen Göstergeler*

Araştırma kapsamında Türkiye yumurta endüstrisinin performansını ortaya koymak amacıyla çeşitli üretim ve ekonomik göstergeler incelenmiştir. Bu doğrultuda yıllık yumurta üretim miktarı sektörün üretim kapasitesini değerlendirmek amacıyla analiz edilmiştir. Ayrıca yumurtacı tavuk sayısındaki değişimler incelenerek üretim altyapısındaki gelişmeler ortaya konulmuştur.

Kişi başı yumurta tüketim miktarı değerlendirilerek toplumun tüketim eğilimleri ve yumurtanın beslenmedeki yeri analiz edilmiştir. Bunun yanında sektörün dış ticaret performansını belirlemek amacıyla yumurta ihracat miktarı ve ihracat gelirleri incelenmiştir.

Sektörün ekonomik sürdürülebilirliği açısından önemli olan karma yem maliyetleri ve üretici fiyatları da araştırma kapsamına dahil edilmiştir. Özellikle yem hammaddesi fiyatlarındaki değişimlerin üretim maliyetleri üzerindeki etkileri değerlendirilmiştir. Ayrıca ithalat–ihracat dengesi incelenerek sektörün dış ticaret yapısı analiz edilmiştir.

İncelenen temel göstergeler aşağıda özetlenmiştir:

- Yumurta üretim miktarı
- Yumurtacı tavuk sayısı
- Kişi başı yumurta tüketimi
- İhracat miktarı ve ihracat değeri
- Yem maliyetleri
- Üretici fiyatları
- İthalat–ihracat dengesi

### *Analiz Yöntemi*

Araştırmada elde edilen veriler tanımlayıcı istatistiksel yöntemler kullanılarak değerlendirilmiştir. Öncelikle 2016–2025 yılları arasındaki veriler yıllara göre sınıflandırılmış ve karşılaştırmalı analiz yöntemiyle incelenmiştir. Üretim, tüketim, ihracat ve ekonomik göstergelerde meydana gelen değişimler yüzdesel değişim oranları kullanılarak değerlendirilmiştir.

Verilerin analizinde tablo ve grafiklerden yararlanılmış, sektörün yıllar içerisindeki gelişim eğilimi görsel olarak ortaya konulmuştur. Özellikle üretim miktarı, ihracat hacmi ve yem maliyetlerindeki değişimler karşılaştırmalı tablolar yardımıyla değerlendirilmiştir.

Ayrıca sektörde yaşanan ekonomik dalgalanmalar, yem fiyatlarındaki artışlar, biyogüvenlik riskleri ve ihracat politikalarının sektör performansı üzerindeki etkileri literatür bulguları doğrultusunda tartışılmıştır. Elde edilen sonuçlar önceki çalışmalarla karşılaştırılarak yorumlanmış ve sektörün mevcut durumu ile geleceğe yönelik gelişim potansiyeli değerlendirilmiştir.

## Bulgular ve Tartışma

### *Türkiye'de Yumurta Üretiminin Gelişimi*

Türkiye yumurta sektörü 2016–2025 döneminde üretim kapasitesi açısından önemli bir gelişim göstermiştir. Modern üretim sistemlerinin yaygınlaşması, entegre tesis yatırımlarının artması ve ihracat talebinin yükselmesi

üretim miktarındaki artışta etkili olmuştur. Özellikle 2016–2023 yılları arasında üretimde düzenli bir büyüme eğilimi görülmüştür. Ancak 2024 ve 2025 yıllarında yem maliyetleri, kuş gribi riskleri ve ihracat düzenlemeleri nedeniyle üretimde gerileme meydana gelmiştir.

Türkiye, yıllık yaklaşık 20 milyar adedin üzerindeki üretim kapasitesi ile dünya yumurta üretiminde ilk 10 ülke arasında yer almaktadır. Özellikle Orta Doğu ve yakın coğrafyalara yapılan ihracat sektörün büyümesine katkı sağlamıştır. Üretimdeki artış aynı zamanda modern kümes sistemleri ve otomasyon teknolojilerinin yaygınlaşması ile ilişkilendirilmektedir.

Tablo 1. Türkiye'de Yumurta Üretim Miktarının Yıllara Göre Değişimi (2016–2025)

| *Yıl* | *Üretim (Milyar Adet)* | *Değişim (%)* |
|---|---|---|
| 2016 | 18.5 | — |
| 2017 | 19.0 | 2.7 |
| 2018 | 19.6 | 3.2 |
| 2019 | 20.1 | 2.6 |
| 2020 | 20.6 | 2.5 |
| 2021 | 21.1 | 2.4 |
| 2022 | 21.8 | 3.3 |
| 2023 | 22.3 | 2.2 |
| 2024 | 21.2 | -4.9 |
| 2025 | 19.9 | -6.0 |

**Tavuk Varlığı ve İşletme Kapasitesi**

Yumurtacı tavuk sayısı, üretim kapasitesinin temel göstergelerinden biridir. Türkiye'de yumurtacı tavuk varlığı 2016–2023 döneminde sürekli artış göstermiştir. Özellikle büyük ölçekli entegre işletmelerin yaygınlaşması üretim kapasitesini artırmıştır. Ancak son yıllarda yüksek maliyetler ve hastalık riskleri nedeniyle tavuk varlığında azalma görülmüştür.

Türkiye'de özellikle kafes sistemleri, otomatik yemleme sistemleri ve çevre kontrollü kümes teknolojileri yaygın olarak kullanılmaktadır. Entegre işletmelerin artışı üretim verimliliğini artırırken biyogüvenlik uygulamalarının gelişmesine de katkı sağlamıştır.

Tablo 2. Türkiye'de Yumurtacı Tavuk Sayısı (2016–2025)

| *Yıl* | *Yumurtacı Tavuk Sayısı (Milyon Baş)* |
|---|---|
| 2016 | 110 |
| 2017 | 115 |
| 2018 | 118 |
| 2019 | 121 |
| 2020 | 124 |
| 2021 | 126 |
| 2022 | 129 |
| 2023 | 132 |
| 2024 | 128 |
| 2025 | 122 |

***Kişi Başı Yumurta Tüketimi***

Türkiye'de kişi başı yumurta tüketimi son on yılda artış göstermiştir. Yumurtanın ekonomik ve kaliteli bir protein kaynağı olması tüketim artışında etkili olmuştur. Ayrıca sağlıklı beslenme bilincinin artması da yumurta tüketimini desteklemiştir.

Türkiye'de kişi başı tüketim dünya ortalamasına yakın seviyelere ulaşmıştır. Özellikle pandemi döneminde protein tüketimine verilen önemin artması yumurta tüketiminde yükselişe neden olmuştur. Ancak son yıllarda fiyat artışları tüketim üzerinde baskı oluşturmuştur.

***İhracat Performansı***

Türkiye yumurta ihracatında dünya çapında önemli ülkeler arasında yer almaktadır. Özellikle Irak, Birleşik Arap Emirlikleri, Katar ve Afrika ülkeleri önemli ihracat pazarları arasında bulunmaktadır. İhracat performansındaki artış sektörün ekonomik büyümesine önemli katkı sağlamıştır.

Son yıllarda ihracat kısıtlamaları, ticari düzenlemeler ve bölgesel siyasi gelişmeler ihracat performansını olumsuz etkilemiştir. Özellikle bazı ülkelerde uygulanan ithalat kotaları ve lojistik sorunlar sektör açısından risk oluşturmuştur.

Tablo 3. Türkiye'de Kişi Başı Yumurta Tüketimi (2016–2025)

| *Yıl* | *Tüketim (Adet/Kişi/Yıl)* |
|---|---|
| 2016 | 210 |
| 2017 | 215 |
| 2018 | 223 |
| 2019 | 230 |
| 2020 | 235 |
| 2021 | 242 |
| 2022 | 248 |
| 2023 | 255 |
| 2024 | 250 |
| 2025 | 246 |

Tablo 4. Türkiye'nin Yumurta İhracat Performansı (2016–2025)

| *Yıl* | *İhracat (Milyar Adet)* | *İhracat Geliri (Milyon $)* |
|---|---|---|
| 2016 | 3.2 | 280 |
| 2017 | 3.5 | 300 |
| 2018 | 3.8 | 320 |
| 2019 | 4.0 | 340 |
| 2020 | 4.1 | 355 |
| 2021 | 4.3 | 370 |
| 2022 | 4.5 | 395 |
| 2023 | 4.7 | 420 |
| 2024 | 4.2 | 398 |
| 2025 | 3.8 | 360 |

***Yem Maliyetleri ve Ekonomik Sorunlar***

Yumurta üretim maliyetlerinin en büyük bölümünü yem giderleri oluşturmaktadır. Özellikle mısır ve soya fasulyesi gibi hammaddelerde dışa bağımlılık maliyetleri artırmıştır. Döviz kurundaki yükselişler yem fiyatlarının önemli ölçüde artmasına neden olmuştur.

Enerji maliyetleri, işçilik giderleri ve lojistik harcamalarındaki artışlar da üretim maliyetlerini yükseltmiştir. Bu durum üretici kârlılığını azaltmış ve küçük ölçekli işletmeler üzerinde ekonomik baskı oluşturmuştur.

Tablo 5. Karma Yem Fiyatlarının Yıllara Göre Değişimi

| *Yıl* | *Ortalama Yem Fiyatı (TL/Ton)* |
|---|---|
| 2016 | 850 |
| 2017 | 980 |
| 2018 | 1250 |
| 2019 | 1450 |
| 2020 | 1650 |
| 2021 | 2400 |
| 2022 | 4200 |
| 2023 | 6200 |
| 2024 | 7800 |
| 2025 | 9200 |

**Sektörde Karşılaşılan Temel Problemler**

Türkiye yumurta sektörü son yıllarda çeşitli ekonomik ve biyolojik risklerle karşı karşıya kalmıştır. Özellikle yüksek patojeniteli kuş gribi vakaları sektör açısından önemli tehditlerden biri olmuştur. Hastalık riskleri bazı dönemlerde üretim kayıplarına ve ihracat kısıtlamalarına neden olmuştur.

Özellikle yem maliyetlerindeki artış sektörün en önemli ekonomik sorunu olarak öne çıkmaktadır. Bunun yanında üretici fiyatlarının maliyet artışlarını yeterince karşılayamaması sektörde sürdürülebilirlik sorunlarına yol açmaktadır.

***Sürdürülebilirlik ve Gelecek Perspektifi***

Son yıllarda sürdürülebilir üretim uygulamaları yumurta sektöründe daha fazla önem kazanmıştır. Hayvan refahı uygulamaları, biyogüvenlik önlemleri ve çevre dostu üretim sistemleri sektörün geleceği açısından kritik görülmektedir.

Güneş enerjisi sistemleri, otomatik iklimlendirme teknolojileri ve çevre dostu üretim modelleri sektörde yaygınlaşmaya başlamıştır. Bunun yanında antibiyotik kullanımının azaltılması ve sürdürülebilir yem kaynaklarının geliştirilmesi gelecekte sektör açısından önemli konular arasında yer almaktadır.

Tablo 6. Türkiye Yumurta Sektörünün Başlıca Sorunları

| *Problem* | *Sektöre Etkisi* |
|---|---|
| Yem maliyetleri | Üretim maliyetlerinde artış |
| Kuş gribi riski | Üretim ve ihracat kaybı |
| Döviz kuru dalgalanmaları | Girdi maliyetlerinde artış |
| Enerji maliyetleri | Karlılıkta azalma |
| İhracat kısıtlamaları | Dış ticaret hacminde düşüş |
| Piyasa dalgalanmaları | Fiyat istikrarsızlığı |

Tablo 7. Sürdürülebilirlik Uygulamaları ve Amaçları

| *Uygulama* | *Amaç* |
|---|---|
| Biyogüvenlik sistemleri | Hastalık riskini azaltma |
| Hayvan refahı uygulamaları | Verimlilik ve kalite artışı |
| Yenilenebilir enerji kullanımı | Enerji maliyetlerini azaltma |
| Atık yönetimi | Çevresel sürdürülebilirlik |
| Su tasarrufu sistemleri | Doğal kaynakların korunması |

## Sonuç ve Öneriler

### *Genel Değerlendirme*

Türkiye yumurta endüstrisi, 2016–2025 döneminde üretim kapasitesi, ihracat hacmi ve teknolojik altyapı açısından önemli gelişmeler göstermiştir. Son on yıllık süreçte yumurta üretim miktarında genel olarak artış eğilimi görülmüş, özellikle 2016–2023 yılları arasında sektör istikrarlı bir büyüme süreci yaşamıştır. Modern kümes sistemlerinin yaygınlaşması, entegre işletmelerin artışı ve ihracat kapasitesinin gelişmesi sektörün büyümesine önemli katkılar sağlamıştır.

Türkiye, yıllık yaklaşık 20 milyar adedin üzerindeki yumurta üretimi ile dünya yumurta üretiminde önde gelen ülkeler arasında yer almaktadır. Ayrıca Orta Doğu başta olmak üzere birçok ülkeye gerçekleştirilen ihracat sayesinde dünya yumurta ticaretinde önemli bir konuma ulaşmıştır. Özellikle Irak, Körfez ülkeleri ve bazı Afrika ülkeleri Türkiye'nin başlıca ihracat pazarlarını oluşturmaktadır.

Bununla birlikte son yıllarda sektörde bazı yapısal ve ekonomik sorunlar daha belirgin hale gelmiştir. Yem hammaddelerinde dışa bağımlılık, döviz kuru dalgalanmaları, enerji maliyetlerindeki artış ve lojistik giderleri üretim maliyetlerini önemli ölçüde yükseltmiştir. Özellikle karma yem fiyatlarındaki hızlı artış üretici kârlılığını azaltmış ve küçük ölçekli işletmeler üzerinde ekonomik baskı oluşturmuştur.

Ayrıca kuş gribi gibi biyogüvenlik riskleri, dönemsel ihracat kısıtlamaları ve piyasa dalgalanmaları sektörün sürdürülebilirliği açısından önemli tehditler arasında yer almıştır. Buna rağmen Türkiye yumurta sektörü sahip olduğu üretim kapasitesi, modern teknolojik altyapısı ve ihracat potansiyeli sayesinde küresel pazarda rekabet gücünü korumayı sürdürmektedir.

Sonuç olarak Türkiye yumurta endüstrisi, son on yılda önemli bir gelişim göstermiş ancak sürdürülebilir büyümenin devam edebilmesi için ekonomik, teknik ve yapısal sorunların çözümüne yönelik yeni stratejilere ihtiyaç duyulmaktadır.

### *Sektöre Yönelik Öneriler*

Türkiye yumurta sektörünün sürdürülebilir gelişiminin sağlanabilmesi amacıyla aşağıdaki öneriler geliştirilmiştir:

#### *Yerli Yem Hammaddesi Üretiminin Artırılması*

Yem maliyetleri üretim giderlerinin büyük bölümünü oluşturmaktadır. Bu nedenle mısır, soya fasulyesi ve diğer yem hammaddelerinde yerli üretimin artırılması büyük önem taşımaktadır. Yem hammaddelerinde dışa bağımlılığın azaltılması sektörün ekonomik kırılganlığını düşürecektir.

#### *İhracat Politikalarının Geliştirilmesi*

İhracat sektörün büyümesinde temel unsurlardan biridir. Bu nedenle ihracat süreçlerinde sürdürülebilir ve istikrarlı politikaların uygulanması gerekmektedir. Yeni pazar arayışlarının desteklenmesi ve dış ticaret kısıtlamalarının minimize edilmesi sektörün rekabet gücünü artıracaktır.

***Üretici Desteklerinin Artırılması***

Üreticilere yönelik yem, enerji ve kredi desteklerinin artırılması sektörün ekonomik sürdürülebilirliğine katkı sağlayacaktır. Özellikle küçük ve orta ölçekli işletmelerin korunmasına yönelik teşvik politikaları önem taşımaktadır.

***Biyogüvenlik Yatırımlarının Güçlendirilmesi***

Kuş gribi ve diğer hastalık risklerinin azaltılması amacıyla biyogüvenlik uygulamalarının yaygınlaştırılması gerekmektedir. Modern dezenfeksiyon sistemleri, kontrollü giriş uygulamaları ve düzenli sağlık kontrolleri sektör açısından kritik öneme sahiptir.

***Sürdürülebilir Üretim Modellerinin Yaygınlaştırılması***

Çevresel sürdürülebilirlik açısından yenilenebilir enerji sistemleri, atık yönetimi uygulamaları ve su tasarrufu sağlayan teknolojilerin kullanımının artırılması gerekmektedir. Ayrıca hayvan refahı uygulamalarının geliştirilmesi hem üretim kalitesini hem de uluslararası pazarlardaki rekabet gücünü olumlu yönde etkileyecektir.

***Üniversite–Sektör İş Birliğinin Güçlendirilmesi***

Sektörde verimlilik artışı ve teknolojik gelişimin desteklenmesi amacıyla üniversiteler ile sektör kuruluşları arasındaki iş birliğinin artırılması önemlidir. Bilimsel araştırmaların üretim süreçlerine entegrasyonu sektörün uzun vadeli gelişimine katkı sağlayacaktır.

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# Valorization of Date Sorting Rejects and Date Pits from Mejhoul, Boufeggous, and Jihel Varieties: Processing, Product Development, and Physicochemical Characterization

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## Abstract

Date sorting rejects, corresponding to fruits with visual defects and low market value, together with date pits, are agro-food by-products that remain underutilized despite their nutritional and technological potential. This study focused on the valorization of these by-products from three Moroccan date varieties (Mejhoul, Boufeggous, and Jihel) for the development of innovative food products. First, the raw materials were characterized in terms of their physicochemical and microbiological properties. Based on these characteristics, several value-added products were developed, including date syrup, date paste, date powder, date-enriched biscuits, and a coffee substitute produced from roasted date pits. The results showed that date sorting rejects present favorable characteristics for valorization, with low water activity (0.303–0.342), high soluble solids content (61.6–78 °Brix), and good microbiological stability. The produced date syrups exhibited high yields (>80%), total soluble solids ranging from 69.9 to 72.8 °Brix, pH values between 4.25 and 4.53, and viscosities from 2,924 to 7,929 cP, comparable to commercial products. Date pastes showed yields above 100%, total soluble solids of 63.4–65.6 °Brix, and water activity between 0.55 and 0.70. Date powders were characterized by low moisture content (<10%) and very low water activity (<0.18), ensuring good storage stability. The powder from the Jihel variety proved particularly suitable for biscuit formulation, allowing complete replacement of sucrose and up to 50% substitution of wheat flour while maintaining acceptable sensory quality. In contrast, coffee substitutes produced from date pits exhibited satisfactory physicochemical properties but limited consumer acceptance. Overall, these findings highlight the strong potential of date sorting rejects and date pits as sustainable raw materials for the development of value-added food products, contributing to the reduction of post-harvest losses and the promotion of a circular economy in the date palm sector.

***Keywords:*** *Date sorting rejects; valorization; syrup; paste; Biscuit formulation; coffee substitutes*

## Introduction

The phoeniculture sector in Morocco plays a significant socio-economic role, particularly in oasis regions where date palm cultivation (*Phoenix dactylifera* L.) represents a key source of income and rural stability. Moroccan dates are recognized as an important terroir product due to their nutritional richness in natural sugars, dietary fibers, and bioactive compounds, making them highly suitable for value addition through processing (Harrak et al., 2019; El Janati et al., 2022).

However, a considerable proportion of the production consists of sorting by-products, including downgraded dates and date pits, which remain underutilized despite their promising potential for agro-food applications. These by-products can be effectively valued through the development of diversified products such as syrups, pastes, bakery products, functional flours, and coffee substitutes, thereby contributing to post-harvest loss reduction and increased economic value of the supply chain (Harrak et al., 2019).

Previous studies have highlighted the technological and nutritional potential of dates and their by-products in food formulation, particularly as partial or total substitutes for sugar and wheat flour, as well as producing functional beverages from roasted date pits (Elkatry et al., 2024; Farag et al., 2019). Nevertheless, product quality and consumer acceptance remain strongly dependent on processing conditions and formulation parameters, highlighting the need for further optimization and characterization.

In Morocco, research efforts conducted by the National Institute of Agricultural Research (INRA) have contributed to the development of date-based products and post-harvest technologies (Harrak et al., 2018; Noutfia et al., 2019). However, further work is still required to standardize processing techniques and to improve the physicochemical and sensory quality of the derived products.



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In this context, the valorization of dates and their by-products through transformation into innovative food products represents a sustainable approach to enhance the competitiveness of the date palm sector and meet the growing demand for natural and functional foods.

## Materials and Methods

### *Plant Material*

Sorting rejects of three Moroccan date varieties, namely Mejhoul, Boufeggouss, and Jihel (with two textures: normal and hard), were collected from a date storage and packing unit located in the Drâa–Tafilalet region. Upon arrival at the laboratory, the samples were subjected to physicochemical analyses, including moisture content, ash content, water activity, pH, titratable acidity, total soluble solids (°Brix), and hardness, as well as microbiological analyses involving the determination of total aerobic mesophilic flora and fungal microflora. The samples were then stored at 4 °C until processing.

### *Physicochemical Characterization*

#### *Determination of Moisture Content*

Moisture content, corresponding to the wet fraction of dates, was determined according to AOAC method No. 920.151 by drying a 10 g sample of dates in an oven (WiseVen®) at 70 °C to avoid sample caramelization, until constant crucible weight was reached (AOAC, 1990). Measurements were performed in triplicate for each variety.

#### *Determination of Ash Content*

The dry matter previously obtained from date samples was incinerated at 500 °C for 5 h in a muffle furnace (Ney® VULCAN D-550) until a whitish ash of constant weight was obtained (NF V05-113, 1972). Measurements were performed in triplicate for each sample.

#### *Determination of Water Activity*

Water activity (aw) was measured using an aw-meter (Labswift, Novasina AG®). Five grams of dates cut into small pieces were placed in a sample cup and introduced into the measuring chamber. The aw value was displayed once the air inside the chamber reached thermodynamic equilibrium with the sample (Chirife and Fontan, 1982). Measurements were performed in triplicate for each sample.

#### *Determination of pH*

pH was determined using a pH meter (AD1030 - Adwa®) according to AOAC method No. 981.12 (AOAC, 1990), using a date/distilled water weight ratio of 0.2. Three replicates were carried out for each sample.

#### *Determination of Titratable Acidity*

Titratable acidity (expressed as g of malic acid per 100 g fresh weight of dates) was determined according to the acid–base titration method described by the French standard NF V05-101 (AFNOR, 1974). Titration was performed using 0.1 N sodium hydroxide (NaOH) in the presence of phenolphthalein as an indicator. Analyses were conducted in triplicate for each variety.

#### *Determination of Total Soluble Solids (°Brix)*

Total soluble solids content, expressed as degrees Brix (1 °Brix corresponding to 1% sugars), was determined using a digital refractometer (ATAGO® model PR-201α) according to AOAC method No. 932.12 (AOAC, 1990). Analyses were conducted in triplicate for each variety.

#### *Hardness Measurement*

Date firmness was determined using a CT3-4500 texture analyzer (Brookfield®) equipped with a cylindrical puncture probe (6 mm diameter) moving at a speed of 30 mm/min to a depth of 5 mm. The compression force exerted by the probe on the date pulp was expressed in Newtons (Hazbavi et al., 2014). Analyses were performed in ten replicates for each variety.

### *Microbiological Characterization*

A 25 g sample of dates from each variety was suspended in 225 mL of sterile distilled water. Subsequently, a series of decimal dilutions was prepared from stock suspension. Then, 0.1 mL of each dilution was spread onto the surface of two agar media (PCA and PDA), using two Petri dishes per dilution and per medium. PCA medium was used for the enumeration of total aerobic mesophilic flora, with incubation at 37 °C for 24 h, whereas PDA medium was used for fungal microflora enumeration, with incubation at 30 °C for 72 h. Results were expressed as colony-forming units per gram (CFU $g^{-1}$).

***Processing of Dates and Characterization of Derived Products***

***Processing***

The date sorting rejects analyzed, including their pits, were used as raw materials to produce several value-added products, namely date syrup, date paste, date flour, and a coffee substitute prepared from date pits. The different processing steps involved in the production of these products are summarized in Figure 1.

The date powder from the Jihel variety (hard texture) was used to prepare a dry biscuit according to the formulation detailed in Table 1, with the aim of substituting sucrose and partially replacing soft wheat flour. Baking was carried out at 180 °C for 10 min.

Table 1. Formulation used for the preparation of dry biscuits based on Jihel date flour

| *Ingredients* | *Control biscuit 1 (100% soft wheat flour)* | *Biscuit with 10% date powder* | *Biscuit with 30% date powder* | *Biscuit with 50% date powder* | *Biscuit with 70% date powder* | *Control biscuit 2 (100% date powder)* |
|---|---|---|---|---|---|---|
| Soft wheat flour | 250g | 225g | 175g | 125g | 75g | 0 |
| Date powder | 0 | 25g | 75g | 125g | 175g | 250g |
| Sugar | 125g | 0 | 0 | 0 | 0 | 0 |
| Butter | 125g | 125g | 125g | 125g | 125g | 125g |
| Egg | 1 | 1 | 1 | 1 | 1 | 1 |
| Salt | 1 pinch | 1 pinch | 1 pinch | 1 pinch | 1 pinch | 1 pinch |

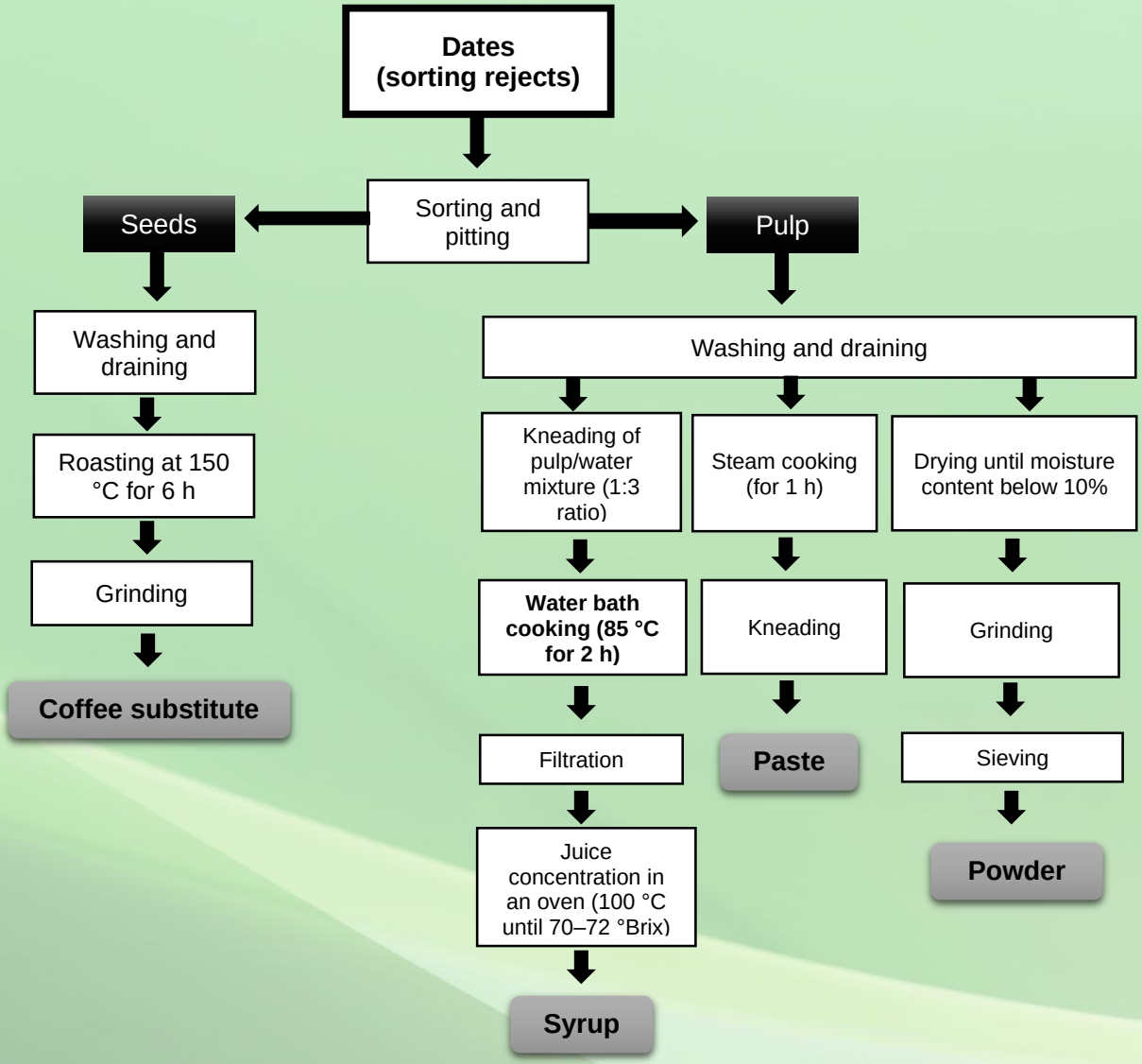



Figure 1. Operational steps to produce date-derived products

***Characterization and Sensory Analysis***

The syrups, pastes, coffee substitutes, and powders were subjected to the physicochemical analyses described above, namely moisture content, ash content, water activity (aw), pH, and total soluble solids (°Brix), in addition to color indices L* (lightness), a* (green–red), and b* (blue–yellow), using a spectrocolorimeter (HunterLab, MiniScan EZ) in ten replicates (Al-Hooti et al., 1997).

For the date syrups obtained, density and viscosity were also measured in ten replicates. Kinematic viscosity (in cSt) was determined using a rotational viscometer (Fungilab Smart Series) (Yücel et al., 2013), equipped with an L2 spindle rotating at 2.5 rpm at approximately 25 ± 2 °C. Results were expressed in centipoise (cP).

Regarding the formulated date pastes, firmness was determined in ten replicates using a CT3-4500 texture analyzer (Brookfield®), as previously described.

Finally, a sensory panel composed of 10 assessors evaluated the manufactured products (syrup, paste, coffee substitute, and biscuits) using evaluation sheets based on taste, odor, color, and texture, on a global 20-point scale.

Some quality attributes of syrups and pastes obtained from the three date varieties were monitored over a two-month storage period at 4 °C. Hermetically sealed glass bottles and plastic trays were used as packaging for the syrups and pastes, respectively.

***Statistical Analysis***

Statistical analyses were carried out using GraphPad Prism software (version 8.0.2 for Windows). The physicochemical parameters were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

**Results**

***Quality of Date Sorting Rejects***

***Physicochemical Characterization***

The mean values of the physicochemical parameters determined for date sorting rejects from the three varieties Mejhoul, Boufeggouss, and Jihel (normal and hard textures) are presented in Table 2. The aim of this characterization was to assess the initial quality of the dates and to guide their processing and valorization.

Table 2. Physicochemical characteristics of sorting rejects from the three date varieties studied

| *Parameter* | *Mejhoul* | *Boufeggous* | *Jihel (normal texture)* | *Jihel (hard texture)* |
|---|---|---|---|---|
| Moisture (%) | 18,54 ± 1,22 $^{b}$ | 22,95 ± 0,725 $^{a}$ | 16,09 ± 1,24 $^{b}$ | 11,67 ± 0,827 $^{c}$ |
| Asch (%) | 2,41 ± 0,388 $^{a}$ | 2,56 ± 0,544 $^{a}$ | 2,01 ± 0,06 $^{a}$ | 2,4 ± 0,108 $^{a}$ |
| $a_w$ | 0,338 ± 0,001 $^{a}$ | 0,342 ± 0,0015 $^{a}$ | 0,321 ± 0,0025 $^{b}$ | 0,303 ± 0,0023 $^{c}$ |
| pH | 6,26 ± 0,005 $^{b}$ | 6,42 ± 0,011 $^{a}$ | 5,68 ± 0,011 $^{c}$ | 5,35 ± 0,005 $^{d}$ |
| Titratable acidity (g malic acid/100 g of dates) | 0,201 ± 0,0096 $^{b,c}$ | 0,247 ± 0,029 $^{a,b}$ | 0,195 ± 0,005 $^{c}$ | 0,285 ± 0,019 $^{a}$ |
| Total soluble solids (°Brix) | 78 ± 0,6 $^{a}$ | 63,8 ± 0,346 $^{c}$ | 69,2 ± 0,346 $^{b}$ | 61,6 ± 0,346 $^{d}$ |
| Firmness (N) | 4,31 ± 0,146 $^{c}$ | 2,84 ± 0,0819 $^{d}$ | 12,16 ± 0,149 $^{b}$ | 35,93 ± 0,098 $^{a}$ |

Values are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Means in the same row bearing different superscript letters differ significantly at $p < 0.05$.

***Physicochemical Quality of Date Sorting Rejects***

The moisture contents obtained for the three studied date varieties allowed classifying Boufeggous as a semi-soft date (20% < moisture < 30%) and Mejhoul and Jihel as dry dates (moisture < 20%) according to Lobo et al. (2014). These values are lower than those reported by Bouhlali et al. (2017) for the same varieties from the same region. Several factors may influence date moisture content within the same variety, including maturity stage (Al-Shahib and Marshall, 2003), geographical origin (Elhoumaizi et al., 2023), climatic conditions (Hasnaoui et al., 2011; Outghouliast et al., 2019), and storage duration and conditions (Hazbavi et al., 2015; Aleid et al., 2014). In the present study, and according to Harrak et al. (2005), Boufeggous, being the most humid variety, is the most suitable for syrup extraction and paste production, whereas Jihel (hard texture, lowest moisture content) is the most appropriate for flour production. It has been reported that one of the main criteria for selecting date varieties for syrup extraction is fruit consistency, which should be soft, semi-soft, or capable of becoming so after soaking (Houssni et al., 2022). In a previous study, Harrak et al. (2018) used sorting rejects and semi-soft Moroccan date varieties of medium commercial quality (Bouittob and Bousthammi) for paste production. Moreover, other researchers used Mejhoul sorting rejects to develop a stable paste for potential incorporation into food products (Sánchez-Zapata et al., 2011). The suitability of Jihel for flour production has also been confirmed in a previous study by Harrak and Jaouan (2010).

Regarding water activity (aw), values ranged from 0.303 (hard Jihel) to 0.342 (Boufeggous). These values are lower than those reported by Hasnaoui et al. (2011), who also showed that aw can vary depending on maturity stage and climatic conditions. This is likely due to dehydration resulting from water evaporation during storage. Noutfia et al. (2018) reported that some Moroccan date varieties spontaneously lose part of their moisture/weight depending on initial water content and storage conditions (temperature, relative humidity, packaging, etc.).

Ash content reflects the total mineral content of a sample. The results showed values ranging from 2.01% to 2.56%, consistent with literature data (Hasnaoui et al., 2010; Harrak, 2022). Mineral content depends on soil type, fertilizers, irrigation water quality, and cultivation practices (Ismail et al., 2006). However, ash content is not a strong varietal marker but may help characterize geographical origin (Harrak and Boujnah, 2012).

All three varieties exhibited slightly acidic pH values. Boufeggous showed the highest pH, followed by Mejhoul and Jihel. Mejhoul dates are reported to have a pH close to neutrality, indicating good commercial quality (Harrak and Hamouda, 2005).

Titratable acidity ranged from 0.195 to 0.285 g malic acid/100 g dates, consistent with previous studies (Noutfia et al., 2019; Harrak, 2022). Some authors reported that malic acid content increases during storage due to continued postharvest ripening (Al-Qurashi and Awad, 2011; Mahmood et al., 2012).

Total soluble solids were highest in Mejhoul (78%), while the other varieties ranged from 61.6% to 69.2%. Differences among varieties were significant ($p < 0.05$). Previous studies on 20 Moroccan date varieties reported values ranging from 63.9% to 82.8% (Harrak et al., 2005). These values may increase during storage due to continued ripening, moisture loss, or polysaccharide hydrolysis into simple sugars (Hazbavi et al., 2015; Noutfia et al., 2019). High sugar content is technologically valuable for syrup and sugar extraction (Harrak and Boujnah, 2012).

Regarding texture, Jihel was the hardest variety (35.93 and 12.6 N for hard and normal texture batches, respectively), followed by Mejhoul (4.31 N) and Boufeggous (2.84 N), with $p < 0.05$. Strong correlations have been reported between texture properties and date chemical composition (Singh et al., 2015). Fruit firmness is generally negatively correlated with moisture content; thus, moisture loss leads to fruit hardening (Afoakwa and Sefa-Dedeh, 2001).

Overall, the physicochemical characterization indicates that the studied sorting rejects are of medium quality and suitable for transformation into paste and syrup, while Jihel (hard texture) appears most appropriate for flour production.

***Microbiological Characterization***

Microbial enumeration of Mejhoul, Boufeggous, and Jihel date samples revealed a complete absence of yeasts and molds on PDA medium, and the presence of only a few bacterial colonies (not exceeding 30 CFU) on PCA plates corresponding to the $10^{-1}$ dilution. These results indicate that the date sorting rejects exhibit very good hygienic quality. Due to their richness in phenolic compounds (AlFaris et al., 2021), dates are generally protected against microbial contamination. In addition, the microbiological safety of the raw materials used in this study is ensured by their slightly acidic pH and low water activity (aw).

It should be noted that the processing methods applied in this study include thermal treatments, which further contribute to the high hygienic quality of the derived products**.**

***Yield, Quality, Evaluation and Stability of Derived Products***

***Date Syrup***

Date syrups obtained from the three varieties Mejhoul, Boufeggous, and Jihel are illustrated in Figure 2. The results of date syrup yields and their physicochemical properties are presented in Table 3.

The three date varieties used in this study yielded syrups comparable to commercial products, with densities ranging from 1.15 to 1.26. These results are consistent with those reported by Djafri et al. (2021) but lower than those obtained by other authors (Mimouni and Siboukeur, 2011; Boussaid et al., 2020; Laouar et al., 2021) for Algerian date syrups. The yields obtained exceeded 80% and were higher than those reported by Djafri et al. (2021). Significant differences were observed among the three syrups.

The Mejhoul syrup showed the highest moisture content (47.12%), followed by Boufeggous (45.72%) and Jihel (39.68%) ($p < 0.05$). These values are in agreement with those reported by Bouhlali et al. (2020), who found moisture contents ranging from 30.4% to 45.9% in Moroccan date syrups. Moisture content depends on variety, processing conditions, added water volume, temperature, and cooking time (Laouar et al., 2021). It is a key factor affecting syrup stability and susceptibility to fungal spoilage (Bellaouchi et al., 2017). Despite high moisture levels, the high sugar content reduces water availability, as reflected by water activity (aw). Mejhoul syrup showed the highest aw (0.735), followed by Jihel (0.631) and Boufeggous (0.602) ($p < 0.05$). These values inhibit most bacterial and fungal growth, which generally require aw > 0.9 and 0.7, respectively (Tapia et al., 2020).

Total soluble solids (°Brix) were consistent with values reported for Algerian date syrups (64–75%) (Laouar et al., 2021). A °Brix range of 70–75% is considered optimal for high-quality syrups (Mimouni and Siboukeur, 2011). Syrup pH values ranged from 4.25 to 4.53, in agreement with previous studies (Boussaid et al., 2020; Bouhlali et al., 2020). The low pH, combined with low aw and phenolic compounds, contributes to microbial inhibition and extends shelf life (Hamad, 2012).

Boufeggous syrup exhibited the highest ash content (2.45%) ($p < 0.05$), likely due to greater mineral diffusion during extraction. However, ash contents were lower than those reported for Moroccan date syrups (3.66–5.21%) (Bouhlali et al., 2020).

Table 3. Physicochemical characterization of the three obtained date syrups

| | *Mejhoul* | *Boufeggous* | *Jihel* |
|---|---|---|---|
| Yield (%) | 86,75 | 80,23 | 86,14 |
| Density | 1,16 | 1,15 | 1,26 |
| Moisture (%) | 47,12 ± 0,01 [a] | 45,72 ± 0,29 [b] | 39,68 ± 0,05 [c] |
| Ash (%) | 1,68 ± 0,07 [b] | 2,45 ± 0,43 [a] | 1,81 ± 0,037 [b] |
| $a_w$ | 0,735 ± 0,003 [a] | 0,602 ± 0,014 [c] | 0,631 ± 0,005 [b] |
| pH | 4,53 ± 0,015 [a] | 4,25 ± 0,02 [b] | 4,49 ± 0,01 [a] |
| Total soluble solids (°Brix) | 70,8 ± 0,2 [b] | 69,86 ± 0,11 [c] | 72,8 ± 0,2 [a] |
| L* | 9,88 ± 1,13 [b] | 7,21 ± 2,07 [c] | 12,8 ± 1,04 [a] |
| b* | 12,9 ± 0,81 [b] | 6,99 ± 0,62 [c] | 14,53 ± 0,8 [a] |
| a* | 11,99 ± 0,86 [a] | 5,91 ± 0,7 [c] | 9,87 ± 0,51 [b] |
| Viscosity (Cps) | 2924,45 ± 126,26 [c] | 7929,44 ± 839,57 [a] | 3729,76 ± 9,33 [b] |

Values are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Means in the same row bearing different superscript letters differ significantly at $p < 0.05$.




Figure 2. Date syrups of (1) Mejhoul, (2) Boufeggous, and (3) Jihel

Mineral content varies widely depending on variety and extraction techniques (Mimouni and Siboukeur, 2011; Boussaid et al., 2020; Djafri et al., 2021). Date syrups are generally richer in minerals than high-fructose syrups (~0.3%) (Mimouni and Siboukeur, 2011).

Viscosity is a key property for syrup quality and application. Boufeggous syrup showed the highest viscosity (7929.44 cP), followed by Jihel (3729.76 cP) and Mejhoul (2924.45 cP) ($p < 0.05$). These values are higher than those reported by Djafri et al. (2021) (419.01–803.7 cP). Viscosity is inversely related to moisture content and depends on soluble solids and temperature (Guérin et al., 1978; Gabsi et al., 2013). Enzymatic treatments can significantly reduce viscosity by degrading polysaccharides (Julai et al., 2023).

Color is a key quality attribute. Boufeggous syrup was the darkest (L* = 7.21), likely due to raw material color and browning reactions during concentration (Julai et al., 2023). Jihel syrup showed the highest lightness (L* = 12.8) and yellowness (b* = 14.53), consistent with previous findings (Bouhlali et al., 2020). Mejhoul syrup exhibited the highest redness (a* = 11.99), possibly due to carotenoids and anthocyanins (Al-Qarni et al., 2020).

Some quality attributes of the syrups obtained from the three date varieties were monitored over a two-month storage period at 4 °C. The samples were stored in hermetically sealed glass bottles. The results are presented in figure 3.

During storage, moisture content decreased in Mejhoul syrup from the first month, while changes appeared from the second month for Boufeggous and Jihel ($p < 0.05$). Water activity remained stable in Boufeggous but decreased in Mejhoul and increased in Jihel. These results differ from Naknean et al. (2013) and Hajmeer et al. (2000), who reported stable moisture and aw during storage. Variations may be attributed to packaging permeability (glass bottles) and initial moisture content.

pH decreased during storage, in agreement with Naknean et al. (2013), likely due to sugar degradation and organic acid formation. °Brix decreased in Mejhoul and Boufeggous but increased in Jihel, consistent with previous studies (Hajmeer et al., 2000; Chauhan et al., 2018). Viscosity decreased over time, as also reported by Hajmeer et al. (2000), due to polysaccharide degradation.

Color changes indicated progressive browning during storage, associated with Maillard reactions (Naknean et al., 2013). Variations in a* and b* values depended on variety and storage conditions.

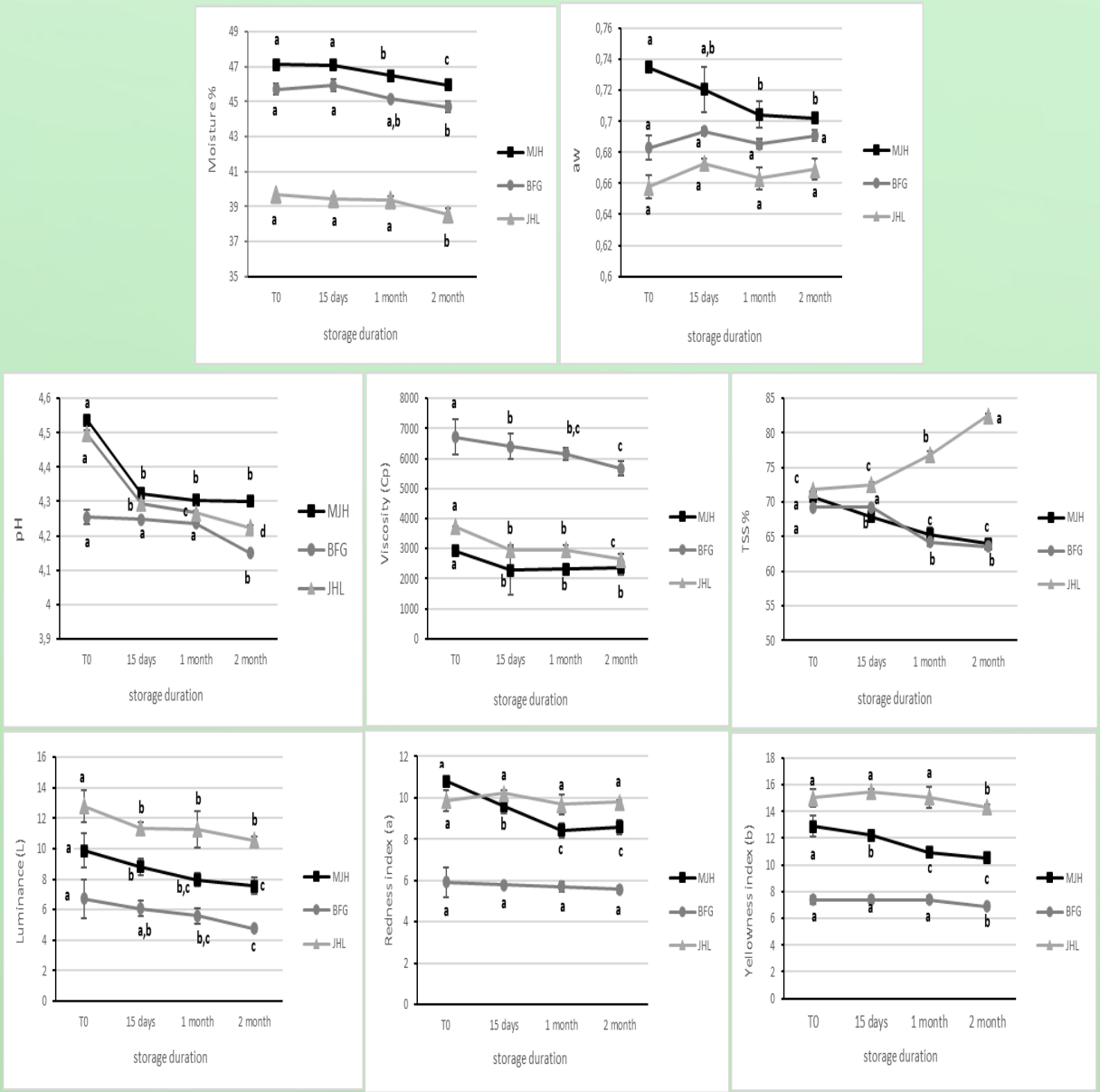



Figure 3. Evolution of quality attributes of the three syrups extracted from Mejhoul, Boufeggous, and Jihel dates

**Date Paste**

Date pastes obtained from the three varieties Mejhoul, Boufeggous, and Jihel are illustrated in Figure 4. The results of the yields obtained for date paste from the three studied varieties and their physicochemical properties are presented in Table 4.

The adopted process allows the production of date pastes with yields exceeding 100%, which can be explained by the hygroscopic nature of dates during steam cooking. No significant difference ($p > 0.05$) was observed in the moisture content between the paste of the Mejhoul variety (30.98%) and that of the Boufeggous variety (31.18%). However, the Jihel variety paste exhibited a lower moisture content (26.08%). These values exceed the limit specified by the Codex Standard (2013) and the Moroccan Ministerial Decree (MAPMDREF, 2018), which is around 20%. This highlights the need for incorporating other ingredients such as flours and oils, which could both improve the quality of the paste and adjust its moisture content. Indeed, Harrak et al. (2017) reported acceptable moisture levels in date pastes derived from two Moroccan varieties (Bouittob and Bousthammi) when using an improved formulation incorporating cereal flours, olive oil, and citric acid. However, it should be noted that the addition of additives in date paste is restricted by Moroccan standards.It has also been reported that the moisture content of date pastes depends on both the date variety and the cooking conditions (Al-Abid et al., 2007).The three pastes exhibited a slightly acidic pH and a moderate water activity (aw), in agreement with the findings of Ahmed and Ramaswamy (2006). Moreover, the three formulated pastes showed statistically similar lightness (L*), whereas redness (a*) and yellowness (b*) values were significantly higher in the Jihel variety paste.Similar L*

values have been reported for pastes prepared from Emirati date varieties (Khalas, Lulu, and Bumaan), with values of approximately 25, 21, and 22, respectively. However, the a* and b* values reported in that study were considerably higher than those obtained in the present work. These differences can be attributed to varietal effects as well as to the initial colour characteristics of the raw date material used for paste preparation (Ahmed and Ramaswamy, 2006). Although the developed pastes were well accepted by consumers, with sensory scores of 15.4, 14.2, and 13.4/20 for Mejhoul, Boufeggous, and Jihel, respectively, their rheological and textural properties still require improvement. Indeed, it has been reported that hardness decreases after processing dates into paste (Ahmed and Ramaswamy, 2006) and is negatively correlated with moisture content (Rahman and Al-Farsi, 2005).

During the two-month storage period at 4 °C (Figure 5), the date pastes of the three varieties showed a progressive decrease in weight, attributed to water loss during storage, which is confirmed by the decrease in moisture content. Water activity showed a slight decrease, with no statistically significant difference ($p > 0.05$), suggesting limited changes in water availability within the sugar-rich matrix during storage. Indeed, total soluble solids increased, likely due to a concentration effect resulting from water loss. An increase in hardness was also observed, indicating a progressive hardening of the date pastes, a phenomenon generally associated with reduced moisture content and the decrease in the plasticizing effect of water in sugar-rich matrices (Alhamdan and Hassan, 1999; Karazhiyan & Razavi, 2012). Finally, colour indices changed during storage, reflecting progressive modifications in the visual properties of the date pastes over the storage period.

Table 4. Physicochemical characterization of the pastes obtained from the three date varieties

| | *Mejhoul* | *Boufeggous* | *Jihel* |
|---|---|---|---|
| Yield (%) | 105,51 | 108,12 | 107,64 |
| Moisture (%) | 30,98 ± 0,204 $^{a}$ | 31,18 ± 0,85 $^{a}$ | 26,08 ± 0,52 $^{b}$ |
| Ash (%) | 2 ± 0,05 $^{a}$ | 1,99 ± 0,12 $^{a}$ | 2,03 ± 0,002 $^{a}$ |
| $a_w$ | 0,697 ± 0,013 $^{a}$ | 0,571 ± 0,003 $^{b}$ | 0,551 ± 0,003 $^{b}$ |
| pH | 5,36 ± 0,03 $^{b}$ | 5,58 ± 0,02 $^{a}$ | 5,12 ± 0,02 $^{c}$ |
| Titratable acidity (g malic acid/100 g of dates) | 0,041 ± 0,04 $^{b}$ | 0,094 ± 0,03 $^{a}$ | 0,134 ± 0,02 $^{a}$ |
| Total soluble solids (°Brix) | 64,8 ± 0,34 $^{b}$ | 63,4 ± 0,35 $^{c}$ | 65,6 ± 0,35 $^{a}$ |
| L* | 21,98 ± 1,34 $^{a}$ | 21,87 ± 0,8 $^{a}$ | 22,33 ± 0,82 $^{a}$ |
| b* | 3,77 ± 0,34 $^{b}$ | 3,47 ± 0,59 $^{b}$ | 4,86 ± 0,72 $^{a}$ |
| a* | 6,94 ± 0,51 $^{b}$ | 6,25 ± 0,57 $^{c}$ | 8,67 ± 1,05 $^{a}$ |
| Firmness (N) | 0,606 ± 0,081 $^{b}$ | 0,9208 ± 0,11 $^{a}$ | 0,889 ± 0,12 $^{a}$ |

Values are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Means in the same row bearing different superscript letters differ significantly at $p < 0.05$.




Figure 4. Date pastes prepared from (1) Mejhoul, (2) Boufeggous, and (3) Jihel

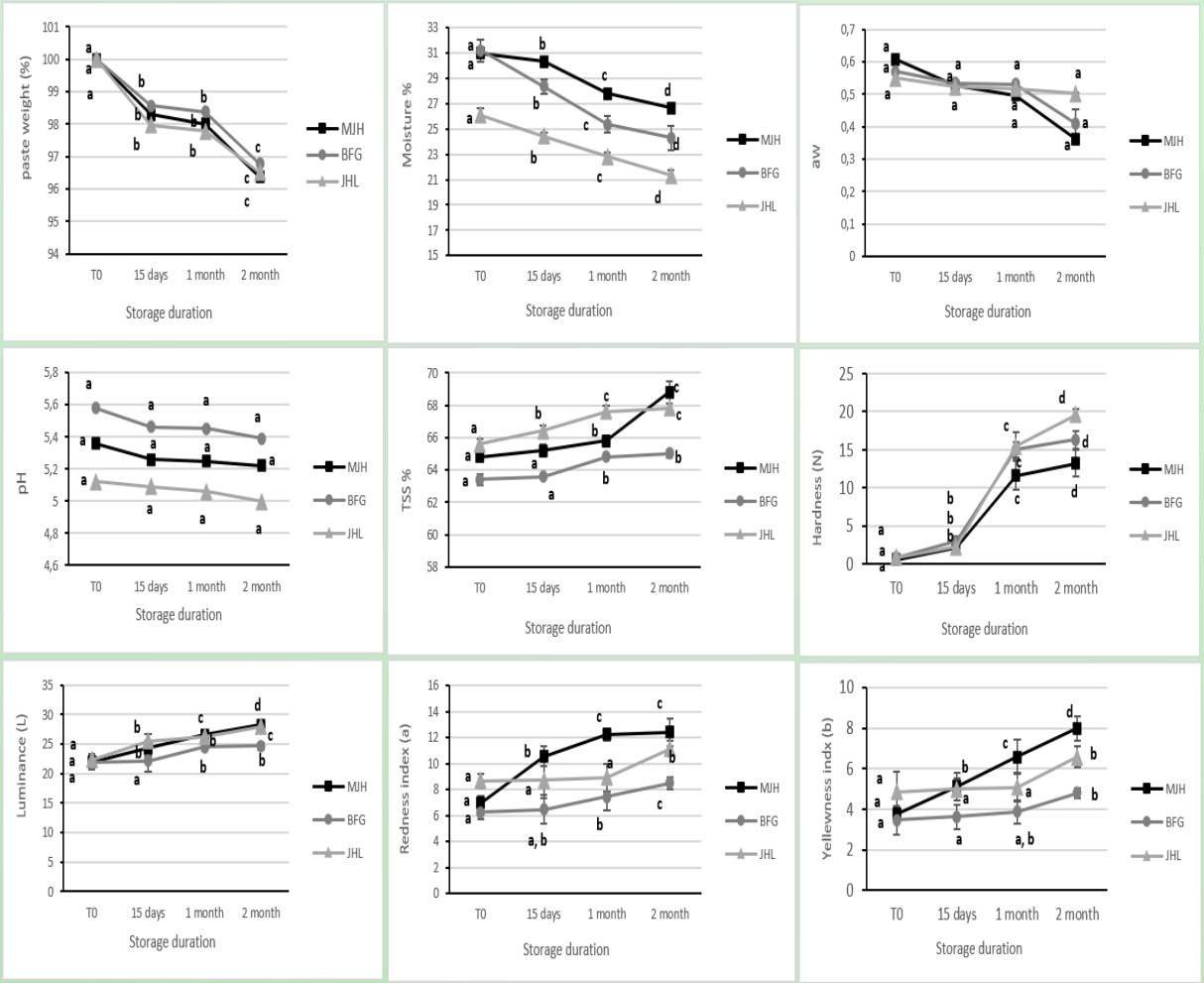



Figure 5. Evolution of quality attributes of the three pastes prepared from Mejhoul, Boufeggous, and Jihel dates

***Date powder***

Figure 6 illustrates the powders obtained from the date samples Mejhoul, Boufeggous, Jihel (normal texture), and Jihel (hard texture). The results of yields obtained for date powder and physicochemical properties of the powders produced from the three studied date varieties are summarized in Table 5. The date powders obtained in this study exhibited a high content of soluble sugars (70.8 to 83 °Brix), reflecting their natural richness in sugars such as glucose and fructose. They were also slightly acidic, with pH values ranging from 4.29 to 4.81, which is typical of date-derived products and contributes to their microbiological stability. In addition, these powders were characterized by a low moisture content (below 10%), associated with a very low water activity (aw < 0.18), which strongly limits microbial growth and ensures good long-term storage stability. Regarding colour, only the flour obtained from the Jihel variety with hard texture (batch 2) showed satisfactory visual characteristics, with a luminance (L*) of 56.5 ± 0.69, indicating a lighter colour and therefore better acceptability for food applications. Consequently, this flour was selected and used as an ingredient in the formulation of dry biscuits at different proportions. Several studies have also highlighted the influence of extraction and processing methods on the physicochemical, nutritional, and technological properties of date powders, particularly in terms of sugar composition, water-holding capacity, and functional properties (Manickavasagan et al., 2015; Moghbeli et al., 2019; Assous et al., 2021; Sourki et al., 2022; Muñoz-Bas et al., 2025).

***Dry Biscuit Based on Date Powder***

Date powder obtained from the Jihel variety (characterized by its hard texture) was used in the preparation of a dry biscuit as a substitute for both sucrose and white flour. Figure 7 illustrates the biscuits formulated with different substitution levels. Sensory evaluation of the prepared biscuits showed that replacing 50% of the white flour and 100% of the sucrose with date flour was highly effective. Elkatry et al. (2024) demonstrated the effectiveness of substituting sugar with Khalas date flour at a level of 25% in dry biscuit formulations. They reported that the addition of date powder significantly increased the polyphenol and flavonoid contents as well as the antioxidant activity, thereby slowing the rancidity process and extending the shelf life of the biscuits. Due to their high fiber content, these biscuits may represent a nutritionally suitable option for diabetic individuals.

Table 5. Physicochemical characterization of the prepared date powders

| | *Mejhoul* | *Boufeggous* | *Jihel (normal texture)* | *Jihel (hard texture)* |
|---|---|---|---|---|
| Yield (%) | 83,63 | 71,57 | 75,24 | 87,67 |
| Moisture (%) | 8,12 ± 0,01 $^{a}$ | 8,89 ± 0,02 $^{b}$ | 6,97 ± 0,05 $^{c}$ | 5,81 ± 0,32 $^{d}$ |
| Ash (%) | 2,55 ± 0,05 $^{b}$ | 2,76 ± 0,12 $^{a}$ | 2,3 ± 0,03 $^{c}$ | 2,34 ± 0,02 $^{c}$ |
| $a_w$ | 0,145 ± 0,001 $^{d}$ | 0,183 ± 0,004 $^{a}$ | 0,173 ± 0,003 $^{b}$ | 0,155 ± 0,002 $^{c}$ |
| pH | 4,41 ± 0,005 $^{c}$ | 4,29 ± 0,00 $^{d}$ | 4,57 ± 0,00 $^{b}$ | 4,81 ± 0,005 $^{a}$ |
| Acidity (g of malic acid / 100 g of dates) | 0,402 ± 0,01 $^{b}$ | 0,482 ± 0,02 $^{a}$ | 0,317 ± 0,00 $^{c}$ | 0,402 ± 0,01 $^{b}$ |
| TSS° | 83 ± 0,35 $^{a}$ | 75,2 ± 0,35 $^{b}$ | 70,8 ± 0,6 $^{d}$ | 73,4 ± 0,35 $^{c}$ |
| L* | 24,86 ± 1,02 $^{c}$ | 26,07 ± 0,04 $^{c}$ | 38,41 ± 0,51 $^{b}$ | 56,5 ± 0,69 $^{a}$ |
| b* | 20,49 ± 0,58 $^{c}$ | 21,15 ± 0,16 $^{c}$ | 34,59 ± 0,44 $^{b}$ | 35,75 ± 0,48 $^{a}$ |
| a* | 16,26 ± 0,31 $^{b}$ | 15,96 ± 0,05 $^{b}$ | 17,84 ± 0,13 $^{a}$ | 12,4 ± 0,16 $^{c}$ |

Values are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Means in the same row bearing different superscript letters differ significantly at $p < 0.05$.

Table 6. Physicochemical characterization of coffee substitutes prepared from date pits

| | *Mjehoul* | *Boufeggous* | *Jihel* |
|---|---|---|---|
| Yields (%) | 83,18 | 83,29 | 80,26 |
| Moisture (%) | 1,35 ± 0,003 $^{b}$ | 1,876 ± 0,04 $^{a}$ | 0,88 ± 0,1 $^{c}$ |
| Ash (%) | 1,28 ± 0,01 $^{b}$ | 1,34 ± 0,02 $^{a,b}$ | 1,4 ± 0,02 $^{a}$ |
| $a_w$ | 0,103 ± 0,008 $^{a,b}$ | 0,108 ± 0,003 $^{a}$ | 0,054 ± 0,007 $^{b}$ |
| pH | 3,74 ± 0,005 $^{c}$ | 3,78 ± 0,005 $^{b}$ | 3,82 ± 0,006 $^{a}$ |
| Acidity (g of malic acid/ 100g of dattes) | 0,308 ± 0,023 $^{a}$ | 0,335 ± 0,023 $^{a}$ | 0,322 ± 0,00 $^{a}$ |
| TSS° | 3,8 ± 0,35 $^{a,b}$ | 4,4 ± 0,35 $^{a}$ | 3,6 ± 0,00 $^{b}$ |
| L* | 24,75 ± 0,34 $^{b}$ | 25,79 ± 0,15 $^{a}$ | 20,65 ± 0,301 $^{c}$ |
| b* | 17,87 ± 0,19 $^{b}$ | 18,62 ± 0,15 $^{a}$ | 13,62 ± 0,22 $^{c}$ |
| a* | 14,1 ± 0,07 $^{b}$ | 15,46 ± 0,14 $^{a}$ | 12,56 ± 0,05 $^{c}$ |

Values are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Means in the same row bearing different superscript letters differ significantly at $p < 0.05$.

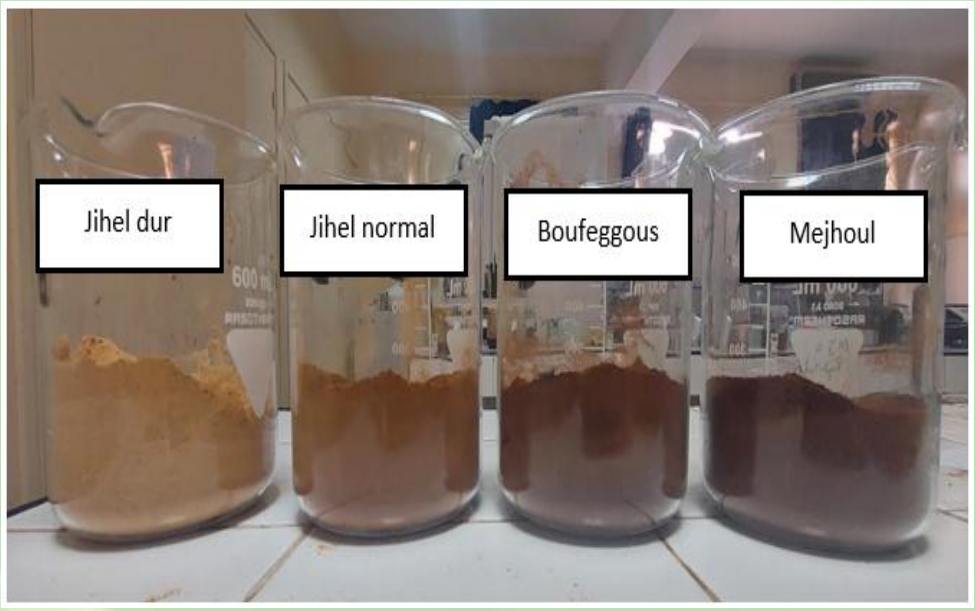



Figure 6. Powders obtained from the four date samples studied

Similarly, Latif and Pik Wan (2023) showed that date powder can be incorporated into biscuit formulations as a substitute for sucrose, improving nutritional quality and extending shelf life. These authors also demonstrated that incorporation levels above 30% negatively affected the sensory properties of the final product, particularly in terms of color and taste.

**Coffee Substitute from Date Pits**

The coffee substitutes produced from the date pits of the three varieties, Mejhoul, Boufeggous, and Jihel, are illustrated in figure 8. The results of the yields obtained for coffee substitutes from date pits of the three varieties, as well as their physicochemical properties, are presented in Table 6.

Although these products exhibited desirable physicochemical quality characteristics like those of real coffee, they were not well accepted by consumers. The latter reported that the taste was far from what would justify the designation "coffee substitute" and suggested emphasizing the potential therapeutic benefits of this beverage to encourage its consumption as an infusion rather than as a coffee substitute.

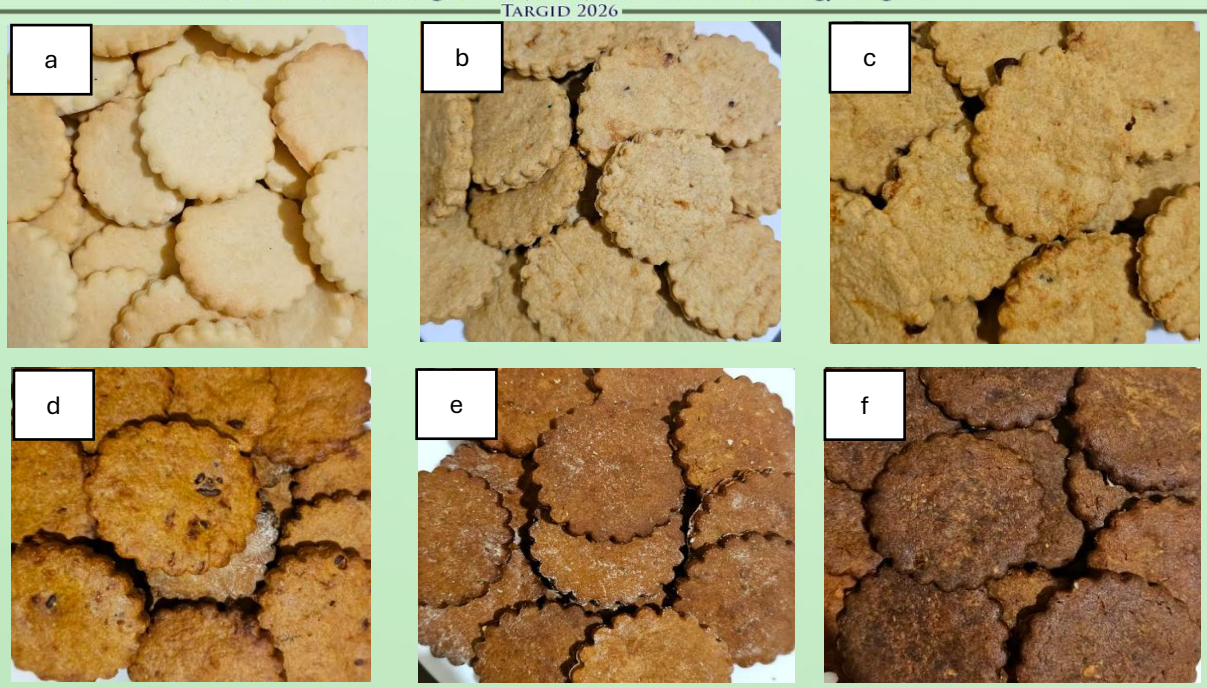



Figure 7. Appearance of dry biscuits prepared with (a) wheat flour (control), (b) 10% date powder, (c) 30% date powder, (d) 50% date powder, (e) 70% date powder, and (f) 100% date powder.




Figure 8. Coffee substitutes prepared from the date pits of the Mejhoul, Boufeggous, and Jihel varieties.

Indeed, Farag et al. (2019) highlighted the abundance of furans, pyrones, terpenoids, and sulfur-containing compounds in roasted date pits, whereas pyrroles and caffeine—typical metabolites of roasted coffee—were absent. Other authors have demonstrated the potential use of roasted date pit powder as a healthy coffee alternative (Venkatachalam & Sengottian, 2016). However, Ghnimi and Almansoori (2015) called for further studies on the composition of this product before recommending it for human consumption and emphasized the need to improve its manufacturing process.

**Conclusion**

The results of this study demonstrate that date sorting by-products and date pits represent a promising and sustainable resource for the development of high-value-added food products. The different date varieties exhibited distinct technological potentials, with Boufeggous showing suitability for syrup and paste production, while the hard-texture Jihel proved to be the most appropriate for date powder manufacturing and bakery applications. The

developed products displayed satisfactory physicochemical and microbiological qualities, as well as good storage stability.The valorization of these underutilized by-products contributes to reducing post-harvest losses, enhancing the sustainability and competitiveness of the Moroccan date palm sector, and diversifying agro-food products derived from dates. Furthermore, the incorporation of date powder into biscuits demonstrated its potential as a natural alternative to refined sugar and wheat flour, offering opportunities for the development of nutritionally improved food products. Nevertheless, further research is required to optimize processing conditions and improve the sensory acceptability of certain products, particularly date-pit coffee substitutes and date pastes. Such efforts would strengthen the commercial viability of these products and support the wider adoption of date by-product valorization strategies within a circular and sustainable agri-food economy.

## Declarations

### *Author Contribution Statement*

MISBAH Asmae: Conceptualization, methodology design, supervision, data analysis and interpretation, statistical analysis, valorization of results, and writing of the original draft. The author also contributed to the review and editing of the manuscript.

ZYADI Fatimzehra and AZIZ Siham: Equal contribution. Both authors performed laboratory experiments, sample preparation, and data collection as part of their internship work.

BENAMAR Houria: Provided technical assistance, laboratory support, and contributed to the execution of experimental procedures.

OUHEJJOU Bouchra: Critical review and revision of the manuscript, validation of the methodology and results, and contribution to the improvement of the final version of the manuscript.

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Effect of diatomite and seashell on ammonium nitrogen in aquarium wastewater

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## Abstract

In aquarium systems, nitrogenous compounds such as ammonium/ammonia are produced through the decomposition of fish metabolic wastes, feces, and uneaten feed, thereby adversely affecting water quality. In this study, the effects of powdered and gravel-sized diatomite and seashell, which can be used as natural substrate materials, on ammonium removal from aquarium wastewater were investigated. The study was conducted in five groups—control, powdered diatomite, gravel-sized diatomite, seashell, and powdered seashell—with three replicates per group. Each experimental container was filled with 600 mL of aquarium wastewater, and 6 g $L^{-1}$ of natural material was added to all groups except the control. Water temperature, pH, $NH_3$, $NH_4^+$-N, and TAN values were monitored for 53 hours. At the end of the experiment, no significant differences were observed among the groups in terms of water temperature and pH ($p>0.05$). However, $NH_3$ values in the powdered and gravel-sized diatomite groups were significantly lower than those in the control group ($p < 0.05$). $NH_4^+$-N values decreased in all treatment groups compared with the control. Diatomite and seashell contributed to the reduction of ammonium levels in aquarium wastewater. In conclusion, diatomite and seashell can be used in aquaria as low-cost, natural, and decorative substrate materials; however, diatomite was found to be more effective, particularly in ammonia control.

***Keywords:*** *Diatomite, seashell, adsorption, aquaculture, ornamental fish culture*

**Diyatomit ve deniz kabuğunun akvaryum atık suyunda amonyum azotu üzerine etkisi**

## Özet

Akvaryum sistemlerinde balık metabolik atıkları, dışkı ve tüketilmeyen yemlerin ayrışması sonucunda amonyum/amonyak gibi azotlu bileşikler oluşmakta ve su kalitesi olumsuz etkilenmektedir. Bu çalışmada, doğal substrat materyali olarak kullanılabilen diyatomit ve deniz kabuğunun toz ve çakıl boyutlarının akvaryum atık suyundaki amonyum giderimi üzerine etkisi araştırılmıştır. Çalışma; kontrol, toz diyatomit, çakıl diyatomit, deniz kabuğu ve toz deniz kabuğu olmak üzere beş grupta, üç tekerrürlü olarak yürütülmüştür. Her kaba 600 mL akvaryum atık suyu konulmuş, kontrol dışındaki gruplara 6 g $L^{-1}$ doğal materyal eklenmiştir. Su sıcaklığı, pH, $NH_3$, $NH_4^+$-N ve TAN değerleri 53 saat boyunca izlenmiştir. Deneme sonunda sıcaklık ve pH açısından gruplar arasında anlamlı fark belirlenmemiştir ($p>0.05$). Buna karşın, toz ve çakıl diyatomit gruplarında $NH_3$ değerleri kontrol grubuna göre anlamlı düzeyde düşük bulunmuştur ($p<0.05$). Tüm uygulama gruplarında $NH_4^+$-N değerleri kontrol grubuna kıyasla azalmıştır. Diyatomit ve deniz kabuğu, akvaryum atık suyunda amonyum düzeylerinin azaltılmasına katkı sağlamıştır. Sonuç olarak, diyatomit ve deniz kabuğunun akvaryumlarda düşük maliyetli, doğal ve dekoratif substrat materyali olarak kullanılabileceği; özellikle diyatomitin amonyak kontrolünde daha etkili olduğu belirlenmiştir.

***Anahtar Kelimeler:*** *Diyatomit, Deniz kabuğu, Adsorpsiyon, Su ürünleri, Süs balıkları yetiştiriciliği*

## Giriş

Süs balıkları yetiştiricilik sistemlerinde optimum su kalitesini sağlamak en önemli kriterlerdendir. Su kalite parametrelerini balık metabolik atıkları ve tüketilmeyen yemler olumsuz yönde etkilemektedir. Çeşitli çalışmalarda, yoğun yetiştiricilik koşullarında tüketilmeyen yemlerden kaynaklanan atık miktarının yaklaşık %10–30 arasında değiştiği bildirilmiştir (Kibria vd., 1997; Devi vd., 2017; Hlordzi vd., 2020).

Daha önce yapılan çalışmalarda akvaryum su kalitesinin düzenlenmesi için doğal adsorban maddelerden yararlanılabileceği bildirilmiştir. Bu doğal adsorban maddeler filtre içerisinde kullanılabileceği gibi, akvaryum zemin materyali olarak da kullanımı yaygındır. Zemin materyali olarak kullanıldığında çevresel zenginleştirmenin yanı sıra su parametreleri üzerine de olumlu yönde katkı sağladıkları daha önceden yapılan çalışmalarda belirlenmiştir (Öz vd., 2021; Öz, 2024).

Çakıl, kum, toprak veya kırılmış mercan gibi birçok farklı malzeme, bir akvaryum için substrat (taban malzemesi) olarak kullanılabilmektedir. Balık türleri, omurgasızlar ve sucul bitkilere göre substrat seçimi farklılık gösterir. Örneğin kırılmış mercan, nispeten yüksek pH, alkalinite ve sertlik seven Afrika çiklit türü veya bazı canlı

doğuran türleri için uygun olabilirken, melek balığı, çöpçü balığı, cüce vatoz türleri veya tetra türleri için uygun değildir (Öz vd., 2024). Ekolojik istekleri farklı birçok sucul canlı türünün uyum içerisinde yaşamını sürdürebilmesi için akvaryum düzenlemelerinde birçok doğal materyal kullanılmaktadır. Bu doğal materyaller kum, çakıl, kaya parçaları, toprak, turba, lav taşı, omurgasız kabukları, mercan kumu, zeolit, bentonit, leonardit, diatomit gibi doğal adsorbanlar, ağaç kökleri, dalları, yaprakları gibi hem su parametrelerinin düzenlenmesine katkı sağlayan hem de dekoratif amaçlı kullanılabilen materyallerdir (Öz vd., 2025). Bu materyaller özellikle sudaki azotlu bileşiklerin (amonyum/amonyak, nitrit, nitrat) gideriminde ve pH değerlerinin düzenlenmesinde etkili olabilmektedir (Öz vd., 2016).

Amonyağın uzaklaştırılmasında kullanılan yöntemlerden biri biyolojik ve kimyasal arıtımdır. Bakteriyel amonyak giderim süreçlerinde substrat oluşturmak amacıyla deniz ve tatlı su kumu, kabuklu canlı materyalleri ve aktif karbon gibi materyaller kullanılmaktadır (Aly vd., 2016). İyon değişimi, katı bir yüzeye bağlı bulunan iyonların çözeltideki iyonlarla yer değiştirdiği geri dönüşümlü bir kimyasal reaksiyondur. Katı yüzeyde bulunan iyonlar çözeltiye geçerken, çözeltideki iyonlar elektrostatik kuvvetler aracılığıyla katı yüzeye bağlanmaktadır. Bu değişim süreci, yüzeyde ve çözeltide bulunan iyon türlerinin konsantrasyonları dengeye ulaşıncaya kadar devam etmektedir (Zain vd., 2018; Aly vd., 2016).

Akvaryum suyundaki amonyumun uzaklaştırılmasında kullanılan zeolit, bentonit, leonardit gibi doğal adsorbanların yanı sıra diatomların kabuklarının uzun yıllar boyunca çökelmesi ile oluşmuş olan diatomitin de kullanımına yönelik araştırmaların son yıllarda araştırıldığı belirlenmiştir (Öz vd., 2022; Öz Ü., 2024). Diyatomite benzer şekilde deniz kabukları ve kumu akvaryumlarda zemin materyali olarak kullanılabilmektedir. Birbirine benzer özellikteki bu iki materyalin filtrasyon, dekorasyon ve bitki tabanı materyali olarak kullanım özelliklerinin belirlenmesi, akvaryum ekosistemlerinin düzenlenmesine çok yönlü katkılar sağlayacaktır. Özellikle yoğun yetiştiricilik için kullanılan resirkülasyon sistemlerinde bu doğal materyaller, su parametreleri ile sucul organizmalar arasındaki gerekli dengenin sağlanması ve korunması açısından çeşitli avantajlar sunmaktadır. Bu avantajlar; ekonomik olmaları, uygun fizikokimyasal özellikler taşımaları, kolay temin edilebilmeleri, işleme ve boyutlandırma ile verimliliklerinin artırılabilmesi, suda bulanıklık oluşturmamaları ve farklı dekoratif özelliklerle kullanılabilmeleri şeklinde sıralanabilir (Eroğlu vd., 2017; AbuKhadra vd., 2020).

Diyatomit bol miktarda bulunan, düşük maliyetli, yumuşak sedimanter kayaç yapısında silika esaslı bir materyaldir. Düşük yoğunluğa sahip gözenekli bir yapı göstermekte olup, temel olarak silisyum dioksitten oluşmaktadır. Bununla birlikte diyatomit; toksik olmayan, kokusuz, doğada yüksek saflıkta ve bol miktarda bulunan, dolayısıyla düşük maliyetle temin edilebilen bir materyaldir (Bello vd., 2014). Bu çalışmada kullanılan deniz kabuğu akvaryumlarda bütün hali ile kullanılabileceği gibi toz/kırık hali de zemin materyali olarak kullanılmaktadır. Bu araştırmanın amacı, doğal zemin materyali olarak kullanılabilen diatomit ve deniz kabuğunun toz ve çakıl boyutunun akvaryum atık suyu üzerine etkisinin belirlenmesidir.

## Materyal ve Yöntem

Çalışma kontrol, toz diyatomit (TD), çakıl diyatomit (ÇD), deniz kabuğu (DK) ve Toz Deniz Kabuğu (TDK) ndan oluşan beş farklı deneme grubundan oluşmuştur. Her bir grup 3 tekerrürlü olmak üzere toplam 15 deneme kabı kullanılmıştır. Her bir deneme kabına tatlı su akvaryumlarının atık suyundan 600 mL eklenmiştir. Bu atık su balık yemi, balık metabolik atığı (dışkı, üre, balık ölüsü), sucul bitki parçaları vb. organik maddelerden oluşmaktadır. Kontrol grubu hariç diğer deneme gruplarına 6 g $L^{-1}$ doğal adsorbanlardan ilave edilerek deneme grupları oluşturulmuştur.

Deneme akvaryumlarındaki suyun fiziko-kimyasal kalitesi belirli periyotlarla (başlangıç, 30. dk., 24. saat, 29. saat, 53.saat) izlenmiştir. Deneysel veriler, YSI Professional çok parametreli ölçüm cihazı kullanılarak belirlenmiştir. Deneme, oda sıcaklığı koşullarında yürütülmüş olup, deneme süresince herhangi bir havalandırma veya karıştırma işlemi uygulanmamıştır. Çalışmada kullanılan diyatomitler Nanotech İnşaat Kimya Maden ve Lojistik firmasından, deniz kabukları ise Sinop-Türkiye kıyılarından temin edilmiştir.

Çalışma süresince $NH_3$ ve TAN (toplam amonyak azotu; TAN = $NH_3$ + $NH_4^+$) düzeyleri hesaplanmış (Purwono vd., 2017); ayrıca $NH_4^+$, pH ve su sıcaklığı değerleri de belirlenmiştir.

$$\mathrm{pK}(NH_3) = \frac{2726.3}{273+°C} + 0.0963 \quad (1)$$

$$NH_3\text{-N} = 10^{(pH-\mathrm{pK}(NH_3))} \times NH_4^+\text{-N} \quad (2)$$

$$TAN = NH_3\text{-N} + NH_4^+\text{-N} \quad (3)$$

*İstatistiksel Analizler*

Su kalite parametreleri, tek yönlü varyans analizi (ANOVA) ile istatistiksel olarak değerlendirilmiş ve ortalamalar Tukey testi kullanılarak %5 önem düzeyinde ($p<0,05$) karşılaştırılmıştır. Elde edilen sonuçların istatistiksel analizleri "Minitab Release 17 for Windows" yazılımı kullanılarak gerçekleştirilmiştir (Nanda vd., 2021).

**Bulgular**

Çalışmanın başlangıcında tüm gruplarda kullanılan atık suyun su sıcaklığı, pH ve amonyum değerleri sırasıyla 23,1±0,01 °C, 7,65±0,02 ve 7,94±0,00 mg $L^{-1}$ olarak belirlenmiştir.

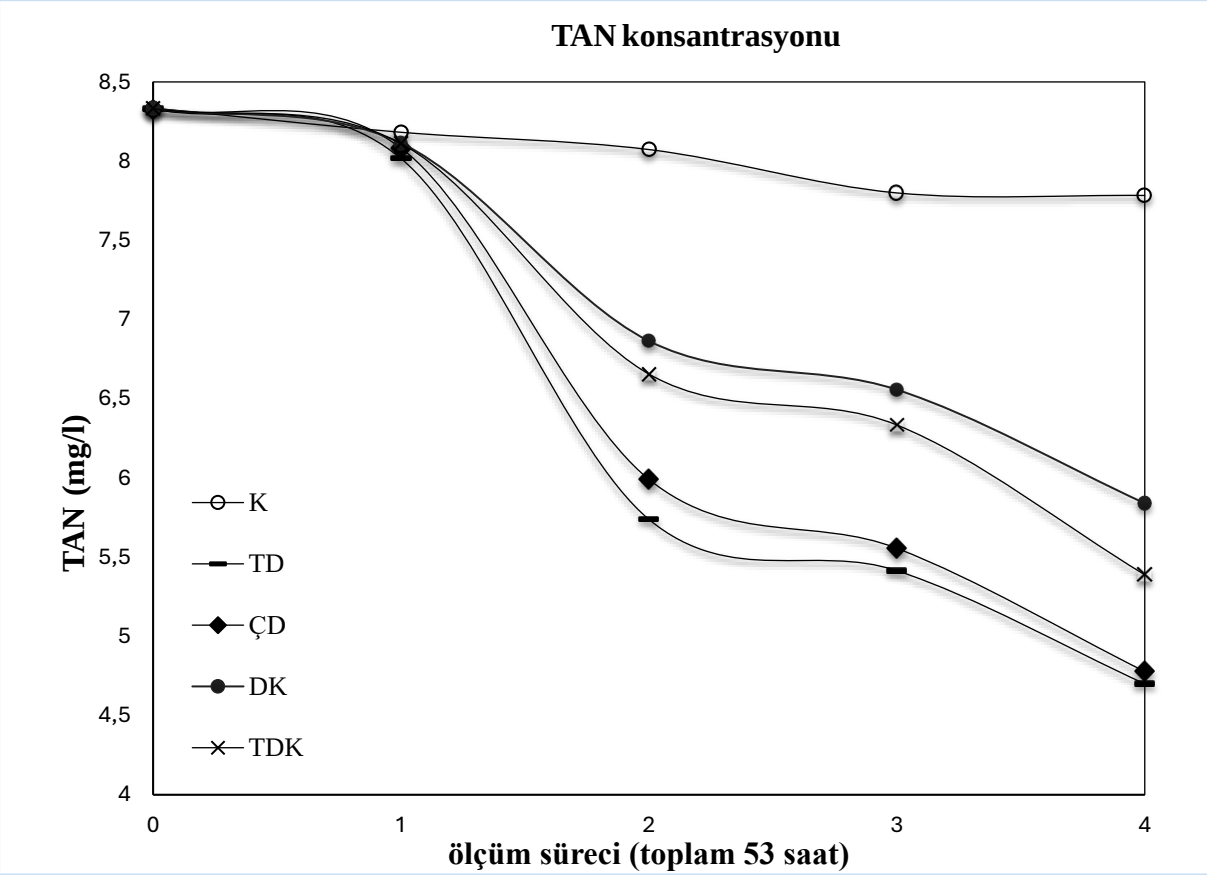
Deneme sonunda belirlenen su kalite parametreleri Tablo 1'de özetlenmiştir. Denemedeki tüm gruplarda su sıcaklığı ve pH değerleri açısından istatistiki bir farkın oluşmadığı ($p>0,05$) tespit edilmiştir. Deneme sonunda diyatomitin toz ve çakıl boyutu kullanılarak oluşturulan deneme gruplarında (TD, ÇD) gruplarından elde edilen $NH_3$ değerleri kontrol grubuna kıyasla istatistiki açıdan farklı bulunurken ($p<0,05$), deniz kabuğu ve toz deniz kabuğu (DK, TDK) gruplarından elde edilen $NH_3$ değerleri kontrol grubu ile istatistiki açıdan benzerlik ($p>0,05$) göstermiştir. Diyatomit ve deniz kabuğunun her iki formu $NH_3$ değerleri açısından istatistiki olarak incelendiğinde ise aradaki farkın önemsiz olduğu ($p>0,05$) tespit edilmiştir. Deneme sonunda elde edilen $NH_4^+$-N değerleri incelendiğinde tüm deneme gruplarının kontrol grubundan istatistiki açıdan farklı ($p<0,05$) olduğu belirlenmiştir.

Tablo 1. Deneme süresince (53 saat) elde edilen su parametre değerleri

| *Deneme Grupları* | *Su sıcaklığı (°C)* | *pH* | *$NH_3$ (mg $L^{-1}$)* | *$NH_4^+$-N (mg $L^{-1}$)* |
|---|---|---|---|---|
| Kontrol | 19,59±0,55 | 8,11±0,08 | 0,40±0,04$^{a}$ | 7,54±0,10$^{a}$ |
| Toz Diyatomit (TD) | 19,53±0,55 | 8,09±0,08 | 0,27±0,02$^{b}$ | 5,70±0,39$^{b}$ |
| Çakıl Diyatomit (ÇD) | 19,52±0,56 | 8,09±0,08 | 0,28±0,02$^{b}$ | 5,83±0,39$^{b}$ |
| Deniz Kabuğu (DK) | 19,53±0,55 | 8,10±0,08 | 0,33±0,03$^{ab}$ | 6,51±0,28$^{b}$ |
| Toz Deniz Kabuğu (TDK) | 19,52±0,50 | 8,11±0,08 | 0,32±003$^{ab}$ | 6,30±0,32$^{b}$ |

*Aynı sütundaki üst simge harfler, gruplar arasındaki istatistiksel olarak anlamlı farklılıkları göstermektedir ($p\leq0,05$).

Deneme süresince elde edilen TAN (Toplam Amonyak Azotu) değerleri ise Şekil 1 de sunulmuştur.




Şekil 1. Deneme sonunda belirlenen TAN değerleri

**Tartışma**

Akvaryumlarda tüm su ürünleri yetiştiricilik sistemlerinde olduğu gibi su parametre değerlerinin optimum şartlarda tutulması oldukça önemlidir. Ayrıca akvaryumlardaki substrat malzemeleri de su parametre değerlerini doğrudan veya dolaylı olarak etkileyebilmektedir. Yapısal özellikleri dolayısıyla adsorpsiyon ve iyon değişiminin

yanısıra geniş mikroskopik yüzey alanlarına sahip olması nitrifikasyon bakterilerinin yaşam alanlarının oluşması açısından önem taşımaktadır (Pungrasmi vd., 2016; Vanderzwalmen vd., 2022; Setijaningsih vd., 2024). Akuakültür sularında azotlu bileşiklerin kirliliği oldukça yaygın olup, başlıca azot kaynaklarını balık dışkıları ve tüketilmeyen yemler oluşturmaktadır (Zhang vd., 2022). Azotlu bileşikler suda $NH_4^+/NH_3$, nitrit ve nitrat gibi farklı formlarda bulunur. Amonyum ($NH_4^+$) ve amonyak ($NH_3$), pH ve su sıcaklığına bağlı olarak değişen bir denge hâlindedir ve bu iki formun toplamı toplam amonyak azotu (TAN) olarak adlandırılır. Özellikle iyonize olmamış form olan $NH_3$ balıklar için daha toksiktir (Wright &Wood, 2012). Amonyak, mevcut seviyelere bağlı olarak balıkları hem doğrudan hem de dolaylı olarak etkiler; bazı türler amonyak toksisitesine diğerlerinden daha duyarlıdır. Amonyak toleransı türler arasında değişir. Yaklaşık 0,05 mg/L gibi düşük konsantrasyonlarda, iyonize olmamış amonyak balık türleri için zararlıdır ve zayıf büyüme ve yem dönüşüm oranlarına, azalmış doğurganlığa ve üreme yeteneğine, stres artışına ve bakteriyel enfeksiyonlara ve hastalıklara karşı duyarlılığın artmasına neden olabilir Hargreaves & Tucker, 2004). Amonyak toksisitesi, $LC_{50}$ değeri esas alınarak değerlendirildiğinde, tatlı su balıklarında ortalama 2.79 mg $L^{-1}$ $NH_3$, deniz balıklarında ise 1.86 mg $L^{-1}$ $NH_3$ düzeyinde ortaya çıkmaktadır (Randall & Tsui, 2002). Mevcut çalışmada amonyak konsantrasyonları toksik seviyelerin oldukça altında kalmıştır. TAN'ın bileşimi sıcaklık ve pH'ya bağlıdır, sıcaklık ve pH'daki artış, iyonize olmamış amonyak düzeyinin yükselmesine neden olmaktadır (Emerson vd., 1975).

Yapılan bu çalışmada TAN değerleri incelendiğinde, deneme grupları arasında istatistiksel olarak anlamlı farklılık olduğu belirlenmiştir ($p<0,05$). Kontrol grubunun ortalama TAN değerlerinin, diyatomit ve deniz kabuğundan daha yüksek (7,94 ± 0,06) olduğu belirlenmiştir. Hem diyatomit hem de deniz kabuğu genel olarak kendi içerisinde değerlendirildiğinde toz halindeki substrat malzemelerin TAN değerleri karşılaştırıldığında, toz boyuttakilerin TAN değerlerinin daha düşük olduğu görülmüştür. Benzer şekilde Öz Ü, 2024 tarafından yapılan çalışmada da toz boyuttaki diyatomit uygulamalarında TAN konsantrasyonları düşük bulunmuştur. Sucul sistemlerde toplam amonyak azotu giderim veriminin değerlendirildiği Öz vd. (2022) çalışmasında diyatomitli gruplarda verimin control grubuna göre %27,21olduğu, benzer şekilde bu araştırmada da verimin toz diyatomitli grupta control grubuna göre %39,59 olduğu belirlenmiştir.

Tatlı su ürünleri sistemlerinde amonyum giderimi üzerine diyatomitle yapılmış sınırlı sayıda çalışmaya rastlanmasına ragmen deniz kabuğu ile yapılmış herhangi bir çalışmaya rastlanılmamıştır. Bu çalışmada belirlenen TAN ve pH değerleri göz önüne alındığında tatlı su akvaryumlarında her iki materyalinde zemin materyali olarak değerlendirilebileceği ve bu yönüyle de literature katkı sağlayacağı düşünülmektedir.

## Sonuç

Çalışma verilerinin değerlendirilmesi sonucunda akvaryumlarda substrat malzemesi olarak sıkça kullanılan deniz kabuklularından oluşan kum ve çakılların yanısıra, diatomların fosilleşmiş hali olan diyatomitlerin akvaryum sularındaki amonyumun uzaklaştırılmasında katkı sağladıkları ortaya konmuştur. Mevcut sonuçlara dayanarak, yoğun akuakültür sistemlerinde veya akvaryum koşullarında her iki malzemenin de toksik olmayan, kokusuz, doğada yüksek saflıkta ve bol miktarda bulunan, dolayısıyla düşük maliyetle temin edilebilen materyal olmaları nedenleri ile akvaryumlarda zemin materyali olarak kullanımlarının ortama görsellik katmalarının yanında su parametrelerinin optimum seviyelerde tutulabilmelerine de katkı sağlayacakları tespit edilmiştir.

## Beyanlar

***Etik Kurul Onay Belgesi***

Bu çalışma için herhangi bir etik kurul onayı gerekmemektedir.

***Yazar Katkı Beyanı***

DŞ: Kavramsallaştırma, araştırma, denemenin tasarlanması ve yürütülmesi, verilerin toplanması, verilerin analiz edilmesi, yazma

MÖ: Araştırma, verilerin toplanması, verilerin analiz edilmesi, yazma

ÜÖ: Verilerin analiz edilmesi, istatistiki hesaplamalar

Tüm yazarlar, makalenin yayımlanacak son hâlini okumuş ve onaylamıştır.

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Bu araştırmada herhangi bir fon desteği bulunmamaktadır.

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Yazarlar herhangi bir çıkar çatışması olmadığını beyan etmektedirler.

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*ID: 804*

# Biosynthesis Of Zno-Cuo Nanoparticles of *Viola Betonicifolia* L. Flower Extract And Investigate Their Therapeutic Potential

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## Abstract

Current study investigated the green synthesis of bimetallic CuO and ZnO nanoparticles mediated by *Viola betonicifolia* L. plant extract as a reducing and stabilizing agent. The synthesized nanoparticles were analyzed by Ultra Violet Spectroscopy, X- rays spectrum as well as by Scanning Electron Microscope and the results revealed the formation of well dispersed small sized nanoparticles of the target metal ions. Biological activities of CuO-ZnO nanoparticles were recognized via several tests. The synthesized nanoparticles showed significant zone of inhibition of 19mm,15mm,13mm and 12 mm by using *Escherichia coli, S.aureus, K.pneumoniae, B.subtilius, Candida and Aspergillus species* respectively. The obtained results revealed potent bactericidal and fungicidal activities. Additionally, these nanoparticles also demonstrated enhanced catalytic performance in degrading textile dye, attributed to increase surface area and electron transfers capabilities. Thus, these CuO-ZnO NPs were found effective multifunctional agent as antimicrobial, antifungal and environmental remediation particularly in removal of dye.

***Keywords:*** *CuO-ZnO, Nanoparticles, Bactericidal, plant, Violabetonicifolia L. ,Antibacterial, Antifungal, Dye-degradation, Therapeutic potential, Green synthesis*

*ID: 806*

# The effect of leonardite on pH in aquaculture and its stabilization with clinoptilolite

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## Abstract

In aquaculture systems, pH values between 6.5 and 8.5 are generally considered suitable; however, the optimum range may vary depending on the cultured species, water alkalinity, temperature, and production system. In the present study, the effects of the natural adsorbents leonardite and zeolite on pH were investigated. Two different particle sizes of leonardite, namely powder and gravel forms, were used at three different concentrations (3, 6, and 9 mg $L^{-1}$), while zeolite was added in gravel form (3–5 mm) at three different concentrations (3, 6, and 9 mg $L^{-1}$). The experiment was conducted for a total of six days, during which pH values were recorded. During the first three measurements, the effect of leonardite on water pH was determined. Following this stage, gravel-sized zeolite was added to the experimental groups, and its influence on pH values in terms of acidic or basic shifts was evaluated. At the end of the experiment, the differences among groups in terms of pH values were found to be statistically significant ($p<0.05$). Final pH values ranged between 6.50 and 7.64, indicating that the recorded values remained within a suitable range for many freshwater species. In conclusion, the combined use of the natural adsorbents leonardite and clinoptilolite may be considered suitable for pH stabilization in freshwater aquaculture.

***Keywords:*** *Leonardite, clinoptilolite, pH, aquaculture, ornamental fish culture.*

## Su ürünleri yetiştirciliğinde leonarditin pH üzerine etkisi ve klinoptilolit ile dengelenmesi

## Özet

Akuakültür sistemlerinde pH genellikle 6,5–8,5 aralığında uygun kabul edilir; ancak ideal aralık yetiştirilen türe, suyun alkalinitesine, sıcaklığa ve üretim sistemine göre değişebilir. Bu çalışmada doğal adsorbanlardan leonardit ve zeolitin pH üzerine etkileri incelenmiştir. Çalışmada leonarditin iki farklı boyutu (toz ve çakıl) 3 farklı oranda (3, 6, 9 mg $L^{-1}$) kullanılmış, zeolit ise çakıl boyutta (3-5 mm) 3 farklı oranda (3, 6, 9 mg $L^{-1}$) eklenmiştir. Çalışma toplam 6 gün sürdürülmüş ve pH değerleri kaydedilmiştir. İlk üç ölçümde leonarditin sudaki pH üzerine etkisi belirlenmiş ve bu aşamadan sonra deneme gruplarına çakıl boyutta ilave edilen zeolitin pH değerlerini hangi yönde (asidik/bazik) etkilediği tespit edilmiştir. Deneme sonunda pH değerleri açısından gruplar arasındaki fark istatistiki olarak anlamlı ($p<0.05$) bulunmuştur. Çalışmanın sonunda pH değerleri 6,50-7,64 aralığında kaydedilmiş ve bu değerlerin birçok tatlı su türü için uygun aralık olduğu belirlenmiştir. Sonuç olarak, doğal adsorban olan leonardit ve klinoptilolitin birlikte kullanımının tatlı su yetiştiriciliğinde pH dengelenmesi için uygun olabileceği tespit edilmiştir.

***Anahtar Kelimeler:*** *leonardit, klinoptilolit, pH, su ürünleri, süs balıkları yetiştiriciliği*

## Giriş

Su kalitesi parametreleri arasında suyun pH'sı, sıcaklığı, $CO_2$ (karbondioksit) konsantrasyonu, $NH_3$ (iyonlaşmamış amonyak), $NO_2^-$ (nitrit), $NO_3^-$ (nitrat), çözünmüş oksijen gibi değişkenler; balık sağlığı, yaşama oranı ve genel üretim dinamikleri üzerinde doğrudan ve ölçülebilir etkiler oluşturan temel belirleyiciler olarak sayılmaktadır (Boyd, 2015). Su kalitesi, tüm canlı organizmaların optimum şekilde işlev gösterebilmesine olanak tanıyacak belirli sınırlar içerisinde olmalıdır. Akuakültür sistemlerinde amonyak, balıkların protein metabolizması sonucu oluşan atıkların (dışkı ve üre) yanı sıra, tüketilmeyen yemler ve diğer organik maddelerin (bitki atıkları gibi) bakteriyel ayrışması sonucu oluşmaktadır. Sucul ortamlarda amonyak; sucul canlılar için yüksek derecede toksik olan iyonlaşmamış amonyak ($NH_3$) ile daha az zararlı olan iyonlaşmış amonyum ($NH_4^+$) arasında bir denge halinde bulunur. Bu iki form birlikte toplam amonyak azotu (TAN) olarak ifade edilmektedir (Verma vd., 2022). İyonlaşmamış amonyak konsantrasyonu yaklaşık 0,05 mg $L^{-1}$ değerini aştığında, birçok tatlı su balığı türünde olumsuz fizyolojik etkiler görülebilmekte; daha yüksek konsantrasyonlarda ise akut toksisite ortaya çıkabilmektedir (Francis-Floyd vd., 2012; Öz vd., 2026).

Yoğun tatlı su akuakültür sistemlerinde, yüksek yemleme oranları ve sınırlı su değişimi nedeniyle azot birikimi giderek artan bir sorun haline gelmektedir (Boyd, 1998; Hargreaves, 1998). Bu tür sistemlerde toplam amonyak azotu (TAN) hızla yükselebilmekte ve toksik iyonlaşmamış amonyak ($NH_3$) oranı özellikle pH'a bağlı olarak kritik seviyelere ulaşabilmektedir. Genel olarak balıklar için su pH'ının 6,5–8,5 aralığında olması, iyi bir büyüme

performansı açısından optimum aralık olarak kabul edilmektedir. Optimum olmayan pH değerlerinin veya pH dalgalanmalarının balıklarda letarji, stres, deri irritasyonu/lezyonları, davranış değişiklikleri örneğin akvaryumdan dışarı atlamaya çalışma veya sürtünme davranışı, korneal ödem, deri renginde değişim, solungaç irritasyonu, mukus üretiminde artış, solunumsal belirtiler ve mortaliteye neden olabileceğini bildirilmiştir. Birçok balık türü düşük pH değerlerine karşı toleranssızdır; bazı türler ise daha toleranslı olsalar dahi mümkün olduğunda düşük pH ortamlarından kaçınma eğilimi göstermektedir Bunun yanında yem alımı, üreme, hareket ve dağılım gibi biyolojik aktiviteler de su sıcaklığından önemli ölçüde etkilenmektedir (Roberts & Palmeiro, 2008; Sahu & Datta, 2018).

Tatlı su parametrelerinin dengelenmesinde doğal adsorban maddelerin kullanımı araştırmalara konu olmaktadır. Akvaryum düzenlemelerinde kullanılan zeolit, leonardit, bentonit ve diyatomit gibi doğal hammaddeler; tarım, akuakültür ve atık su arıtımı gibi birçok sektörde kullanılmaktadır. Zeolitler, özellikle su arıtımı ve yem katkı maddesi olarak kullanımları açısından çok sayıda araştırmaya konu olmuş mineral maddelerdir (Alshameri vd., 2014). Buna karşın, leonardit ise son yıllarda su arıtımı ve yem katkı maddesi olarak kullanılmaya başlanmıştır (Şahin, 2022; Zengin, 2013).

Zeolitler; alkali ve toprak alkali metallerin kristal yapıya sahip hidratlı alüminosilikatları olup, kafes silikatlar grubunda yer alan mineral maddelerdir. Doğal zeolitler doğada yaygın olarak bulunan düşük maliyetli materyaller olup yüksek katyon değişim kapasitesine sahiptir (Cataldo vd., 2021).

Leonardit, yüzey koşullarına maruz kalma sonucunda oksidasyona uğramış linyitten elde edilen özel nitelikli düşük dereceli bir kömür türü olup, hümik maddeler bakımından zengin tortul bir materyal olarak da tanımlanmaktadır (Ausavasukhi, 2016). Leonardit, hümik maddelerde bulunan karboksilik ve hidroksilik fonksiyonel gruplar ile çeşitli inorganik minerallerin oluşturduğu kompleks yapılar nedeniyle yüksek miktarda hümik madde içermektedir (Chammui vd., 2014).

Akuakültür ortamlarında amonyak gibi organik atıkların uzaklaştırılmasında doğal ve çevre dostu adsorbanların kullanımı, sucul organizmaların sağlığını destekleyen ekolojik bir yaklaşım olarak değerlendirilmiştir (Öz, 2024a; Öz Ü, 2024; Şahin vd., 2025). Daha önce yürütülen araştırmalarda leonardit ve zeolit uygulamasının bireysel ve birlikte kullanımının amonyak değerlerinin azaltılmasına katkı sağladığı ve pH değerlerinde leonarditli gruplarda bir düşüş olduğu belirtilmiştir. Ancak leonardit miktarının pH değerleri üzerine farklı boyut ve miktarlarda kullanımının etkisi araştırılmamıştır. Bu çalışmada ise; sudaki pH değişimlerinde leonarditin ve klinoptilolitin etkilerinin araştırılması hedeflenmiştir.

## Materyal ve Yöntem

Çalışma iki aşamalı yürütülmüştür. Birinci aşama 500 ml çeşme suyuna 3,6,9 g/L olacak şekilde toz ve çakıl boyuttaki leonardit eklenmesiyle oluşturulmuştur. Üçer tekerrürlü yürütülen çalışmada toplam 18 adet deneme düzeneği kullanılmıştır. İlk üç gün düzenli bir şekilde sudaki pH değerleri ölçülmüş ve 4. gün deneme kaplarına 4-5 mm boyutlarında leonardit ile aynı miktarda olacak şekilde klinoptilolit türü zeolit eklenerek ikinci aşama oluşturulmuştur. Toplam 6 gün süren çalışma süresince kaplarda herhangi bir havalandırma, karıştırma, aydınlatma vb. işlemler uygulanmamıştır. Ayrıca deneme düzeneğinde herhangi bir amonyak kaynağı (balık, balık dışkısı, yem vb.) bulunmadığından deneme süresince sadece pH ve su sıcaklığı parametreleri YSI Professional Plus Multiparameter cihazı ile kaydedilmiştir.

Çalışmada kullanılan leonardit ve zeolit sırasıyla Kütahya Kimya (Kütahya, Türkiye) ve Rota Madencilik AŞ (Manisa, Türkiye) firmalarından temin edilmiştir.

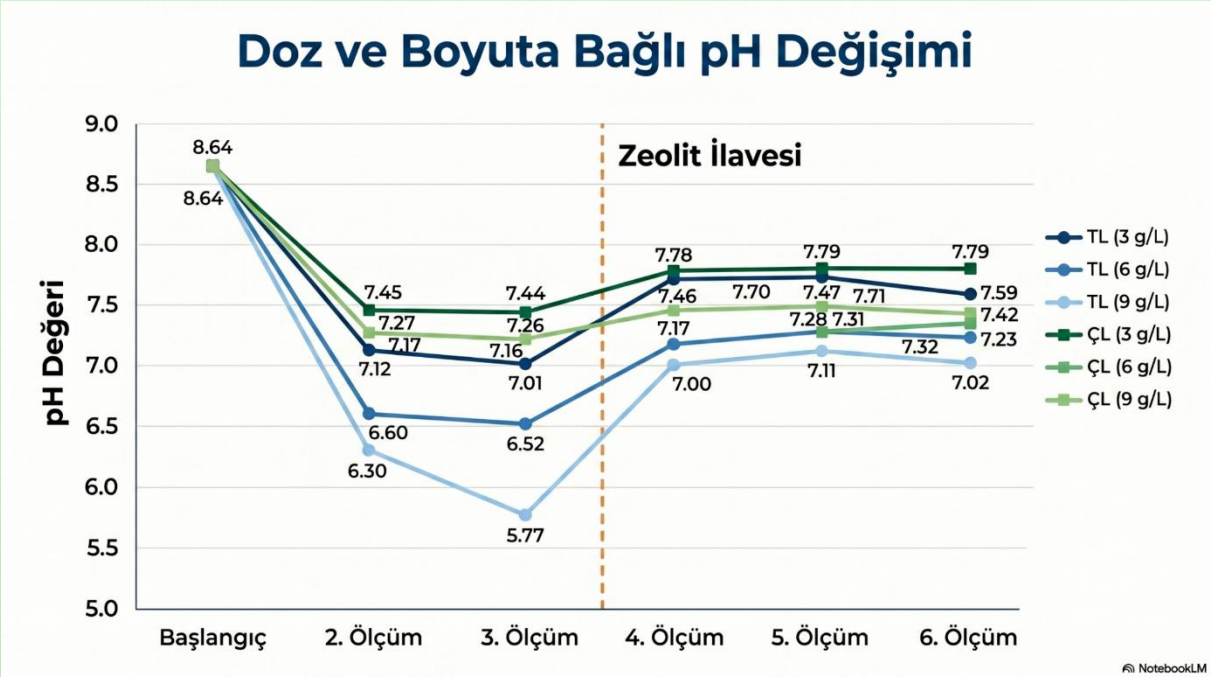
### *İstatistiksel Analizler*

Su kalite parametreleri, tek yönlü varyans analizi (ANOVA) ile istatistiksel olarak değerlendirilmiş ve ortalamalar Tukey testi kullanılarak %5 önem düzeyinde ($p<0,05$) karşılaştırılmıştır. Elde edilen sonuçların istatistiksel analizleri "Minitab Release 17 for Windows" yazılımı kullanılarak gerçekleştirilmiştir (Nanda vd., 2021).

## Bulgular

Çalışmanın başlangıcında tüm gruplarda kullanılan suyun su sıcaklığı ve pH değerleri sırasıyla 26±0,01 °C ve 8,64±0,01 olarak kaydedilmiştir.

Denemedeki tüm gruplarda su sıcaklığı açısından istatistiki bir farkın oluşmadığı ($p>0,05$) tespit edilmiştir. Birinci ve ikinci aşama süresince oluşan pH değişimleri Şekil 1'de gösterilmiştir. Deneme başlangıcında her bir kapta ölçülen 8,64 değerindeki pH, ilk aşamadaki 2 farklı boyutta 3 farklı miktarda leonardit ilavesi ile düşmeye başlamış, daha sonra ortama eklenen klinoptilolit ile bazik yönde artış göstermiştir. Toplam 210 saat sürdürülen çalışmanın 162. saatinde leonardit içeren deneme kaplarına zeolit ilavesi yapılmıştır. Leonarditin etkisinin incelendiği 138. saatin sonunda pH değerleri min 5,77 ile 9 gram/l toz leonardit içeren grupta ve maksimum 7,44 ile 3 g/l çakıl leonardit grubunda belirlenmiştir. Zeolit ilavesinden 210 saat sonra ise minimum pH 7,02 ile 9 gram/l toz leonardit ve zeolit içeren grupta ve maksimum pH 7,79 ile çakıl leonardit ve zeolit içeren grupta belirlenmiştir.




Şekil 1. Deneme süresince (210 saat) elde edilen pH değişimleri

**Tartışma**

Balıklar, fizyolojik işlevlerinin düzgün bir şekilde yerine getirilmesi için hayati önem taşıyan iç asit-baz dengelerini korumak için gelişmiş mekanizmalara sahiptir. Ortamlarındaki pH değişiklikleri bu dengeyi bozarak asit-baz düzenlemesinde aksamalara yol açabilir. Asidik veya alkali koşullar, balık homeostazını zorlayarak iyon düzenlemesini, enzim aktivitesini ve metabolik süreçleri etkileyebilir. pH değişiklikleri, balıkların solunum sistemini etkileyerek oksijen taşınmasını ve dokulara iletimini etkiler. pH, balıkların fizyolojik süreçlerini ve genel sağlığını etkileyen çok önemli bir çevresel faktördür. pH seviyelerindeki dalgalanmalar balık popülasyonları üzerinde önemli etkilere sahip olabilir. Çoğu tatlı su balık türü için optimal pH aralığı genellikle 6,5 ile 9,0 arasındadır ve maksimum büyüme ve üremeye olanak tanır. 6,5-9,0 Çoğu tatlı su balık türü için optimaldir ve maksimum büyüme ve üremeye olanak tanır. Su ürünleri yetiştiriciliği için havuzlardaki su kalitesi. 6,5 ile 9,0 arasında değişen pH seviyeleri çoğu tatlı su balığı türü için en uygun seviyedir. Bu pH aralığı, büyümeleri ve üremeleri için elverişli koşullar sağlayarak sağlıklı popülasyonlara katkıda bulunur (Marium vd., 2023). Suyun sıcaklığı ve pH'ı, sudaki ($NH_4^+$) :($NH_3$) oranını etkiler. Daha düşük sıcaklıklarda ve daha düşük pH'ta amonyağın iyonize olmayan (toksik)formunun ($NH_3$) yüzdesini azaltır (Emerson vd., 1975).

Yapılan bu çalışmada zeolit ilavesinden 210 saat sonra ise minimum pH değerinin 7,02 ile 9 gram/l toz leonardit ve zeolit içeren grupta olduğu belirlenmiştir. Benzer şekilde Şahin vd. (2026) nin bildirdiğine göre, pH, hem balık sağlığını hem de sucul sistemlerdeki amonyak–amonyum dengesini etkileyen kritik bir parametredir. Yaptıkları çalışmada çeşme suyuna ilave edilen leonardit (7,22) ve zeolit+leonardit (7,36) gruplarından elde edilen pH değerlerinin diğer deneme gruplarına kıyasla daha düşük değerlerde olduğu gözlemlemişlerdir. Öz (2024b) yaptığı çalışmada zeolit+leonardit karması içeren deneme gruplarında pH değerlerinin 7,01-7,66 aralığında olduğunu ve bu değerlerin kontrol gruplarına göre istatistiki olarak farklı olduğunu bildirmiştir. Yine Şahin vd. (2025) yaptıkları çalışmada zeolit+leonardit karması içeren deneme gruplarında pH değerlerinin 7,45-7,49 aralığında olduğunu ve bu değerlerin kontrol gruplarına göre istatistiki olarak farklı olduğunu bildirmiştir.

Balık yetiştiriciliğinde türe özgü su koşullarının oluşturulması yetiştiriciliğin başarısında doğrudan etkilidir. Suyun fizikokimyasal özelliklerinin düzenlenmesinde doğal materyallerin kullanılması akvaryum uygulamalarında oldukça yaygındır. Leonardit suyun pH değerlerini düşürücü etkisi ile tetra, melek, cüce vatoz, beta ve gurami türleri gibi düşük pH değerleri, humik maddeler, tanenler gibi sucul ortam koşullarına sahip habitatların oluşturulmasında dikkat çekici bir rol oynama potansiyeline sahiptir. Ayrıca, Leonarditin zeolit ile birlikte kullanımında pH değerlerinde daha nötr ve bazik değerlere ulaşım sağlandığında; daha nötr değerlere yakın veya yüksek pH değerleri isteyen canlı doğuran balık türleri, bazı çiklit türleri ve koi - japon gibi türler için uygun değerler oluşturulabilir.

Bu amaç ile ilk aşamada leonarditin pH değerlerinin düşürme oranı belirlenerek ikinci aşamada zeolitin eklenmesiyle pH değerleri değişiminin belirlenmesi hedeflernmiştir.

## Sonuç

Çalışma verilerinin değerlendirilmesi sonucunda akvaryumlarda substrat malzemesi olarak sıkça kullanılan deniz kabuklularından oluşan kum ve çakılların yanısıra, diatomların fosilleşmiş hali olan diyatomitlerin akvaryum sularındaki amonyumun uzaklaştırılmasında katkı sağladıkları ortaya konmuştur. Mevcut sonuçlara dayanarak, yoğun akuakültür sistemlerinde veya akvaryum koşullarında her iki malzemenin de toksik olmayan, kokusuz, doğada yüksek saflıkta ve bol miktarda bulunan, dolayısıyla düşük maliyetle temin edilebilen materyal olmaları nedenleri ile akvaryumlarda zemin materyali olarak kullanımlarının ortama görsellik katmalarının yanında su parametrelerinin optimum seviyelerde tutulabilmelerine de katkı sağlayacakları tespit edilmiştir.

## Beyanlar

***Etik Kurul Onay Belgesi***

Bu çalışma için herhangi bir etik kurul onayı gerekmemektedir.

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DŞ: Kavramsallaştırma, araştırma, denemenin tasarlanması ve yürütülmesi, verilerin toplanması, verilerin analiz edilmesi, yazma

MÖ: Araştırma, verilerin toplanması, verilerin analiz edilmesi, yazma

ÜÖ: Verilerin analiz edilmesi, istatistiki hesaplamalar

Tüm yazarlar, makalenin yayımlanacak son hâlini okumuş ve onaylamıştır.

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# Probiotics In Poultry Nutrition: A Comprehensive Bibliometric and Thematic Analysis of Two Decades of Research (2000-2025)

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## Abstract

This study presents a comprehensive bibliometric and thematic analysis of two decades of global research on probiotics in poultry nutrition (2000-2025) using the R bibliometric library version 4.4.3 and VOSviewer software version 1.6.20. Based on a documentary corpus of 4 447 publications from 317 scientific journals indexed in Scopus and Web of Science (WoS), it maps the evolution of scientific production, major actors, international collaboration networks, and thematic structure of the field. According to the analyses, China, the United States, and Egypt are the most productive countries in this field, and the United States appears as a central actor, with collaborations connecting several continents. Wang Y., Wang J., and Liu Y appear as major contributors to research on probiotics in poultry nutrition, and Zagazig University, an Egyptian university, appears as the most influential institution. The results also reveal exponential growth in publications particularly from 2016 onwards, as well as progressive structuring of the field around dominant themes such as “broilers”, “growth-performance”, “performance”, “probiotics”, and “supplementation”, shifting from primarily zootechnical approaches towards more integrated investigations, including interactions between intestinal microbiota, growth performance, and immune response. Despite this apparent maturity, the field remains fragmented, with dispersion of themes and approaches. This study thus constitutes one of the first bibliometric analyses specifically dedicated to probiotics in avian nutrition and highlights the need for better conceptual integration to guide future research and optimize the use of these additives in poultry production.

***Keywords:*** *probiotics, chicken, feed additives, poultry nutrition, Biblioshiny, VOSviewer, bibliometric analyses*

## Introduction

The global poultry industry plays a decisive role in global food security. According to FAO (2021), global poultry meat production increased from 9 to 133 million tons between 1961 and 2020, representing 40% of total global meat production. In 2019, the global poultry flock exceeded 27 billion birds (Yıldız, 2021). Furthermore, the demand for animal proteins continues to grow under the combined effect of population growth, urbanization, and rising incomes (Henchion et al., 2017). In this dynamic, the poultry sector is expected to represent 43% of proteins from all meats consumed by 2033 (OECD & FAO, 2024), thus establishing itself as the most dynamic segment of contemporary livestock production.

This unprecedented growth in the poultry sector has, however, relied on farming practices that are now being questioned. Until 2006, poultry farming systems widely used antibiotics as growth promoters (AGP). Added to animal feed for more than 60 years at subtherapeutic doses (Castanon, 2007), they act by reducing intestinal microbial load and associated inflammation, which limits energy losses and allows the animal to devote more energy to its growth (Niewold, 2007).

Although this practice significantly improved zootechnical performance in animals (Gadde et al., 2017; Wickramasuriya et al., 2024), its prolonged use has favored the emergence of antibiotic-resistant bacteria, compromising their effectiveness in both veterinary and human medicine (Ben et al., 2019; World Health Organization, 2000). This awareness led to the progressive ban of AGP in the European Union from January 1, 2006 (Regulation (EC) No 1831/2003, 2003), then in California from 2015 (CA State Legislature, 2015) before extending to other countries.

While this ban responds to a public health imperative, it has profoundly modified production strategies, requiring the search for alternatives capable of maintaining zootechnical performance in the absence of AGP. In this context, probiotics have gradually emerged as a promising solution in avian nutrition. They are defined as live microorganisms that, when administered in adequate amounts, exert a beneficial effect on the host's health (FAO/WHO, 2002). In poultry, probiotics mainly include strains of Lactobacillus, Bifidobacterium, Bacillus, and Enterococcus (Chennoun & Zitouni, 2020). Their interest lies in their ability to modulate the intestinal microbiota, improve the integrity of the digestive barrier, and stimulate immune responses, thus contributing to the optimization of growth performance in the absence of AGP (Idowu et al., 2025; Naeem & Bourassa, 2025; Yosi & Metzler-Zebeli, 2023).

Since the early 2000s, scientific research on probiotics in avian nutrition has experienced considerable expansion, reflecting the sustained interest of the scientific community and industry in these feed additives. However, although Wickramasuriya et al. (2024) conducted a bibliometric analysis on alternatives to antibiotic growth promoters for poultry, no comprehensive bibliometric study has yet been exclusively devoted to mapping the evolution of research on the use of probiotics in avian nutrition over the past two decades. In this context, bibliometric analysis appears as an appropriate approach to analyze scientific production in this field. It allows the identification of research trends, major contributions, as well as interactions between authors and themes over time (F.-H. Liu et al., 2022; Nicolaisen, 2010). Based on statistical indicators and reproducible methods, it provides a synthetic view of the evolution of knowledge (Wallin, 2005).

This study therefore aims to analyze the temporal evolution of global scientific production, identify the main research actors (authors, institutions, countries, journals), and map international collaboration networks. Finally, it also intends to reveal the thematic structure of the field through the analysis of keywords and co-occurrences, as well as identify the most influential publications and foundational works that have structured this research field.

## Materials and Methods

### *Databases, search strategies and article selection procedure*

To analyze global scientific production on probiotics in avian nutrition between 2000 and 2025, two international bibliographic databases were used to constitute the documentary corpus. These include Scopus (Elsevier) and Web of Science (WoS) (Clarivate). These databases were selected because they offer broad and complementary coverage of international scientific literature. They provide complete bibliographic metadata necessary for bibliometric analyses (Birkle et al., 2020; Martín-Martín et al., 2018). To constitute the documentary corpus of this study, a search strategy was developed by combining keywords related to probiotics and avian species. The Boolean query was constructed according to the following model to capture all relevant publications while minimizing documentary noise: (probiotic* OR prebiotic* OR symbiotic* OR “direct-fed microbial*” OR “beneficial bacteria” OR “lactic acid bacteria” OR Lactobacillus* OR Bacillus* OR Bifidobacterium* OR Saccharomyces* OR Enterococcus* OR “microbial supplement*”) AND (poultry OR chicken* OR broiler* OR “laying hen*” OR avian OR “broiler chicken*”) NOT (human* OR patient* OR “clinical trial*” OR swine OR pig* OR porcine OR cattle OR bovine OR sheep OR ovine OR fish OR aquaculture OR shrimp)”. The search terms were applied in the title, abstract, and keywords fields of publications in Scopus. In WoS, these terms were searched in all fields. The covered period extends from January 1st, 2000 to December 31, 2025. Regarding the article selection process, this procedure was carried out in several stages as shown in Figure 1. After applying inclusion and exclusion criteria and removing duplicates, a total of 4,447 documents were used to conduct this study. Only documents in English, published in journals as articles, and available in open access were retained. Furthermore, to avoid excessive documentary noise, certain research categories were excluded as shown in Figure 1.

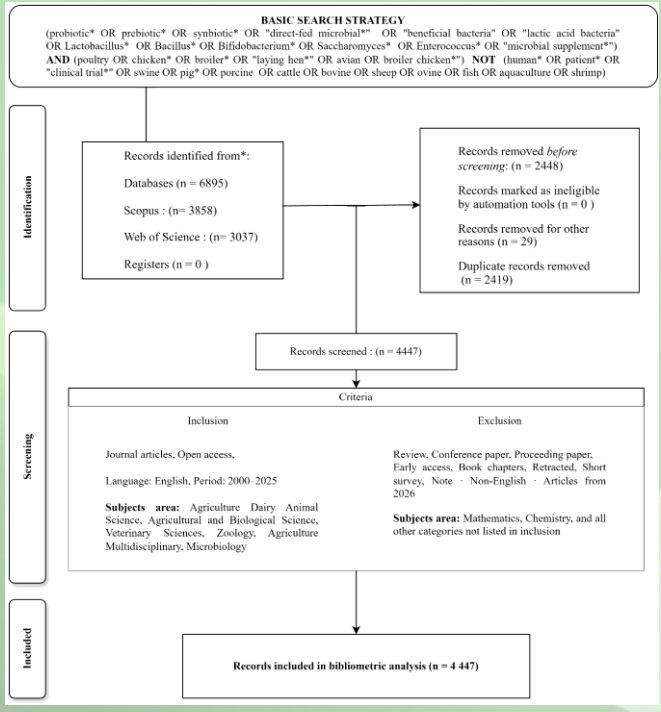



Figure 1: Data collection PRISMA flow diagram

***Bibliometric analysis tools and bibliometric indicators analyzed***

Two complementary software programs were mobilized to conduct this bibliometric analysis. The RStudio program (version 4.4.3) was used via the *biblioshiny()* interface of the *bibliometrix* package (Aria & Cuccurullo, 2017) for data processing and analysis. In complement, VOSviewer (version 1.6.20) (van Eck & Waltman, 2017) enabled the construction and visualization of bibliometric networks, particularly keyword co-occurrence.

To ensure data compatibility with *biblioshiny*, the documentary corpus was exported from each database according to a specific format. For Scopus, the export was performed in *.csv* format, while for WoS the Plain Text *(.txt)* format was used. In total, 17 bibliometric analyses were performed in this analytical environment.

**Results**

***Main information***

Table 1 presents the main information relating to scientific publications devoted to the use of probiotics in poultry nutrition between 2000 and 2025.

Table 1: Main Information

| *Description* | *Results* |
|---|---|
| MAIN INFORMATION ABOUT DATA | |
| Timespan | 2000:2025 |
| Sources (Journals, Books, etc) | 317 |
| Documents | 4447 |
| Annual Growth Rate % | 15,95 |
| Document Average Age | 6,42 |
| Average citations per doc | 19,9 |
| DOCUMENT CONTENTS | |
| Keywords Plus (ID) | 8144 |
| Author's Keywords (DE) | 7296 |
| AUTHORS | |
| Authors | 13716 |
| Authors of single-authored docs | 52 |
| AUTHORS COLLABORATION | |
| Single-authored docs | 84 |
| Co-Authors per Doc | 6,27 |
| International co-authorships % | 18,1 |
| DOCUMENT TYPES | |

From a targeted search strategy combining keywords related to probiotics and poultry, the Scopus and Web of Science databases identified a total of 4447 articles published between 2000 and 2025. Analysis of this documentary corpus reveals that these works were published in 317 specialized scientific journals, with an annual publication growth rate of 15.95%. Furthermore, the average age of publications is 6.42 years, while each article records an average of 19.9 citations. In terms of scientific collaboration, the analysis reports 13716 contributing authors, among whom 52 published without co-authors. Regarding international collaboration, it represents 18.1% of all publications.

***Annual status and average annual citations of scientific publications***

Figure 2 presents the joint evolution of the annual number of publications (blue curve) and the average number of citations per article (orange curve) on probiotics in avian nutrition over the period 2000-2025.

This superimposed representation allows observation of the inverse relationship between these two bibliometric indicators. Indeed, according to this figure, it is easy to note exponential growth in the number of publications, particularly marked from 2016 onwards. The annual volume increases from 17 articles in 2000 to 687 in 2025, with notable acceleration during the last five years where the number of publications rises from 337 in 2020 to 687 in 2025, representing a doubling of scientific production volume. Conversely, this same figure reveals a decreasing trend in the average number of citations per article. While publications from 2000 total approximately 91 citations on average, this number fluctuates between 35 and 71 citations during the period 2002-2014, before beginning a progressive decline. Recent years show the lowest values with an average number of citations around 27 citations in 2019. A drop to less than 10 citations for publications from 2023 to 2025 follows, with less than 2 citations on average per article in 2025. This figure clearly shows a contrasted dynamic. Indeed, the quantitative expansion of scientific production is accompanied by a decrease in the average number of citations per article.

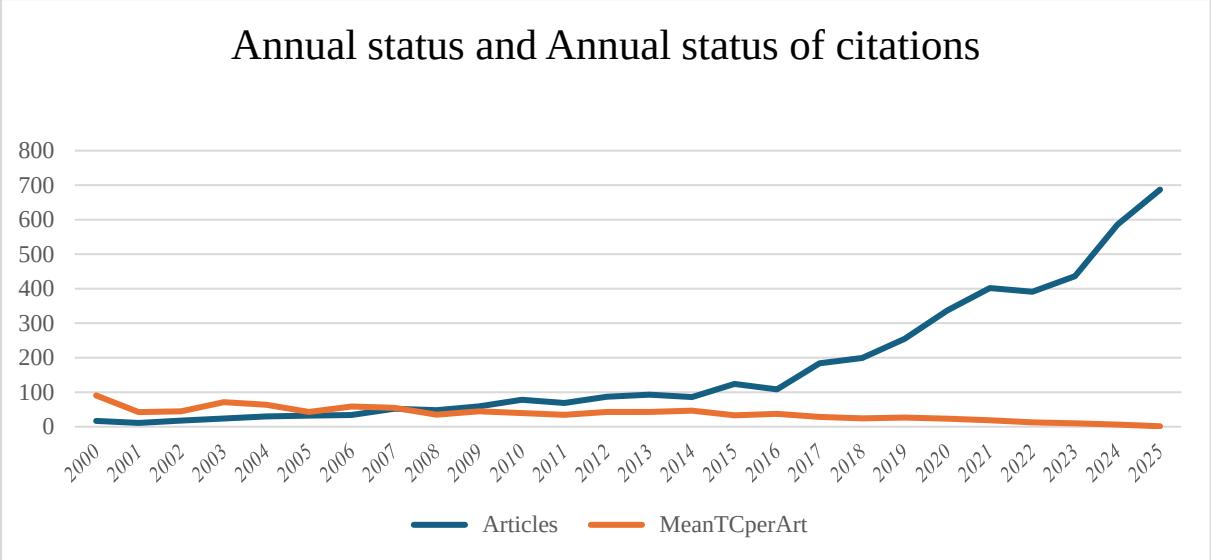



Figure 2: Annual status and annual status of citations

***Sankey diagram***

Figure 3 highlights the relationships between three key bibliometric dimensions: authors' countries of affiliation, the most productive authors, and the main keywords.

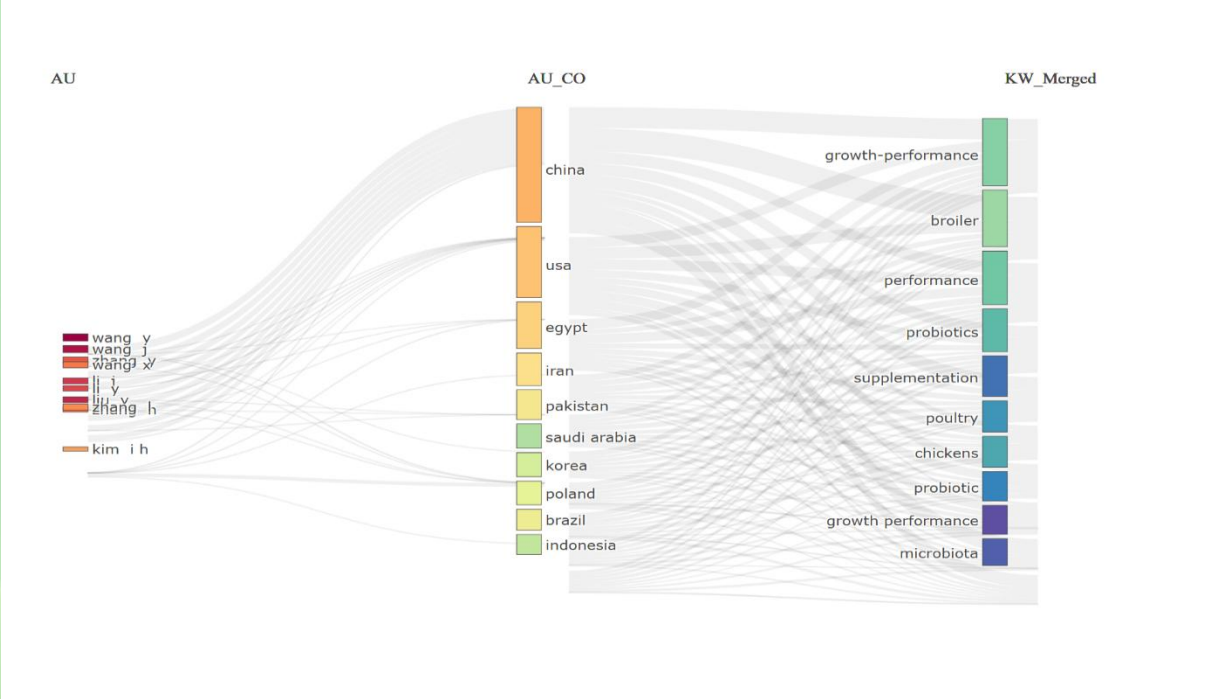



Figure 3: Three-Field Plot

Created using Biblioshiny, this figure allows identification of the most productive geographical areas and researchers as well as the most dominant keywords. Thus, geographically, China stands out clearly as the most contributing country in terms of scientific production related to the use of probiotics in avian nutrition between 2000 and 2025. Among the most prolific authors, Wang Y. appears as a major contributor to the production of scientific documents in this field. Keyword analysis reveals that "growth-performance", "broilers", "performance", "probiotics", and "supplementation" constitute the most recurrent terms for article indexing, thus reflecting the main research axes favored by authors in this field.

***Most productive scientific journals***

Table 2 presents the 10 most recurrent scientific journals in the publication of articles related to themes concerning the use of probiotics in avian nutrition between 2000 and 2025.

Table 2: Most important journals

| *Sources* | *Articles* |
|---|---|
| POULTRY SCIENCE | 1166 |
| ANIMALS | 359 |
| JOURNAL OF APPLIED POULTRY RESEARCH | 168 |
| FRONTIERS IN VETERINARY SCIENCE | 149 |
| BRAZILIAN JOURNAL OF POULTRY SCIENCE | 108 |
| INTERNATIONAL JOURNAL OF POULTRY SCIENCE | 95 |
| ITALIAN JOURNAL OF ANIMAL SCIENCE | 95 |
| EFSA JOURNAL | 85 |
| VETERINARY WORLD | 73 |
| ASIAN-AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES | 63 |

According to the analysis of this table, it appears that the main publication journals in the studied field are "Poultry Science", "Animals", and "Journal of Applied Poultry Research". The journal Poultry Science ranks widely first with 1,166 articles, followed by Animals with 359 publications. Journal of Applied Poultry Research occupies third position with a total of 168 articles.

***Most productive authors***

Figure 4 presents the ranking of the most relevant authors according to their scientific production in the field of probiotics in avian nutrition from 2000 to 2025.

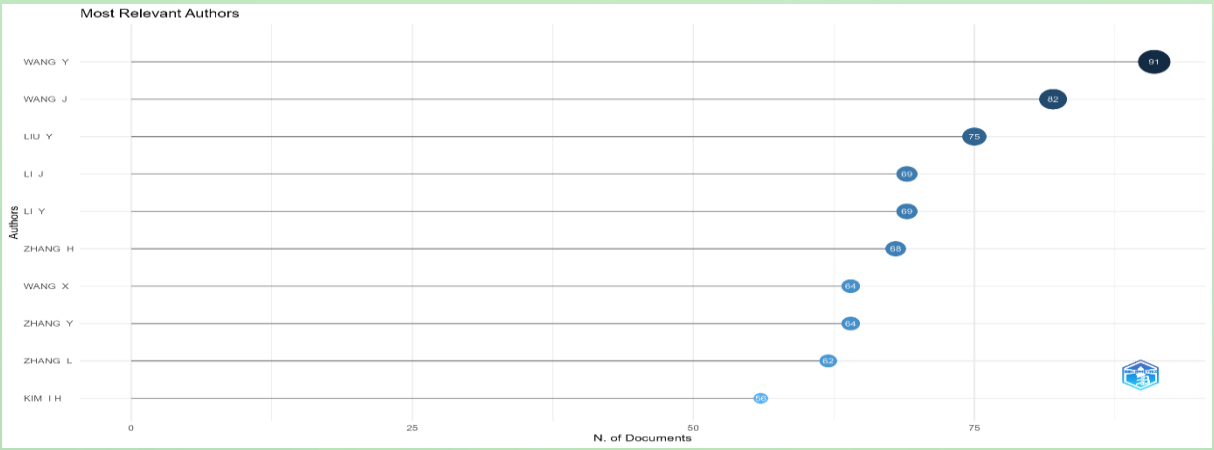



Figure 4 : Most prolific Authors

Wang Y. positions himself as the most prolific author with 91 publications, followed by Wang J. with 82 documents. Liu Y. occupies third position with 75 publications. These three authors total 248 publications, representing 35.4% of the cumulative production of the ten main authors, while the seven other authors contribute 452 publications, representing 64.5%. Although the first three authors are more productive, the difference with the other authors remains modest. Indeed, the three most productive authors present an individual production of 82.7 publications, compared to 64.6 for the other authors.

***Universities with the most publications***

Table 3 presents the ranking of the 10 most productive institutions in the field of probiotics in avian nutrition.

Table 3: Most important universites

| *Affiliation* | *Articles* |
|---|---|
| ZAGAZIG UNIVERSITY | 186 |
| ISLAMIC AZAD UNIVERSITY | 159 |
| UNIVERSITI PUTRA MALAYSIA | 158 |
| CHINA AGRICULTURAL UNIVERSITY | 137 |
| UNIVERSITY OF ARKANSAS SYSTEM | 125 |
| UNIVERSITY OF ARKANSAS FAYETTEVILLE | 122 |
| KING SAUD UNIVERSITY | 120 |
| UNITED STATES DEPARTMENT OF AGRICULTURE (USDA) | 102 |
| UNIVERSITY OF VETERINARY AND ANIMAL SCIENCE - PAKISTAN | 93 |
| UNIVERSITY OF GEORGIA | 90 |

Zagazig University ranks first with 186 publications, followed by Islamic Azad University (159 articles) and Universiti Putra Malaysia (158 articles). China Agricultural University ranks fourth with 137 publications, while the University of Arkansas System completes the top 5 with 125 articles. This distribution highlights geographical diversity of institutions, with contributions from Africa (Egypt), Asia (Iran, Malaysia, China), and North America (United States).

**Scientific Production by Country**

Figure 5 illustrates the geographical distribution of global scientific production related to the use of probiotics in avian nutrition between 2000 and 2025.

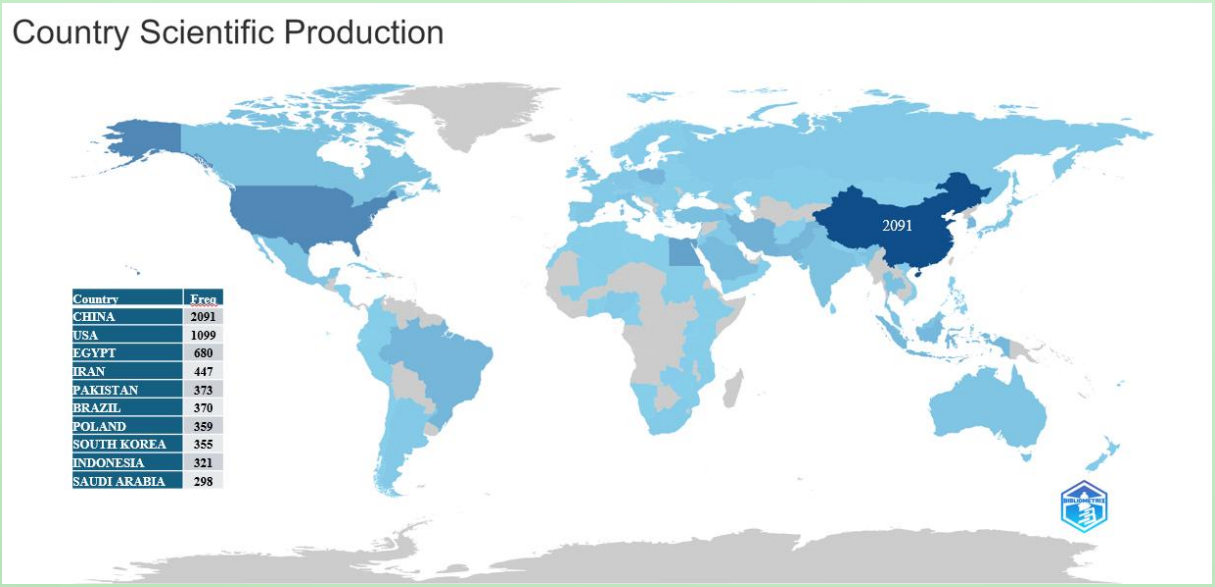



Figure 5: Most productive countries

The map is based on a gradient of three colors indicating the volume of publications by country. Dark blue corresponds to high production, light blue to moderate production, while gray indicates low or almost non-existent production (Hodonou et al., 2025).

China positions itself as the undisputed world leader with 2,091 publications, nearly double American production. The United States occupies second position with 1099 articles, followed by Egypt with 680 publications, Iran with 447 publications, and Pakistan with 373 publications. These five countries have a total of 4549 publications, representing a significant share of global scientific production in this field. The map reveals a concentration of research in four major regions, namely East Asia (dominated by China), the Middle East (Iran, Saudi Arabia), North Africa (Egypt), and North America (United States). Europe, South America, and sub-Saharan Africa show lighter color intensities, reflecting more modest or even non-existent scientific production.

***Scientific collaboration between countries***

Figure 6 represents the world map of bilateral scientific collaborations in the field of probiotics in avian nutrition from 2000 to 2025.

Collaboration flows materialized by lines connecting partner countries, while the intensity of the red color indicates the degree of involvement of countries in these international collaborations. Thus, the three most active bilateral partnerships are: the Egypt-Saudi Arabia collaboration with 75 joint publications, the China-United States partnership with 41 publications, and the United States-Mexico collaboration with 31 publications. Other notable partnerships include collaborations between China and Egypt, China and Pakistan, as well as between Pakistan and Saudi Arabia. Furthermore, the United States appears as a central actor, with collaborations established with Canada, Egypt, Mexico, South Korea, and Iran. These cross-border scientific collaborations underscore the priority nature of research on probiotics in avian nutrition on a global scale during the last two decades.

***Most cited countries***

Table 4 presents the ranking of the 10 most cited countries according to the total number of citations received by their publications in the field of probiotics in avian nutrition from 2000 to 2025. This indicator reflects the scientific impact and international visibility of research work produced by each country.

China dominates widely with 21,185 total citations, representing 55% more than the United States, which occupies second position with 13,617 citations. South Korea completes third position with 4,732 citations. These three countries alone total 39,534 citations, representing 69% of the cumulative total of the ten countries presented and more than double the citations of the seven other countries combined (17,769 citations). This thus reflects the quality and international visibility of the work produced and published by these nations.

***Most important documents***

Table 5 presents the ranking of the 10 most cited publications in the field of probiotics in avian nutrition between 2000 and 2025. The total number of citations (TC) reflects the academic influence and scientific scope of each article within the research community.

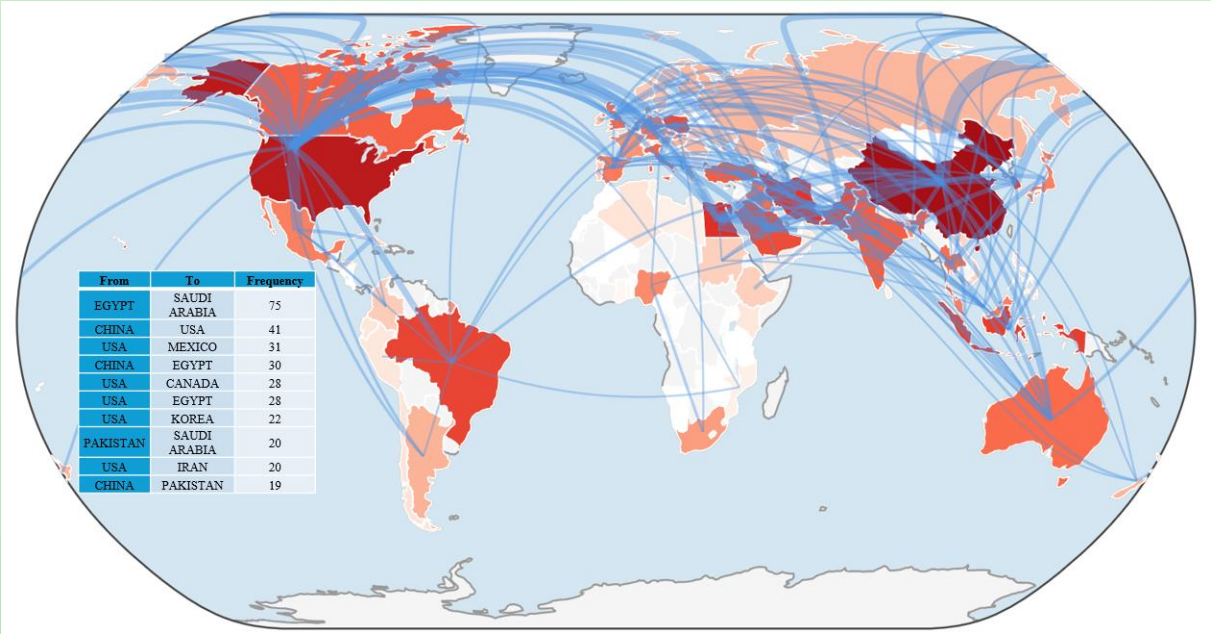



Figure 6: Cooperation map of countries

Table 4: Most Cited Countries

| *Country* | *TC* |
|---|---|
| CHINA | 21185 |
| USA | 13617 |
| KOREA | 4732 |
| EGYPT | 3267 |
| CANADA | 2911 |
| IRAN | 2878 |
| POLAND | 2633 |
| MALAYSIA | 2135 |
| SPAIN | 2050 |
| AUSTRALIA | 1895 |

Table 5: Most cited publications

| *Paper* | *DOI* | *TC* |
|---|---|---|
| XU Z, 2003, POULTRY SCI | https://doi.org/10.1093/ps/82.6.1030 | 663 |
| AWAD W A, 2009, POULTRY SCI | https://doi.org/10.3382/ps.2008-00244 | 626 |
| SPRING P, 2000, POULTRY SCI | https://doi.org/10.1093/ps/79.2.205 | 573 |
| WEI S, 2013, POULTRY SCI | https://doi.org/10.3382/ps.2012-02822 | 412 |
| SONG J, 2014, POULT SCI | https://doi.org/10.3382/ps.2013-03455 | 401 |
| CROSS D E, 2007, BRIT POULTRY SCI | https://doi.org/10.1080/00071660701463221 | 380 |
| VIVEROS A, 2011, POULTRY SCI | https://doi.org/10.3382/ps.2010-00889 | 376 |
| MOUNTZOURIS K C, 2010, POULTRY SCI | https://doi.org/10.3382/ps.2009-00308 | 365 |
| ZHANG A, 2005, POULT SCI | https://doi.org/10.1093/ps/84.7.1015 | 354 |
| BAURHOO B, 2007, POULTRY SCI | https://doi.org/10.1093/ps/86.6.1070 | 345 |

The article by Xu et al. (2003), published in Poultry Science, ranks first with 663 citations. Entitled "Effects of Dietary Fructooligosaccharides on Digestive Enzyme Activities, Intestinal Microflora and Morphology of Male Broilers", this pioneering work likely laid the foundations for the study of prebiotics in avian nutrition. Awad et al. (2009), also published in Poultry Science, rank second with 626 citations thanks to their article entitled "Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens". Spring et al. (2000) complete this ranking in third position with 573 citations for their study entitled "The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks", which is also published in Poultry Science. Collectively, these three foundational articles accumulate 1,862 citations and share the distinction of having been published in the same journal, confirming the central role of the journal "Poultry Science" in the dissemination of research on the use of probiotics in poultry nutrition during these two decades.

***Top 5 most cited articles by decade***

Table 6 presents the top 5 most cited articles across three distinct periods.

Table 6 : Most cited articles by publication period

| *Year* | *Paper* | *DOI* | *TC* |
|---|---|---|---|
| 2000 | XU Z, 2003, POULTRY SCI | https://doi.org/10.1093/ps/82.6.1030 | 663 |
| - | AWAD W A, 2009, POULTRY SCI | https://doi.org/10.3382/ps.2008-00244 | 626 |
| 2009 | SPRING P, 2000, POULTRY SCI | https://doi.org/10.1093/ps/79.2.205 | 573 |
| | CROSS D E, 2007, BRIT POULTRY SCI | https://doi.org/10.1080/00071660701463221 | 380 |
| | (Amit-Romach et al., 2004)HOO B, 2007, POULTRY SCI | https://doi.org/10.1093/ps/86.6.1070 | 345 |
| 2010 | WEI S, 2013, POULTRY SCI | https://doi.org/10.3382/ps.2012-02822 | 412 |
| - | VIVEROS A, 2011, POULTRY SCI | https://doi.org/10.3382/ps.2010-00889 | 376 |
| 2019 | MOUNTZOURIS K C, 2010, POULTRY SCI | https://doi.org/10.3382/ps.2009-00308 | 365 |
| | SOHAIL M U, 2012, POULTRY SCI | https://doi.org/10.3382/ps.2012-02182 | 308 |
| | SVIHUS B, 2014, J APPL POULTRY RES | https://doi.org/10.3382/japr.2014-00937 | 293 |
| 2020 | REHMAN A, 2020, POULTRY SCI | https://doi.org/10.1016/j.psj.2020.09.043 | 149 |
| - | HU R, 2021, J ANIM SCI BIOTECHNO | https://doi.org/10.1186/s40104-020-00532-4 | 148 |
| 2025 | REDA F M, 2020, ANIMALS-BASEL | https://doi.org/10.3390/ani10050754 | 143 |
| | XU Y, 2021, POULTRY SCI | https://doi.org/10.1016/j.psj.2021.101358 | 139 |
| | ALAGAWANY M, 2021, POULTRY SCI | https://doi.org/10.1016/j.psj.2021.101172 | 133 |

Temporal analysis confirms the historical dominance of foundational works from the first decade. Unsurprisingly, the three most cited articles globally (Awad et al., 2009; Spring et al., 2000; Z. Xu et al., 2003), described in Table 5, also dominate their respective period 2000-2009. These alone total 1862 citations. This correspondence would underscore their role as structuring references that shaped the field's orientations for the following two decades. Of the five articles from this period that accumulate 2767 citations, four are published in Poultry Science. The 2010-2019 decade reveals the emergence of new references. Wei et al., (2013) dominate with 412 citations, followed by Viveros et al. (2011) with 376 citations and Mountzouris et al. (2010) with 365 citations. Poultry Science remains the primary publication vehicle for this period, also hosting four of the five most cited articles. The 2020-2025 period shows lower absolute totals, a direct consequence of their lesser seniority. The articles by (Rehman et al., 2020, 149 citations), (Hu et al., 2021, 148 citations), and (Reda et al., 2020, 143 citations) occupy the first three positions respectively. This period is distinguished by a diversification of publication outlets, with the appearance of journals such as "Animals" and "Journal of Animal Science and Biotechnology" alongside "Poultry Science".

***Word cloud***

Figure 7 presents a word cloud generated from all keywords in the analyzed documentary corpus. Created using Biblioshiny, this visualization allows identification of central concepts and dominant themes in the field of probiotics in avian nutrition between 2000 and 2025. In this figure, the size of each term is proportional to its frequency of appearance in the publications.

Five terms stand out clearly by their preeminence. These include "probiotics", "growth-performance", "broiler", "performance", and "chickens", which reflect the major research axes. Intermediate-sized terms reveal keywords such as "supplementation", "microbiota", "lactobacillus", "microflora", and "intestinal morphology".

***Tree map***

Figure 8 presents a tree map (TreeMap) of keywords from the corpus. The area of each rectangle is proportional to the frequency of occurrence of the term in all analyzed publications. This hierarchical visualization allows simultaneous comprehension of the distribution and relative importance of different concepts structuring the field of probiotics.




Figure 7: Word Cloud

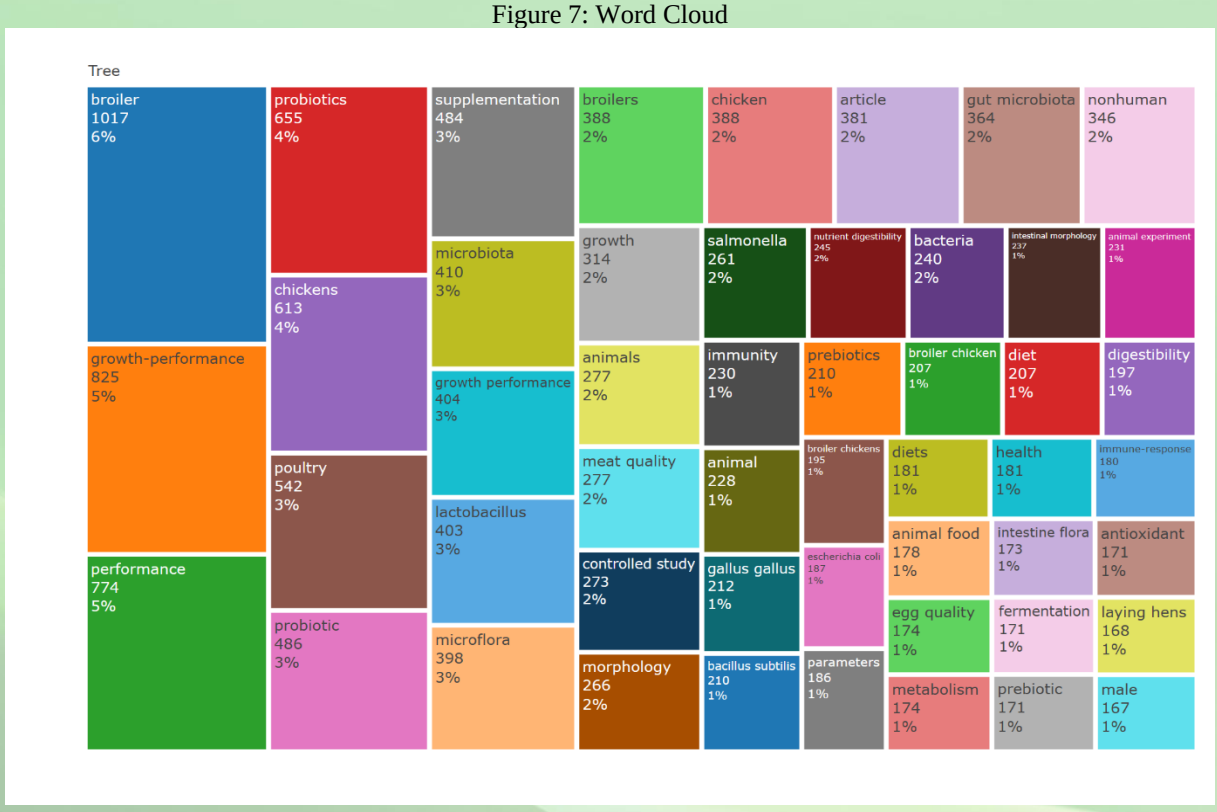



Figure 8: Tree map

Three thematic categories emerge by their predominance. First, terms related to avian species that total a substantial share, with “broiler” (1017 occurrences, 6%), “chickens” (613, 4%), “broilers” (388, 2%), and “poultry” (542, 3%) collectively representing approximately 15% of keywords. Second, performance parameters dominate with “growth-performance” (825, 5%) and “performance” (774, 5%), which total 10% of occurrences. Finally, in third position come the additives studied represented by “probiotics” (655, 4%), “probiotic” (486, 3%), and “prebiotics” (210, 1%), totaling 8% of occurrences.

***Temporal evolution of keywords***

Figure 9 illustrates the temporal evolution of cumulative occurrences of the ten principal keywords over the 2000–2025 period. The curves represent the progressive accumulation of each term over the years.

All curves display a similar two-phase trajectory. The 2000-2015 period shows slow, quasi-linear growth. From 2016 onwards, an exponential acceleration begins for all terms. The term “broiler” clearly dominates with approximately 1000 cumulative occurrences in 2025. “Chickens” and “growth-performance” follow with approximately 850 occurrences each. Then come “performance”, “microbiota”, “probiotics”, “poultry” and “supplementation”, which reach comparable levels, ranging between 500 and 650 occurrences.

***Trend topics***

Figure 10 presents the temporal evolution of 50 emerging keywords over the 2005–2025 period. The temporal evolution of all keywords was analyzed using the «Trend Topics» function of Biblioshiny. A minimum frequency threshold of 25 occurrences was applied to exclude terms that appear very rarely, to focus the analysis on recurring concepts. Furthermore, for each year, only the 5 most frequent keywords were retained to ensure a balanced representation of the different periods while maintaining the readability of the graph. This approach made it possible to identify progressive changes in the major research themes without informational overload. In this figure, each line represents a term, the bubbles indicate the median year of appearance, and their size reflects the total frequency of occurrence.

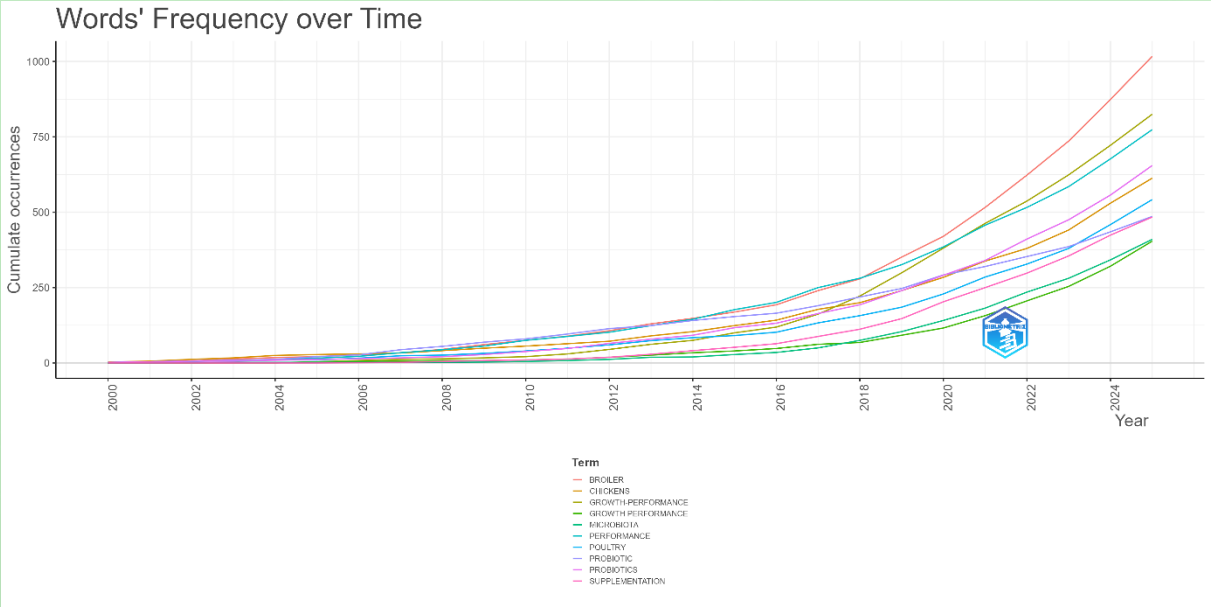



Figure 9: Words' Frequency over Time

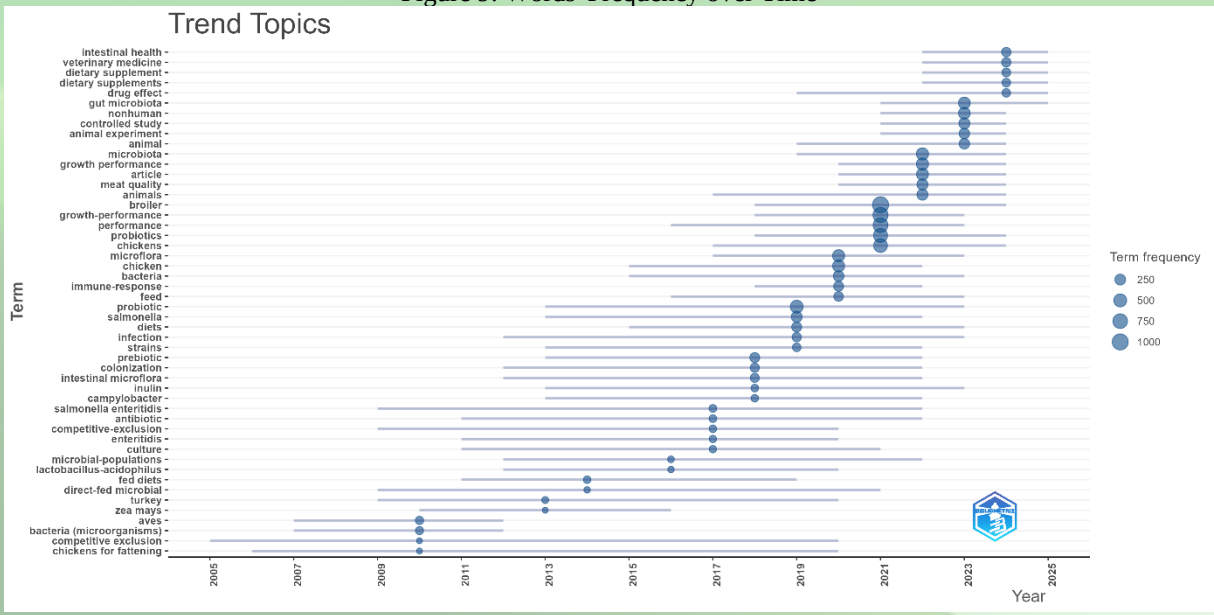



Figure 10 : Trend topics

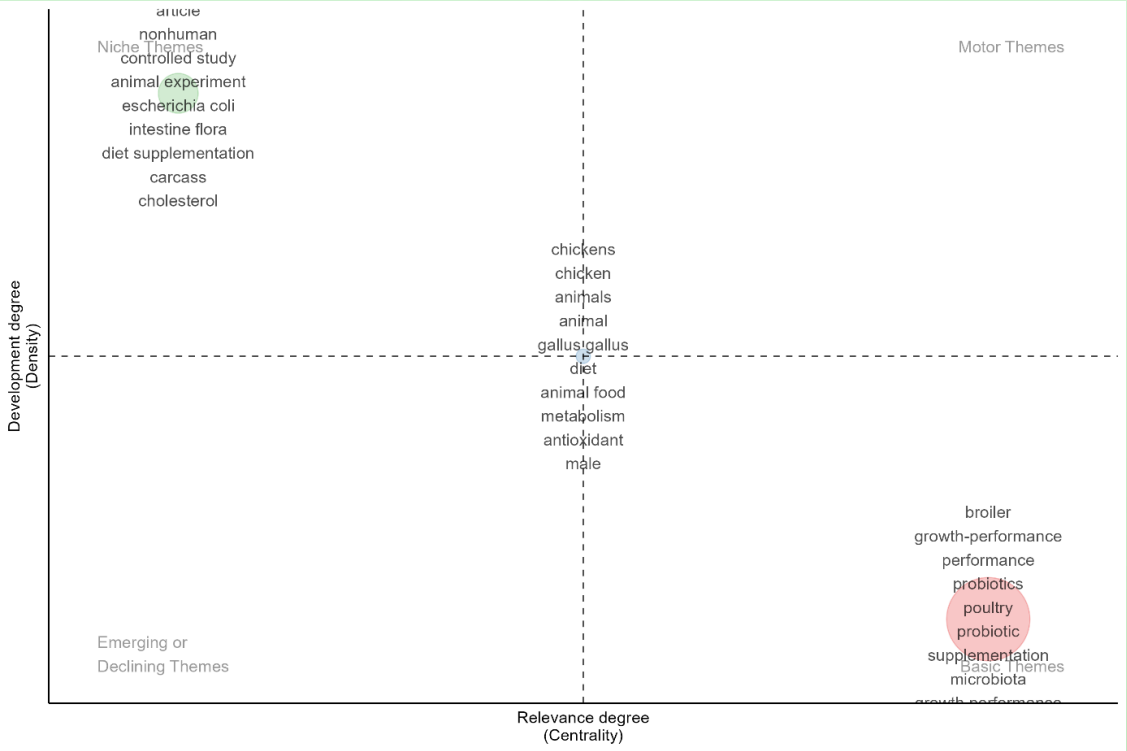



Figure 11 : Thematic map

Over the 2005-2012 period, research focused primarily on microorganisms and pathogenic agents. The terms “Lactobacillus acidophilus”, “direct-fed microbial”, “competitive exclusion”, “Salmonella enteritidis”, “Campylobacter” and “antibiotic” appeared frequently, reflecting an interest in microbial strains, mechanisms of action, and pathogen control in the context of antibiotic use. Between 2013 and 2020, the themes evolved toward the study of the intestinal ecosystem. The keywords “intestinal microflora”, “prebiotic”, “probiotic”, “colonization”, “immune response” and “strains” were widely represented, reflecting growing attention to host-microbiota interactions and immune responses. In the most recent period (2021-2025), research has shifted toward more integrative approaches. The terms “gut microbiota”, “intestinal health”, “veterinary medicine”, “dietary supplement”, “growth performance” and “broiler” appear with high frequency, indicating a focus on intestinal health and zootechnical performance. Overall, these results reflect a progressive evolution of research themes over time.

***Thematic map of author keywords***

Figure 11 illustrates the thematic mapping of keywords according to their centrality and density. The quadrants of the map allow the distinction between basic themes, motor themes, niche themes, and emerging or declining themes.

Basic themes, located in the lower-right quadrant, present high centrality but low density. They represent concepts that are essential to the field but have yet to be extensively developed. Keywords such as “probiotics”, “poultry”, “microbiota”, “growth-performance” and «supplementation» belong to this category. This indicates their central role in the overall understanding of the subject, despite the limited development of research associated with them. Regarding motor themes located in the upper-right quadrant, these combine both high centrality and high density. They constitute the dominant and well-structured axes of research. In this analysis, no group of keywords is visible in this zone. This suggests the current absence of strongly consolidated and central themes. Niche themes, located in the upper-left quadrant, present high density but low centrality. They correspond to specialized or technical subjects, developed in depth by restricted groups of researchers. Among the keywords found in this zone are: “Escherichia coli”, “intestinal flora”, “carcass”, “diet supplementation” and “nonhuman”. As for emerging or declining themes, in the lower-left quadrant, they are poorly central and weakly developed. They indicate research areas either in emergence or losing interest and are here entirely absent. Keywords located at the junction of the vertical and horizontal axes, close to the center of the map, occupy an intermediate position in terms of centrality and density. This position reflects a transversal role. In other words, keywords such as “chickens”, “animals”, “diet” and “metabolism” connect multiple themes and serve as bridges between different research domains, without being themselves strongly developed or dominant themes. Their importance therefore lies in their capacity to connect and structure the overall scientific field under study.

***Co-occurrence analysis of keywords***

Figure 12 presents a network map of the 500 most frequent keywords in the documentary corpus. Of the 13,637 keywords identified, 976 reach the minimum threshold set at 10 occurrences across the entire corpus, of which 500 were retained for visualization with VOSviewer.

The **green cluster** groups terms related to probiotics and performance. “Probiotics”, “probiotic”, “broiler” and “growth-performance” dominate this group. “Supplementation”, “microflora” and “Bacillus subtilis” complete this theme. This cluster represents the applied core of the field, centered on the use of probiotics in poultry production.

The **blue cluster** brings together general methodological and descriptive terms. “Animals”, “microbiology”, “chickens” and “growth” structure this group. “Dietary supplements”, “probiotic agent”, “Gallus gallus”, “diet” and “anti-bacterial agents” also feature here. This cluster constitutes the basic vocabulary common to all research.

The **red cluster** contains experimental and physiological terms. “Article”, “controlled study”, “animal experiment” and “intestinal flora” dominate. “Diet supplementation”, “cholesterol” and “immune response” complete this group. This cluster reflects the methodological approaches and physiological parameters studied.

The **yellow cluster** at the center groups terms including “Escherichia coli”, “Staphylococcus aureus”, “antimicrobial resistance”, “antibiotic resistance”, “Enterococcus faecium”, “Lactobacillus plantarum” and “food safety”. This cluster gathers terms related to pathogens, antibiotic resistance and food safety. The dense connections between clusters reveal strong thematic integration. The green cluster connects intensely to both the blue and red clusters. This structure reveals a coherent field despite its thematic diversity.

***Factorial analysis of keywords***

Figure 13 presents a conceptual map obtained from a factorial analysis of keywords. This representation highlights the organization of the scientific field under study through several distinct thematic groupings identifiable by clusters of terms sharing semantic proximity. The distribution of keywords shows that research is not organized around a single dominant axis, but rather around several complementary thematic poles.

In the **upper-left section**, the **violet thematic group** brings together terms related to physiology, growth and dietary supplements. Terms such as “dietary supplement”, “animal feed”, “physiology”, “growth development” and “veterinary medicine” appear closely associated. This grouping reflects a general approach to the field, centered on the biological and nutritional foundations as well as the physiological mechanisms related to the use of dietary supplements in animals. Adjacent to this group, a second **green thematic group** focuses more on animal health and avian diseases. Terms such as “poultry diseases”, “microbiology”, “bird disease”, “animals” and “diet” indicate a more applied orientation, centered on the study of interactions between pathologies, diet and animal health. This group shows that research is not limited to zootechnical performance but also places considerable importance on sanitary and microbiological aspects.

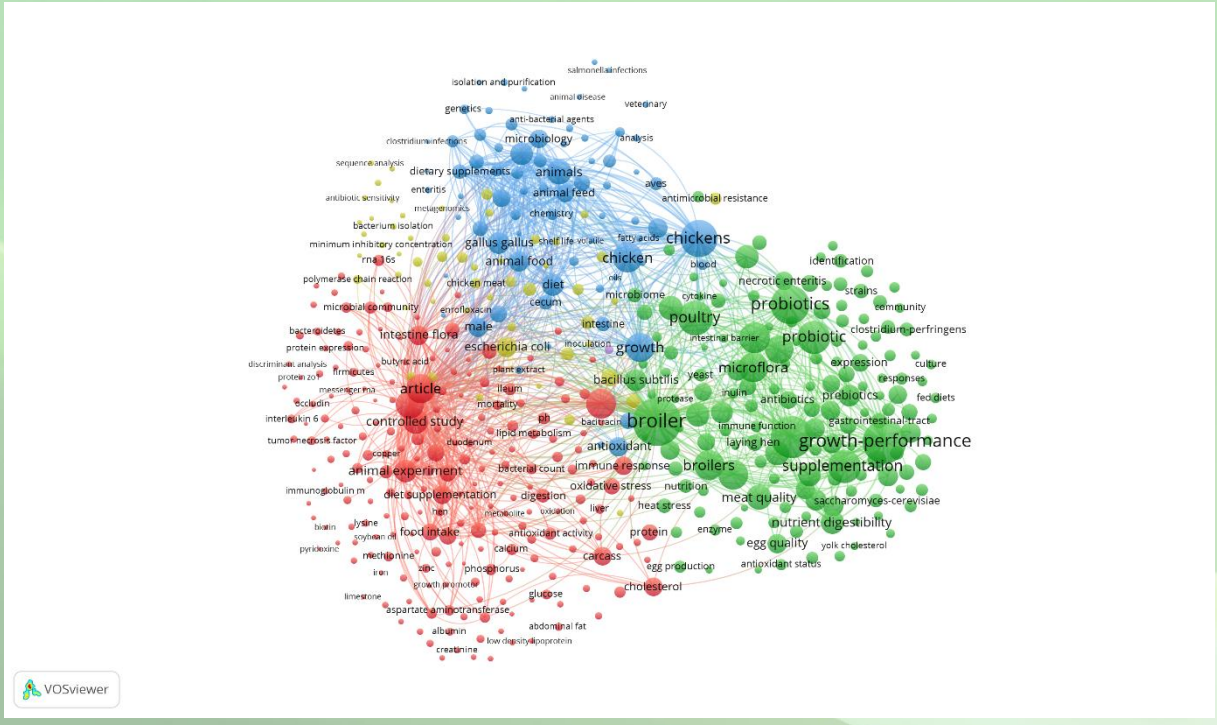



Figure 12: Keyword network map

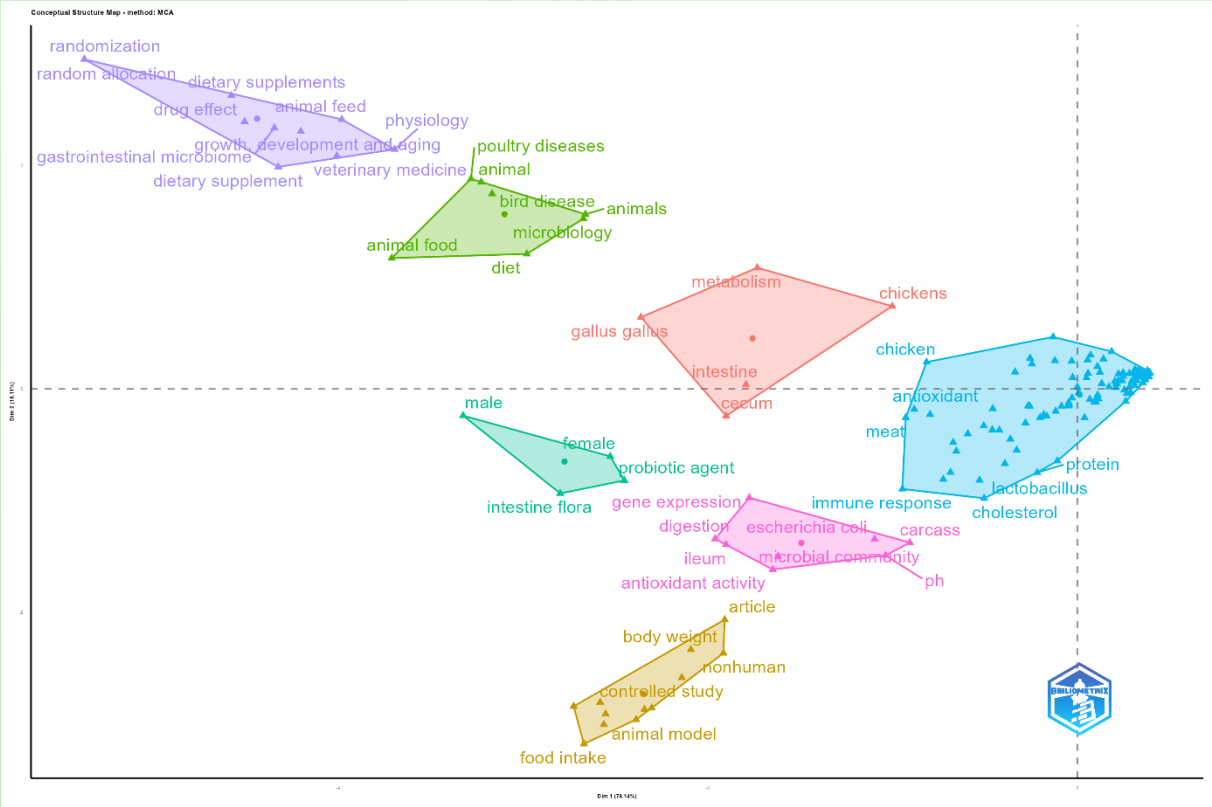



Figure 13: Factor analysis of all-keywords

In the **upper-central section** of the figure, a distinct group identified by the **red color** brings together terms related to digestive anatomy and metabolism. The keywords “intestine”, “cecum”, “metabolism”, “Gallus gallus” and “chickens” indicate a more targeted approach, oriented toward the study of internal physiological mechanisms. This pole highlights the importance of research on the functioning of the digestive system and metabolic processes in poultry, which constitute a central element in the evaluation of probiotic effects.

On the **right side** of the map, a particularly structured group appears around performance and biological responses. Identified by the **blue color**, it clusters keywords such as “protein”, “antioxidant”, “immune response”, “cholesterol”, “Lactobacillus” and “meat”, revealing that research is strongly oriented toward evaluating the effects of probiotics on product quality, growth performance and physiological responses. This grouping reflects an applied and experimental orientation of the field, centered on measurable outcomes and zootechnical performance.

In the **lower-central section**, another group of keywords represented by the **pink color** is associated with molecular and microbial mechanisms. The terms “gene expression”, “microbial community”, “digestive system”, “antioxidant activity”, “ileum” and “carcass” indicate a more recent specialization, oriented toward the study of probiotic mechanisms of action at a deeper level. This group highlights the evolution of the field toward more analytical approaches, based on molecular biology and microbiology.

Another grouping in **turquoise**, located in the **lower-left section**, clusters terms such as “probiotic agent”, “intestinal flora”, “male” and “female”. This grouping shows that some studies focus more on the functional effects of probiotics on intestinal flora and on biological differences between animals. This represents a more specific research axis, but one that remains linked to the other themes of the field.

Finally, in the **lower section** of the figure, a **yellow thematic group** brings together methodological terms such as “animal model”, “controlled study”, “body weight” and “food intake”. This grouping reflects the importance of experimental approaches in the field under study and shows that research relies heavily on controlled protocols designed to precisely measure the effects of probiotics. Keywords located near the center of the figure indicate transversal concepts used across several research axes simultaneously. Conversely, groups located at the periphery correspond to more specialized research axes.

## Discussion

The growing awareness of issues related to animal health, food safety, the emergence of antimicrobial resistance, and the ban on the use of antibiotic growth promoters (AGPs) in 2006 has fundamentally redirected scientific research toward the exploitation of alternative solutions for poultry nutrition. Since then, studies devoted

to probiotics have undergone an unprecedented expansion over the past two decades, as evidenced by the exponential increase in scientific publications in this field. Numerous researchers, countries, institutions and industrial partners have collaborated on studies examining the use of probiotics in poultry nutrition. This dynamic justifies the present bibliometric analysis, which aims to identify the milestones already reached, the identified research gaps, and the future trends of the field.

From 2000 to 2025, annual publications on the use of probiotics in poultry nutrition experienced a significant increase, with a peak observed in 2025 when 687 articles were recorded. The annual number of publications increased slowly from 2000 to 2011, with fewer than 50 articles per year. The increase observed from 2012 onwards could be explained by a growing awareness of the positive impacts of probiotics on intestinal health, growth performance and the modulation of the avian microbiota (Awad et al., 2009; Deng et al., 2012; Higgins et al., 2008; Jin et al., 2000; Lei et al., 2013; Yang et al., 2012). This period also corresponds to the intensification of research on AGP alternatives following the European regulations of 2006 (Regulation (EC) No 1831/2003, 2003). Furthermore, the emergence of concerns regarding antimicrobial resistance and food safety stimulated interest in natural feed additives (Baurhoo et al., 2007, 2009; Jung et al., 2008; Teo & Tan, 2007; World Health Organization, 2000). The last five years have been particularly productive, with more than 300 scientific documents published annually, representing 63.8% of total publications. From 2010 to 2020, the increase in the number of publications was positively associated with the rise in annual mean citations. However, a decrease in the mean number of citations per article over time was observed, particularly for the most recent publications. Whereas articles published in 2000 had accumulated an average of 91 citations, this number progressively declined to fewer than 2 citations per article in 2025. This decreasing trend could be explained by the fact that recent articles have not yet had sufficient time to accumulate citations.

China occupies a dominant position in research on the use of probiotics in poultry nutrition, ranking first in terms of scientific output, citation volume and author productivity. This result is consistent with observations made in other bibliometric analyses covering fields related to poultry science (Hodonou et al., 2025; Uyanga et al., 2023). The analysis of collaboration networks highlights significant interactions between China and several countries, notably the United States, Egypt and Iran. However, in terms of international collaboration, the United States is distinguished by a particularly high centrality, reflecting a structuring role in the scientific networks of the field. Furthermore, this Chinese dominance is not fully mirrored at the institutional level, where Zagazig University, an Egyptian university, appears as the most influential institution. Only one Chinese university, China Agricultural University, occupies the fourth position, while three American institutions the University of Arkansas System, the United States Department of Agriculture and the University of Georgia also figure among the most influential.

Regarding authors, Wang Y., Wang J. and Liu Y. emerge as the major contributors to research on probiotics in poultry nutrition. It should be noted that these authors are affiliated with Chinese institutions, which confirms China's position as a major hub of avian probiotic research. However, these authors appear as co-authors on numerous scientific publications (Y. Liu et al., 2024, 2025; J. Wang et al., 2021, 2023; Y. Wang et al., 2018, 2018). Consistent with this, the international collaboration rate among authors stands at 18.1%, suggesting that inter-institutional and international collaborative efforts in this research field are relatively moderate. This observation aligns with findings reported in the fields of heat stress and factors affecting egg quality in poultry, where international author collaboration rates remain below 25% (Hodonou et al., 2025; Uyanga et al., 2023).

Furthermore, the analysis of the most cited articles between 2000 and 2025 highlights a progressive evolution in the use of probiotics in poultry nutrition, shifting from a primarily zootechnical approach toward an integrated mechanistic understanding. Indeed, studies have demonstrated that probiotics significantly improve growth performance (ADG, FCR and live body weight), intestinal morphology, and exert effects comparable to, or even superior to, AGPs (Awad et al., 2009; Baurhoo et al., 2009; Mountzouris et al., 2010; Rehman et al., 2020; Z. Xu et al., 2003). The foundational phase from 2000 to 2009 also allowed the identification of specific effects, notably the action of prebiotics such as mannan-oligosaccharides (MOS) against *Salmonella* and fructo-oligosaccharides (FOS) on the stimulation of enzymatic activities, intestinal microflora and villous morphology in broiler chickens (Spring et al., 2000; Z. Xu et al., 2003). As for the decade 2010–2019, it is marked by the emergence of new landmark studies. Among these are the works of Wei et al. (2013), who established a phylogenetic census of the avian intestinal microbiome, as well as those of Sohail et al. (2012), who characterized the effect of supplementing broiler diets with a mixture of probiotics and prebiotics on growth performance and intestinal barrier integrity under heat stress conditions. The recent period from 2020 to 2025 marks a turning point toward far more integrated approaches. Indeed, studies now incorporate immunological biomarkers (IgY, IgA, cytokines and anti-inflammatory markers) as well as markers of antioxidant status. The works of Rehman et al. (2020) on the effects of probiotics on broiler immunity, those of Hu et al. (2021) on extracellular vesicles derived from *Lactobacillus reuteri*, and those of Y. Xu et al. (2021) on *Bacillus subtilis* and *B. licheniformis* illustrate this evolution. Overall, the effects of probiotics are essentially based on five major mechanisms: improvement of zootechnical performance, improvement of intestinal morphology, modulation of the caecal microbiota, reduction of intestinal pathogens, and regulation of immune and antioxidant capacities in poultry. Beyond experimental results, the structuring and dissemination of knowledge also relies on scientific journals, the analysis of which makes it

possible to identify the most influential editorial outlets. In the communication of research results on probiotics in poultry nutrition, the journal *Poultry Science* has largely dominated the editorial landscape. Indeed, this journal has produced most of the most cited articles worldwide. The predominance of this journal could be explained by its long editorial history, its international recognition and its high impact factor. These attributes attract leading researchers, who view it as a legitimate vehicle for disseminating their findings.

From a bibliometric perspective, author-supplied keywords constitute an essential tool for analyzing the thematic structuring of a field, as they reflect the core concepts and dominant research orientations. In this study, certain terms stand out by their high frequency of occurrence within the analyzed corpus. These include notably "broilers", "growth-performance", "performance", "probiotics" and "supplementation". Several tools highlight this predominance, including the word cloud (WordCloud), the heat map (TreeMap) and trend analysis (Trend Topics). However, it is interesting to note that the keywords "growth-performance" and "broilers" present a higher occurrence than "probiotics" and other terms. This observation suggests that more researchers are interested in studies addressing growth performance in relation to probiotic supplementation in poultry diets. This same observation is accompanied by another reality: broilers represent the subjects on which many researchers work most of the time. Moreover, the thematic evolution of keywords (Words' Frequency over Time) confirms this dynamic. This consistency is also reflected in the keyword co-occurrence analysis and the factorial analysis of keywords, which confirm and systematize the major research orientations previously identified — namely growth performance, intestinal morphology and microbiota, molecular mechanisms, immunity and metabolites, as well as food safety and antimicrobial resistance (Abdul-Ratha et al., 2024; Al-Khalaifa et al., 2019; Amit-Romach et al., 2004; Baurhoo et al., 2009; Bjerrum et al., 2006; Kim et al., 2011; Nakadira et al., 2025; Sohail et al., 2010; Yang et al., 2012).

The analysis of the thematic keyword map reveals a particular structure that raises questions about the maturity of the field. Indeed, the total absence of motor themes and of emerging or declining terms constitutes an unusual observation. Such an observation suggests a phase of stabilization rather than innovation within the field. One could infer that the field currently favors the deepening of established concepts rather than the exploration of new frontiers. Such a situation may reflect a degree of maturity, but also a risk of conceptual stagnation. Future innovations will likely need to emerge from the convergence between niche themes and central themes.

## Conclusion

This bibliometric analysis highlights the progressive structuring and strong dynamics of research on probiotics in poultry nutrition, in a context marked by the ban on antibiotics as growth promoters. The concentration of scientific output around a few countries — notably China, the USA and Egypt — could be explained by a combination of several factors. Indeed, these countries may benefit from higher levels of funding, incentive-driven science policies, more developed research infrastructures, and better integration into international collaboration networks compared to other countries.

Beyond its quantitative growth, this bibliometric analysis demonstrates that the field of research on probiotics in poultry nutrition is mature in its fundamentals, dynamic in its innovations, but incomplete in its understanding of mechanisms of action and in its operational deployment on farms. The transition toward a microbiota-centered approach has been initiated but requires methodological consolidation and field validation. In the future, research should move toward more targeted and integrative approaches, including the development of precision probiotics, the exploration of postbiotics and synbiotics, as well as the integration of tools derived from advanced biotechnologies. These developments will be decisive in optimizing the efficacy and sustainability of supplementation strategies in poultry production.

## Study limitations

This study presents the methodological limitations inherent to bibliometric analyses. First, the analyzed corpus relies exclusively on the Scopus and Web of Science databases. These databases are known to favor anglophone publications and generally privilege internationally indexed journals with high visibility. Although this methodological choice guarantees data quality and standardization, it induces a selection bias. It underrepresents works published in other languages and excludes grey literature as well as research arising from local contexts. As a result, the obtained findings more closely reflect visible and indexed scientific output, but do not represent the entirety of knowledge actually produced in the field. Furthermore, the analysis relies primarily on bibliometric metadata, and no in-depth qualitative evaluation of article content was conducted. In addition, the methodological choice regarding the search strategy directly influences the composition of the corpus. The use of broad keywords aimed at maximum coverage leads to the inclusion of peripheral articles. Conversely, certain relevant works may be excluded. Similarly, the application of a minimum threshold of 10 occurrences for the keyword co-occurrence analysis has certainly excluded emerging themes that are still underrepresented. Finally, temporal limitations must

also be highlighted. The study focuses on the period from 2000 to 2025, thus excluding earlier pioneering works. Despite these limitations, this study provides a rigorous and systematic mapping of the field.

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# The Planning in Smart Agricultural Production with Artificial Intelligence

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## Abstract

As the population grows and conflicts continue, we need to increase agricultural productivity to ensure food security. Artificial intelligence is now a key part of agricultural and Data-Driven Agricultural Management. It is helping to move modern farming toward smart farming. This leads to more crops, better products, and lasting growth. Today, we see significant progress in AI technologies and the Internet of Things being used for many farming tasks. The goal of this study is to demonstrate some of the modern ways that artificial intelligence can be used to plan smart agricultural production. Adding AI-powered systems to the farming planning process can help address problems with traditional farming and make the system more flexible and efficient. Digital technologies are now changing the agriculture industry in a big way.

***Keywords:*** *smart agriculture, Artificial intelligence, agricultural planning*

## Introduction

Agriculture is one of the most important industries working to solve these problems. It needs to grow in both number and quality. The agricultural industry also has to deal with workers leaving for better-paying jobs and farmers being older on average (Miyake et al., 2022). To solve these problems and meet the nutritional needs of the growing population, scientists and agricultural experts need to find more creative and long-lasting ways to grow food. To solve these problems, agricultural experts have come up with new ways to keep an eye on things, like variable-rate applications, remote sensing systems, global positioning systems, and optimal nutrient monitoring. All of these methods are part of precision farming or precision agriculture (PA). To get the most out of both quantity and quality while cutting down on waste, precision agriculture uses the right actions, such as fertilization or irrigation, in the right way, at the right place, and at the right time (Nowak, 2021). There is also a new idea called "smart agriculture," which uses AI and digital technology in farming (Zinke-Wehlmann & Charvát, 2021).

Artificial intelligence (AI), the Internet of Things, robotics, and big data are all types of information technology that are growing quickly. AI can help smart farming make predictive control models and make the best choices. Robotics can perform high-intensity work that people normally do, and the Internet of Things can collect and analyze large-scale environmental data (Ge et al., 2025). Ultimately, this will render the entire agricultural process secure, economical, and efficient (Yang et al., 2021). Data-driven AI has become the most important part of smart agriculture among these new technologies. Smart agriculture is the precise, automated, and intelligent management of agricultural production (Taha et al., 2025). It is important to use it because the world's population is growing, food security issues are rising, and the demand for high-quality agricultural goods is rising. The main purpose of this study is to show some of the new ways that smart agricultural planning is using AI. Through this review, we also seek to highlight some artificial intelligence technologies used in agriculture and their role in driving agricultural production.

### Artificial Intelligence and smart agricultural production planning

The use of AI technology in agriculture has been the subject of much study in recent years, further supporting the industry's ongoing growth (Bai et al., 2025). AI seeks to enable robots to perform complex tasks such as learning, reasoning, perception, and decision-making by analyzing datasets, identifying patterns, and generating predictions about targets. It is widely used in domains such as social security, autonomous driving, smart healthcare, and industrial automation. AI technologies are changing agricultural production practices through data, algorithms, and equipment, rather than relying on traditional human-experience-based analysis and decision-making or on simple mechanical labor substitution. With applications in agricultural production management, soil and crop monitoring, pest and disease detection, yield prediction, and crop planning, food quality, climate-smart agriculture, supply chain optimization, autonomous farming equipment, mechanical equipment fault analysis, etc., these technologies address nearly every facet of agricultural production, including planting, cultivation, management, and harvesting. However, open, unstructured, and complex agricultural environments, limited and

unevenly distributed high-quality annotated data, multi-source heterogeneous data fusion, and problems like inadequate generalization, poor interpretability, and insufficient real-time performance of AI models pose serious obstacles to the application of AI in agriculture (Ge et al., 2025).

Artificial intelligence (AI) contributes to agricultural sustainability by enabling precision crop management. By analyzing data from sensors, satellites, and drones, AI systems monitor crop health, soil quality, and weather conditions. These insights enable the optimization of irrigation, fertilizer, and pesticide applications, thereby reducing waste and minimizing environmental impact. Consequently, resource use becomes more efficient, leading to increased productivity and the conservation of soil and water (Ali et al., 2025). AI significantly advances sustainable agriculture by increasing efficiency, optimizing resource utilization, and facilitating data-driven decision-making within contemporary farming systems. The adoption of AI technologies, including machine learning, remote sensing, robotics, and the Internet of Things (IoT), enables precision agriculture and supports the progression toward Agriculture 5.0. This evolution seeks to improve productivity while reducing environmental impact (Taha et al., 2025). With predictive analytics and decision-support tools, farmers can spot crop diseases, pest problems, and climate risks early. This early detection enables them to act quickly and use fewer chemicals, helping protect biodiversity. AI monitoring systems also improve water management by indicating when crops need water and helping farmers plan irrigation, which is most important in areas with limited water (Ali et al., 2025).

AI models can predict crop yields, check soil health, and suggest the best crop types for local conditions. These tools help farmers grow more food sustainably, which is important as the world's population grows and the climate changes (Kumari et al., 2025). AI agricultural robots are a state-of-the-art use of robotics and artificial intelligence in contemporary agriculture. In the current era, AI agricultural robots are increasingly being adopted in agricultural production. Their substantial potential to improve food safety, reduce labor shortages, increase production efficiency, and safeguard the environment are the main causes of this development (Wang et al., 2023). Intelligent applications for field operations, particularly in planting, crop management, and harvesting, are now the focus of technical advancements in agricultural robots. Autonomous navigation technology has also become a core pillar of innovative agricultural robots (Ge et al., 2025). AI also supports climate-smart farming. By analyzing greenhouse gas emissions, soil carbon, and other environmental data, AI helps farmers choose methods that lower emissions and increase soil carbon storage. These practices support sustainable farming and align with global goals for food security and environmental protection (Ali et al., 2025). A four-layer intelligent agriculture system that includes cloud computing, edge computing, data transmission, and agricultural data collection was developed by Bu & Wang (2019). This technology enables real-time environmental monitoring, soil moisture and climate analysis, and automatic adjustments for irrigation and fertilizer dosing. Its adaptability to various working environments also makes it easier to incorporate existing expertise into new farming settings. To enhance plant growth conditions and provide real-time notifications to users, Mohamed et al. (2022) developed a fog cultivation system that primarily uses Arduino-based cooling fans to intelligently cool the lettuce root zone based on real-time temperature and humidity data from the surrounding environment.

Qureshi et al. (2025) explored plasma technology for microbial control and plant nitrogen fixation. They developed an The artificial intelligence of things (AIoT)-enabled system that automatically adjusts fertilizer content, spray cycles, and plasma composition to reduce resource use while maintaining crop yield. According to Judijanto et al. (2024), AI can reduce input usage by over 30% and increase productivity by up to 20% compared to traditional methods. The rigidity of conventional approaches is surpassed by technologies such as drones equipped with computer vision, IoT sensors, expert systems, and neural networks, which facilitate continuous monitoring and adaptive responses to changing soil, crop conditions, and climate (Shamshiri et al., 2018). Even with these benefits, using AI in farming poses challenges such as high startup costs, the need for technical skills, and difficulties obtaining sufficient data. Still, as technology improves and researchers, farmers, and policymakers work together, AI could help make farming more sustainable, resilient, and efficient (Ali et al., 2025).

AI technologies have extensive applications in smart agriculture, each offering unique advantages. Nonetheless, substantial challenges persist in their practical implementation (Ge et al., 2025). In the domains of AI detection, fault diagnosis of agricultural machinery, and crop condition forecasting, the majority of existing models rely heavily on large, labeled datasets and substantial computational resources to achieve satisfactory accuracy (She et al., 2024). However, due to the inherent complexity of agricultural environments, acquiring large-scale, balanced, and thoroughly annotated datasets is often costly, labor-intensive, and computationally demanding, thereby significantly limiting their applicability in real-world settings. Furthermore, AI models often lack interpretability, hindering agricultural producers' ability to understand the decision-making processes underlying them. This lack of transparency can undermine trust and confidence in the diagnostic and predictive outcomes generated by these models (Zhou et al., 2023). Future research could develop models capable of handling low-quality, imbalanced data, reducing computational demands, and enhancing generalization and stability under varying agricultural conditions. Exploring lightweight neural network architectures and optimization algorithms can reduce computational demands, enabling high-performance AI models to be directly deployed on agricultural machinery, drones, or field edge computing devices for real-time, online detection and diagnosis (Ge et al., 2025).

Given these advantages, the use of artificial intelligence technology offers significant benefits in agriculture, helping maximize production and improve the quality of agricultural products.

## Conclusion

Through improvements in how people perceive, what they know, and how they make decisions, artificial intelligence has made modern farming much more effective. Finding crops, eliminating pests, making predictions, and sounding alarms for equipment are all things that it assists with. The application of artificial intelligence, particularly in the form of agricultural robots, is rapidly expanding as traditional farming methods become less common. The use of artificial intelligence is replacing traditional farming methods, but there are still issues that need to be resolved. To address these issues, we need to conduct further research and leverage technology.

## Declarations

### *Ethical Approval Certificate*
No need

### *Author Contribution Statement*
Wadah Elsheikh: writing the original draft. Ilknur Ucak: supervision, conceptualization, review, and editing.

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### *Conflict of Interest*
The authors declare no conflict of interest.

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# Distribution areas and medicinal importance of the species of *Cirsium Mill.* found in Azerbaijan

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## Abstract

The article analyzes the geographical distribution areas, ecological characteristics and medicinal importance of the species of the genus *Cirsium* Mill. of the Asteraceae family, which are widespread in Azerbaijan. At the same time, the protection and effective use of species belonging to the genus are discussed. **Problem:** *Cirsium* Mill., one of the widespread medicinal plants in Azerbaijan, is considered an important component of biodiversity. These plants are mainly distributed in foothills, meadows, semi-deserts and roadside areas. *Cirsium* Mill. species are found especially in the Lesser Caucasus, Greater Caucasus, Talysh zone and lowland regions. In the high mountain belts of the area, it is found in rocky, stony, stony-gravel and gravelly vegetation. As a result of the location of *Cirsium* Mill. plant individuals at a certain distance from each other in rocky areas, sparse and poorly developed vegetation is formed. Studies show that *Cirsium* Mill. plants are rich in flavonoids, alkaloids, essential oils and antioxidant substances. In folk medicine, their roots, leaves, and seeds are used to treat liver, biliary tract, digestive system, and inflammatory diseases. **Method:** Field studies and comparative morphological analyses of several species, especially *Cirsium tricholoma*, *Cirsium rhizocephalum* and *Cirsium frickii*, were carried out in the mountainous regions of the Lesser and Greater Caucasus during the vegetation period. Herbarium materials and phytochemical analyses were also used to determine ecological and medicinal characteristics of the species. **Result:** The obtained results indicate that these species are ecologically adapted to subalp and alp meadow ecosystems and possess important pharmacological potential due to the presence of biologically active compounds such as flavonoids, alkaloids and phenolic substances. **Conclusion:** The study emphasizes the ecological role, medicinal value and necessity of protection and sustainable use of *Cirsium* Mill. species in Azerbaijan flora.

***Keywords:*** *Cirsium Mill., flavonoid, folk medicine, areal, subalpine*

## Introduction

Plants form the basis of life for all living organisms on the planet, and the organic world and living life began with plants. The plant kingdom has the ability to directly utilize sunlight through the unique process of photosynthesis, which creates the appearance of the Earth that we observe. It is precisely plants that provide the Earth with oxygen and thereby act as the driving force of evolution. They are a rich reserve of organic matter and a highly valuable natural resource. The vegetation cover of each region is constantly subjected to changes as a result of the continuous complex influence of environmental factors. These changes occur depending on the physical-geographical conditions of the area, global warming, and the disruption of the ecological balance of the atmosphere. Depending on the geobotanical and bioecological characteristics of vegetation types, certain dynamics are observed in the species composition of plant cover, associations and formations, projective cover, and cenotypic features of phytocenoses in the areas where they are distributed. Human beings are not the rulers of nature, but only elements endowed with reason; they must serve nature, help it, and stand by its side. Nature protection is a global and universal task. Biologically active substances secreted by plant roots help improve soil structure and increase its capacity to retain and filter water [4].

Among such plants are the thistle species belonging to the genus *Cirsium* Mill. *Cirsium* Mill. species are distinguished by their ability to adapt to various ecological conditions and play an important functional role in soil–ecosystem relationships. The strong and deep root system of the plant positively affects the improvement of soil structure, the reduction of erosion processes, and the increase of water retention and filtration capacity in the soil. Biologically active substances secreted by the roots of *Cirsium* Mill. enhance the activity of soil microflora and contribute to the preservation of soil fertility. In this regard, *Cirsium* Mill. is considered one of the plant examples with important phytomeliorative significance in ensuring ecological stability [1].

### Materials and Methods

The research material consisted of species belonging to the genus *Cirsium* distributed in the flora of Azerbaijan, especially *Cirsium tricholoma*, *Cirsium rhizocephalum* and *Cirsium frickii*. Samples were collected from the northern part of the Lesser Caucasus, mountainous zones of the Greater Caucasus, as well as subalpine and alpine

meadows. Natural populations of the plants were observed under field conditions during the flowering and vegetation periods (VI–IX months), and herbarium specimens were prepared and identified in the laboratory. During species identification, morphological characteristics such as stem height, leaf shape, degree of spininess, structure of inflorescences and features of the root system were taken as the main criteria [8].

Classical botanical-systematic methods, comparative morphological analysis and phytocenological observations were used as methodological approaches. The distribution areas of the plants were recorded based on GPS coordinates, taking into account altitudinal zonality. Ecological conditions such as soil type, humidity, slope inclination and degree of illumination were documented through field records [5,6].

For the preliminary evaluation of the chemical composition, qualitative reactions were carried out on dried plant materials, and the presence of flavonoids, phenolic compounds, alkaloids and saponins was determined using standard phytochemical methods. At the same time, the pharmacological properties of the species were comparatively analyzed based on literature sources.

**Discussion**

In Azerbaijan, species belonging to the genus *Cirsium* are found particularly in the alpine and subalpine meadow belts, on grassy mountain-meadow soils, at altitudes of approximately 1800–2600 meters above sea level. The subalpine belt, located in the upper part of the forest zone, forms a transitional zone with the alpine belt, and the favorable hydrothermal conditions formed here positively affect the development of *Cirsium* species. Species of the genus *Cirsium* mainly participate in the floristic composition of subalpine meadows as perennial herbaceous plants and play an important role in the formation of phytocenotic biodiversity. These plants spread together with other mesophytic species in tall-herb meadows, contributing to the formation of productive forage areas. They serve to reduce erosion processes and maintain the ecological stability of mountain ecosystems. *Cirsium* species are also considered valuable due to their medicinal properties, in addition to their importance as forage plants in summer pastures [7].

Subalpine meadows are mainly characterized by tall-herb plants as dominant edificators. Among these, species belonging to the genus *Cirsium* occupy an important place. Tall-herb plants develop on mountain-meadow soils that have emerged from forest zones, contributing to the formation of subalpine tall-herb vegetation and forest-edge humid meadows. In the tall-herb layer of subalpine meadows, species of the genus *Heracleum* L. are widely distributed, forming distinct plant formations. In these “hogweed communities,” species such as *Aconitum orientale* Mill., *Delphinium flexuosum* Bieb., *Knautia montana* (Bieb.) DC., *Cephalaria gigantea* (Ledeb.), *Dactylis glomerata* L., *Adenostyles platyphylloides* (Somm. et Levier) Czer., and *Doronicum macrophyllum* Fisch. ex Hornem. are commonly found. In regenerating ruderal areas, species of the genera *Urtica* L., *Rumex* L., *Dryopteris* Adans., and *Cirsium* Mill. form monodominant associations.

*Cirsium* Mill. is represented by 30 species in the flora of Azerbaijan. In this study, the morphological and botanical characteristics, ecological distribution, and pharmacological importance of some species belonging to the genus were analyzed. At the same time, the healing properties of biologically active compounds such as flavonoids, alkaloids, essential oils, and other substances contained in these species were investigated, and information was provided on their use in folk medicine and phytotherapy.

*Cirsium tomentosum* C.A.Mey. 1831 – woolly thistle is a perennial herbaceous plant with a long rhizome and is a hemicryptophyte. The plant reaches a height of 70–100 cm and is mainly distributed in lower, middle mountain, subalpine, and alpine zones, on gravelly slopes and rocky debris areas. The species is considered characteristic for the East Caucasian flora and is widely found in the mountainous regions of the Greater Caucasus in Azerbaijan. It is also distributed in Southwest Asia, particularly in the floras of Turkey and Iran. This species is important not only floristically but also pharmacologically. In *Cirsium tomentosum*, biologically active compounds such as flavonoids, phenolic compounds, alkaloids, tannins, saponins, and essential oils have been identified. These compounds possess antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties. In folk medicine, the aerial parts of the plant are used as a wound-healing, diuretic, and digestive-regulating remedy. At the same time, phenolic and flavonoid complexes found in *Cirsium* species play an important role in reducing the effects of free radicals in the human body.

*Cirsium kosmelii* (Adams.) Fisch. ex Hohen. – Kosmeli thistle is a perennial herbaceous plant with a long rhizome and a hemicryptophyte life form. The plant reaches a height of 50–80 cm and is mainly distributed in mid-mountain and subalpine zones, as well as on dry mountain slopes and foothill areas. The species was described from the Central Caucasus and is also distributed in Southwest Asia, particularly in the flora of Turkey.

This species is believed to contain biologically active compounds such as flavonoids, phenolic compounds, tannins, alkaloids, saponins, and essential oils. These compounds exhibit antioxidant and anti-inflammatory effects, thereby reducing the impact of free radicals in the body. In folk medicine, the aerial parts of *Cirsium* species are reported to be used as diuretic, wound-healing, and digestive-regulating agents.

*Cirsium osseticum* (Adams.) Petr. – Osetian thistle is a biennial herbaceous plant reaching a height of 50–100 cm. This hemicryptophyte species is mainly distributed in mid-mountain and subalpine zones, as well as in forest

clearings and mountain meadows. It is considered characteristic of the Central Caucasian flora and is also found in Turkey and Iran.

Phytochemical investigations have identified flavonoids, phenolic acids, tannins, essential oils, and various antioxidant compounds in this species. These constituents exhibit antimicrobial, anti-inflammatory, and immunostimulatory properties. In folk medicine, different parts of *Cirsium osseticum* are reported to be used in the treatment of inflammatory conditions, skin lesions, and digestive disorders. Its biochemical composition also supports its potential value as a promising medicinal plant.

*Cirsium tricholoma* Fisch. et C.A.Mey. ex DC. – woolly thistle is a biennial herbaceous plant with a hemicryptophyte life form. It reaches a height of 80–110 cm, and its flowering period occurs in June–July. The species is mainly distributed in the northern part of the Lesser Caucasus, in subalpine zones, and in mountain meadows. It belongs to the Caucasian flora and was originally described from the Eastern Caucasus.

The species contains biologically active compounds such as flavonoids, phenolic compounds, alkaloids, saponins, and essential oils. These substances exhibit antioxidant, anti-inflammatory, and antimicrobial activities. In folk medicine, the aerial parts are reported to be used for various inflammatory conditions and for general strengthening of the organism.

*Cirsium rhizocephalum* C.A.Mey. – root-headed thistle is a perennial herbaceous plant with a rhizomatous root system and a hemicryptophyte life form. The plant height ranges between 5–20 cm, and it is mainly distributed in subalpine and alpine zones, particularly in high-mountain meadows. This species is considered a Caucasian subendemic and was described from the Eastern and Central Caucasus.

The chemical composition of the species includes flavonoids, phenolic acids, tannins, saponins, and other antioxidant compounds. These constituents exhibit protective effects at the cellular level, reduce inflammatory processes, and enhance resistance against microorganisms. In folk medicine, both root and aerial parts may be used for regulating the digestive system and for anti-inflammatory purposes.

*Cirsium frickii* Fisch. et C.A.Mey. – Frick's thistle is a perennial herbaceous plant with a rhizomatous root system, reaching a height of 10–20 cm, and it also exhibits a hemicryptophyte life form. It is mainly distributed in the northern part of the Lesser Caucasus, on high mountain slopes and in subalpine zones. The species was originally described from the flora of Turkey and has a distribution range covering the Caucasus–Southwest Asia region.

This species is also presumed to contain biologically active compounds such as flavonoids, phenolic compounds, essential oils, and saponins. These constituents contribute to antioxidant and anti-inflammatory activities, enhancing the medicinal potential of the plant. In folk medicine, it is reported to be used similarly to other *Cirsium* species in the treatment of various inflammatory and skin diseases [3].

## Conclusion

The conducted study shows that species belonging to the genus *Cirsium*, which are widely distributed in the flora of Azerbaijan, particularly *Cirsium tricholoma*, *Cirsium rhizocephalum*, and *Cirsium frickii*, are important components of high-mountain ecosystems, especially subalpine and alpine meadows. These species are mainly distributed in the mountainous regions of the Lesser and Greater Caucasus, as well as across different altitudinal zones, demonstrating a high level of adaptation to environmental conditions. Morphological and ecological analyses indicate that these species are characterized by a hemicryptophyte life form and are resistant to harsh climatic conditions. The root systems of these plants play a significant role in soil stabilization, reducing erosion, and maintaining the stability of mountain ecosystems.

From a phytochemical perspective, the presence of flavonoids, phenolic compounds, saponins, alkaloids, and essential oils in these species confirms their high biological activity. These compounds possess antioxidant, anti-inflammatory, and antimicrobial properties, thereby enhancing the medicinal value of the plants.

Overall, species of the genus *Cirsium* play an important phytocenotic role in ecological systems and are considered promising medicinal plants with significant pharmacological potential.

## Declarations

***Ethical Approval Certificate***

Not applicable.

***Author Contribution Statement***

Ramil Mammadov: Conceptualization, methodology, data collection, formal analysis, writing – original draft, review & editing.

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***Conflict of Interest***

The author declares no conflict of interest.

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*ID: 811*

# Investigation of the Landscape Design of 80 Binde Devr-i Alem Park Using the Eye-Tracking Technique

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## Abstract

Landscape design is a combination of natural and cultural components that directly affect people's spatial perception and aesthetic experiences. The eye-tracking technique, on the other hand, is an innovative method that reveals perception toward the urban and visual environment with qualitative and quantitative data by tracking pupil movements. The purpose of this study is to investigate the landscape design of Konya 80 Binde Devr-i Alem Park through user perception via the eye-tracking technique. Within the scope of the research, landscape images obtained from the site were shown to 30 volunteer participants. In the study, areas of interest (AOIs) were defined in three main categories as vegetative elements, structural elements, and water elements. Based on these defined areas of interest, heat maps, along with total fixation duration and fixation count metrics, were obtained. The quantitative data obtained from eye-tracking metrics were analyzed using the Repeated Measures ANOVA test. The analysis results revealed that there were statistically significant differences between landscape elements (vegetative, structural, water) in terms of both eye-tracking metrics ($p < 0.05$). It was determined that the participants fixated more frequently and for a longer duration on the vegetative elements located within the park compared to structural and water elements. The findings obtained provide important data for designers in terms of the management of visual focal points and the balanced distribution of landscape components in thematic park designs.

***Keywords:*** *80 Binde Devr-i Alem Park, visual perception, landscape design, eye-tracking technique, Konya*

*ID: 812*

# The Urban Image of Konya in the Memory of Selçuk University Students

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## Abstract

The urban image is a reflection of the ways in which individuals perceive, give meaning to, and code urban space in their memories. Due to their intensive spatial interactions and dynamic mobility, university students constitute an important stakeholder group in shaping the urban image. This study aims to examine the Konya urban image formed in the mental processes of Selçuk University students through the components of urban perception and spatial memory. Based on Kevin Lynch's "The Image of the City" theoretical framework, the methodology of the research relies on a questionnaire study conducted with 110 volunteer students studying at Selçuk University. The obtained quantitative data were analyzed using SPSS statistical software; within this scope, the relationships between the demographic characteristics of the participants and the primary elements forming the urban image (paths, edges, districts, nodes, and landmarks) were examined via cross-tabulations and the Chi-square test of independence.

According to the analysis results, it was observed that the Konya urban image in students' memories is predominantly shaped along the axis of the Mevlana Museum and Alaaddin Hill in the historical city center, the Selçuk University Alaeddin Keykubat Campus—which is the daily living space of the students—and the tram line connecting these two nodes. Statistical analyses revealed that the gender variable does not create a significant difference in spatial image preferences ($p > 0.05$), whereas students' duration of residence in Konya creates a highly significant difference in their urban space usage practices and level of perceiving district boundaries ($p < 0.001$). Additionally, it was determined that participants' status of being local (from Konya) or non-local has a statistically decisive effect on their spatial wayfinding abilities within the city ($p = 0.0119$). The obtained findings provide significant recommendations in terms of student-oriented urban planning and landscape design strategies for the future of the city of Konya.

***Keywords:*** *Urban image, urban perception, spatial memory, Konya, Selçuk University*

## Introduction

The urban image embodies the processes of sensation, perception, and cognition in the interaction between the environment and humans, and it is formed in the mind through the experience of the environment. According to the environmental psychology literature, the psychological interaction of humans with the environment primarily begins with the process of perceiving stimuli from the physical environment through sensory channels and transforming them into mental maps. This initial stage of the process carries a universal characteristic, and environmental data are evaluated according to many perceptual evaluation criteria. Kevin Lynch addressed these fundamental perceptual principles put forward by Gestalt in his 1960 book *The Image of the City* within the framework of the concept of "legibility," mentioning that parts can be perceived together as a whole or individually. Differing from the sensory process, perception and cognition, which are treated as the second stage of the interaction between human and environment, vary on an individual basis. The primary reasons for this variation are personal differences such as age, gender, and education, which play an active role in the processes by which people evaluate the information they obtain through their senses. Lynch (1960), Warr and Knapper (1968), Rapoport (1977), and Lang (1987) emphasized that culture and social memory lie at the base of this differentiation, stating that the meanings attributed to the environment vary due to these differences, and therefore, the image in the mind varies from person to person (Eraydın, 2016). Conceptually, an "image" is an abstract dimension of emotion and perception, constituting a psychological reflection of a specific region or place in people's minds; it is a comprehensive arrangement of individuals' impressions, ideas, cognitions, evaluations, and emotions (Su et al., 2023).

Lynch (1960) defines the urban form components that enable the urban image to materialize on the mental map as five core elements. These physical elements, which directly affect the processes of individuals reading and making sense of their environment, are paths, edges, districts, nodes, and landmarks. These landmarks, which shape the urban image and the general character of the city, are defined as objects within the urban form that are easily discernible physically and function as an external wayfinding focus for individuals. Viewed from the perspective of cultural transmission, urban landmarks are an indispensable and inseparable channel for people to perceive urban spaces. Concurrently, these landmarks are also important carriers of urban history and culture, showcasing the unique characteristics of a specific city formed over time (Zhao et al., 2020). Cultural landmarks, seen as the "calling card" of the city, carry more meaning than simple architecture. Embodying the memory of a

city, these structures also exist as symbols that functioned in people's past lives. The well-known cultural landmarks of various countries all have unique and profound meanings. For instance, when it comes to cultural landmarks in Paris, the Eiffel Tower is always the first element that comes to mind. The tower entirely meets modern aesthetic standards, standing as the most eye-catching structure of its time and a symbol of industrial civilization. A cultural landmark should not be a superficial image project or an artificial landscape disconnected from its environment, but a structure that records the development of time, reflects the personality of the city, and is vividly memorized by people (Gao, 2020).

The urban landscape, on the other hand, is a product of human settlement activities, including tangible material elements as well as abstract connotations of value and significance, which are the projection of human-world relations and social relations in cities. This comprehensive cultural phenomenon is an intuitive carrier of urban image characteristics and cultural attributes, serving as an important tool for people to perceive and experience cities. A beautiful urban landscape is the foundation for the formation of collective memory and intensifying "nostalgia" among urban residents; it helps create senses of place and identity (Gao and Liu, 2021). The aesthetics of the urban landscape create a sense of place and identity by forming the basis for the collective memory of city dwellers. At this point, "landscape" means an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors (Anonymus, 2026a).

From the moment it emerged, the theory of the urban image has determined the direction of urban architecture, exerting profound effects on the spatial planning and renewal of university campuses, thereby transforming into a research paradigm. Having become one of the most important theoretical frameworks in the field of urban planning and design today, this theory emphasizes people's subjective perceptions and cognitive approaches to the urban environment; it treats the city not merely as a totality of physical spaces, but also as a place possessing a unique meaning and image in people's minds. The development of this theory stems from in-depth research into the relationship between human cognitive psychology and urban space, and its purpose is to reveal how people construct the form, structure, and characteristic properties of a city in their minds. Studies on urban images allow researchers to better understand a city's identity, characteristics, and cultural connotations; thus providing a more humane and sustainable direction for the planning, design, and management of urban spaces (Wang et al., 2024).

Consequently, cities are not composed of physical spaces alone, but are complex structures shaped by the experiences and perceptions of individuals. The meanings that people assign to cities are formed in line with the individual and collective relationships they establish with that city. In this context, urban images are closely related to how individuals see, feel, and remember a city. Particularly, temporary yet active user groups, such as university students, both leave traces in the city they live in and reconstruct the image of that city through their personal experiences. Konya, with its historical depth, cultural diversity, and spatial character, generates strong urban images in many individuals. In this study, by analyzing the spatial relationship that university students studying in Konya establish with the city along the axis of their demographic characteristics, durations of residence, and statuses of being local, it has been revealed around which physical image elements of Kevin Lynch the perceptions of the city concentrate, and how the paradoxical relationships between the city's objective landscape aesthetics and its subjective social image are shaped.

## Materials And Methods

### *Characteristics of the Study Area*

The province of Konya, which constitutes the geographical focus of the research, is located in the south of the Central Anatolia Region, in the middle of the Anatolian Peninsula. Possessing an average altitude of 1,011 m and a surface area of 38,873 km², the population of the city is 2,343,409 as of the year 2026 (TÜİK, 2026). Continental climate conditions dominate the city, characterized by hot and dry summers, and cold and rainy winters; these climatic factors are of a nature that can directly affect university students' open space usage practices and perceptual processes regarding the urban image.

Selçuk University, which constitutes the institutional universe of the research, is among the most established higher education institutions in Turkey with its 60,287 active students, 493,470 graduates, 3,195 academic, and 4,365 administrative personnel. Incorporating 24 faculties, 7 institutes, 5 schools, 22 vocational schools, and 53 research centers within its structure, the university was founded on April 11, 1975, and possesses a deep-rooted history of 50 years (Anonymus, 2026b). This massive scale and urban dynamism possessed by the university reinforce the representativeness of the student sample over the urban image.

### *Research Materials*

The primary material of the research consists of the raw data obtained from the questionnaire form prepared for Selçuk University students to measure urban perception and spatial experiences. As auxiliary materials, scientific books, articles, theses, and institutional internet resources belonging to the literature on urban image and urban identity were used. In the processes of collecting, organizing, and statistically analyzing the data, MS Office programs and SPSS 27.0 statistical software were utilized.

***Research Methodology***

The research was conducted in four fundamental stages extending from theoretical preparation to statistical reporting:

- Identification of the Problem: The research question and hypotheses were formulated by discussing the role of university youth on urban memory and wayfinding practices.
- Literature Review and Questionnaire Design: The theoretical infrastructure focused on urban image and urban identity was reviewed. In the light of the obtained theoretical inputs, a multidimensional questionnaire form was designed to measure the participants' demographic profiles as well as their mental images regarding the physical, cultural, and social elements of Konya, their cognitive relationships established with urban landmarks, their wayfinding practices, route routines, and feelings of spatial belonging.
- Data Collection and Sample Selection: The questionnaire study was carried out in line with the approval of the Ethics Committee of Selçuk University Faculty of Architecture and Design and by ensuring full compliance with the principle of volunteerism. In the field research, considering the universe size of 60,287 students, the minimum number of participants to be reached at a 95% confidence level and within a 5% margin of error boundary was calculated as 96 (Newbold et al. 2022; Seyhan & Güngör, 2025). However, in order to increase the statistical power and reliability of the research, a total of 110 valid questionnaire forms were reached face-to-face in different open and closed areas of the campus through a convenience sampling approach.
- Statistical Analysis and Evaluation: The collected data were transferred to SPSS software and coded in a digital environment. In the analysis process, first, descriptive statistics containing frequency (n) and percentage (%) distributions were performed to reveal the general trends, spatial preferences, and urban image frequencies of the participants. Then, Cross-tabulations (Crosstabs) were created and the Chi-Square ($\chi 2$) Test of Independence was applied to uncover potential relationships between the demographic characteristics of the students, such as gender, age, education level, and origin (whether from Konya or not), and the components of the urban image as well as wayfinding abilities.

The statistically significant findings obtained from the analyses were conceptualized by linking them with the 5 core elements (paths, edges, districts, nodes, and landmarks) in Kevin Lynch's theory of The Image of the City, and the ground was prepared for the conclusion/recommendations section of the study.

## Research Results and Discussion

***Demographic Profile of the Participants***

A total of 110 valid participants were included in the study. It has been certified in the case processing summary of the sample (Table 1) that there is no missing data. Female students constitute 64.5% (n=71) of the participants, while male students constitute 35.5% (n=39). When the age distribution is examined, it is observed that the vast majority of the sample (74.5%, n=82) consists of the dynamic young population in the 18–24 age range. According to education status, 58.2% (n=64) of the participants are high school graduates (active undergraduate students), and in the occupational distribution, 72.7% (n=80) are directly of student status. The fact that 74.5% (n=82) of the participants came from outside of Konya and started residing in the city lends an original quality to the study in terms of measuring the perception of the "temporary user experiencing the city from the outside" rather than the local population.

Table 1. Case Processing Summary

| | *Valid* | | *Missing* | | *Total* | |
|---|---|---|---|---|---|---|
| | N | Percent | N | Percent | N | Percent |
| What is your gender? | 110 | 100,0% | 0 | 0,0% | 110 | 100,0% |
| What is your age range? | 110 | 100,0% | 0 | 0,0% | 110 | 100,0% |
| What is your education status? | 110 | 100,0% | 0 | 0,0% | 110 | 100,0% |
| What grade are you in? | 110 | 100,0% | 0 | 0,0% | 110 | 100,0% |
| What is your occupation? | 110 | 100,0% | 0 | 0,0% | 110 | 100,0% |
| Are you from Konya? | 110 | 100,0% | 0 | 0,0% | 110 | 100,0% |

***Analysis of Konya's Urban Image According to Lynch's Five Primary Elements***

*Paths and Spatial Routines*

In Kevin Lynch's theory, "Paths" are the channels, transit lines, and street axes along which the observer moves. The research findings demonstrate how the relationship university youth establish with transportation homogenizes their mental maps:

- The majority of the participants (81.8%, n=90) stated that they use "Public transportation (tram, bus, minibus)" the most in Konya.

- In parallel with this, 46.4% (n=51) of the participants replied "agree" and 44.5% (n=49) replied "strongly agree" to the statement "The routes I use while going from the campus to the city center have turned into a routine for me."

Regardless of gender, age, education, and duration of residence, this trend towards routinization carries an absolute weight across all demographic groups. This data clarifies that the most dominant "Path" element in the memories of Selçuk University students is the Campus-Alaaddin Tram Line. The fact that individuals utilize the same transit axis (the tram) every day causes urban perception to become confined around this main backbone, while other spatial components of the city remain in the background of their minds.

*Edges and Perceptual Indecisiveness*

According to Lynch, "Edges" are the linear elements that separate two districts or regions and function as barriers in the mind. Regarding the question of whether there are clear boundaries between districts in Konya, the sample displayed a very distinct variation:

- Females (38%, n=27), males (33.3%, n=13), and particularly young students in the 18-24 age group (42.7%, n=35) remained "Undecided" on this issue. On an educational basis, high school graduates and students showed indecisiveness, whereas postgraduate graduates and working individuals accepted the existence of clear boundaries.
- While 1st-grade (52.2%), 2nd-grade (56.7%), and 3rd-grade (36.8%) students were undecided about the existence of boundaries, 85.8% of 4th-grade students and 71% of graduates stated that there are clear boundaries between districts. Regarding the duration of residence, those living in the city for 3–5 years agree with the existence of boundaries at a high level (77.8%, n=14).

This finding shows that the ability to read urban morphology is entirely "experiential." Lower-grade students who are new to the city struggle to perceive and remain undecided about district boundaries (Edges) within Konya's flat and linear structure; conversely, individuals who transition to upper grades and spend more than 3 years in the city can successfully map the "boundary lines" where district identities diverge in their minds.

*Districts and Functional Clustering*

According to Lynch, "Districts" are the homogeneous urban areas that the observer can mentally enter inside of and that possess unique identifying characteristics. When the urban districts where participants spend time most intensively in the city of Konya are examined in general, the Selçuk University Alaeddin Keykubat Campus (40.0%, n=44), Housing Environment (25.5%, n=28), and Bosna Hersek Neighborhood (24.5%, n=27) come to the fore. This distribution indicates that the homogeneous urban districts in the minds of university youth are predominantly clustered around academic and residential functional areas.

*Nodes and Social Interaction*

Nodes are the urban focuses where people gather and where paths intersect.

- Among the participants who stated that there are frequently used meeting points (Nodes) in the city of Konya, 59.1% (n=26) identified the Selçuk University Alaeddin Keykubat Campus as the area where they spend the most time.
- Participants expressed that in their urban wayfinding practices, they directly reference symbolic/symmetrical structures and meeting points to find their way when lost.

The fact that 52.9% (n=13) of those who evaluated transportation opportunities (tram/paths) positively stated that they easily found their way in the city demonstrates that paths, landmarks, and nodes function as an integrated system feeding one another on the mental map.

*Landmarks and Collective Memory*

According to Lynch, landmarks are the prominent physical focuses chosen by individuals as visual reference points during their orientation processes in urban space. The participants' perceptions regarding the most important feature introducing the city of Konya in relation to their demographic characteristics were examined via a cross-tabulation (Table 2).

- Across all variables of gender, age, education, occupation, and hometown, the common imagery focus was "Mevlana and Sufi Culture" (46.4%, n=51). This rate is 49.3% in females and 41.0% in males.
- Similarly, when asked about the first place they would like to take a guest, the Mevlana Museum and Its Surroundings (58.2%, n=64) was selected as the absolute landmark (60.6% in females, 53.8% in males).
- While upper classes and graduates completely brought the Mevlana culture to the fore, 1st-grade students identified Konya more with natural areas and landscapes (Sille, Meram, etc.) (39.1%, n=9) and were equally divided between the Mevlana Museum (34.8%) and Sille Village (34.8%) when asked about the place they would take a guest.

The findings indicate that those who think there are symbolic and iconic structures in Konya directly identify the city with Mevlana. However, it has been determined that the 1st-grade students newly arriving in the city primarily record tangible green spaces and recreational landscapes (Sille, Meram) in their memories as landmarks, and as the duration of residence in the city extends, this perception of physical objects leaves its place to a deep-rooted and abstract cultural image like "Mevlana."

Table 2. Distribution of Participants' Perceptions Regarding the Most Important Feature Introducing Konya According to Demographic Variables

| | | *What is the most important feature that introduces the city of Konya?* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Bosna-Hersek Neighborhood | Natural landscape areas | Konya cuisine | Mevlana and Sufi culture | Historical and cultural landscape areas | S. U. Alaeddin Keykubad Campus | Other |
| What is your gender? | Male | %2.6 n=1 | %15.4 n=6 | %15.4 n=6 | %41 n=16 | %15.4 n=6 | %2,6 n=1 | %7.7 n=3 |
| | Female | %1,4 n=1 | %18,3 n=13 | %12,7 n=9 | %49,3 n=35 | %8,5 n=6 | %8,5 n=6 | %1,4 n=1 |
| What is your age range? | 18-24 | %2,4 n=2 | %22 n=18 | %13,4 n=11 | %41 n=34 | %11 n=9 | %6,5 n=5 | %3,7 n=3 |
| | 25-34 | - | %6,3 n=1 | %12,5 n=2 | %62,5 n=10 | %6,3 n=1 | %12,5 n=2 | - |
| | 35-44 | - | - | - | %83,5 n=5 | - | - | %16,7 n=1 |
| | 45-54 | - | - | - | %50 n=1 | %50 n=1 | - | - |
| | 55-64 | - | - | %50 n=1 | - | %50 n=1 | - | - |
| | 65 years and above | - | - | %50 n=1 | %50 n=1 | - | - | - |
| What is your education status? | Postgraduate graduate | - | - | - | %100 n=6 | - | - | - |
| | High school graduate | %1,6 n=1 | %23,4 n=15 | %12,5 n=8 | %40,6 n=26 | %10,9 n=7 | %6,3 n=4 | %4,7 n=3 |
| | University graduate | %2,5 n=1 | %10 n=4 | %17,5 n=7 | %47,5 n=19 | %12,5 n=5 | %7,5 n=3 | %2,5 n=1 |
| What grade are you in? | 1. Grade | - | %39,1 n=9 | %13 n=3 | 30,4 n=7 | %13 n=3 | %4,3 n=1 | - |
| | 2. Grade | %3,3 n=1 | %16,7 n=5 | %13,3 n=4 | %30 n=9 | %20 n=6 | %10 n=3 | %6,7 n=2 |
| | 3. Grade | %5,3 n=1 | %10,5 n=2 | %5,3 n=1 | %73,7 n=14 | - | - | %5,3 n=1 |
| | 4. Grade | - | %28,6 n=2 | %28,6 n=2 | %42,9 n=3 | - | - | - |
| | Mezun | | %3,2 n=1 | %16,1 n=5 | %58,1 n=18 | %9,7 n=3 | %9,7 n=3 | %3,2 n=1 |
| What is your occupation? | Not working | | %8,3 n=1 | %16,7 n=2 | %58,3 n=7 | %8,3 n=1 | %8,3 n=1 | - |
| | Retired | - | - | %50 n=1 | %50 n=1 | - | - | - |
| | Public sector employee | - | - | - | %80 n=4 | %20 n=1 | - | - |
| | Student | %2,5 n=2 | %21,3 n=17 | %12,5 n=10 | %41,3 n=33 | %12,5 n=10 | %6,3 n=5 | %3,8 n=3 |
| | Private sector | - | %9,1 n=1 | %18,2 n=2 | %54,5 n=6 | - | %9,1 n=1 | %9,1 n=1 |
| Are you from Konya? | Yes | - | %10,7 n=3 | %25 n=7 | %39,3 n=11 | %25 n=7 | - | - |
| | No | %2,4 n=2 | %4,9 n=4 | %19,5 n=16 | %9,8 n=8 | %48,8 n=40 | %6,1 n=5 | %8,5 n=7 |

*Urban Aesthetics and the Image Paradox*

Lynch's theory examines the psychological barriers that damage the urban image as much as it investigates the positive visual elements of the city:

- Both local participants from Konya (50.0% absolutely beautiful, 28.6% beautiful) and non-local participants (24.4% absolutely beautiful, 45.1% beautiful) find Konya highly successful in aesthetic terms. The most admired features of the city were determined as ease of transportation (72.0%, n=18), being planned/ordered (56.3%), and being calm/clean (56%).
- In response to the question "What is the feature of the city of Konya that you admire the least?", 49.1% (n=54) of the students, who admire the physical structure of the city to this extent, stated that "the people of Konya are closed-minded in terms of views," while 25.5% (n=28) specified the lack of social activities. The areas whose absence is felt the most in the city became cultural and artistic event spaces (42.7%, n=47).

These obtained findings clearly reveal the contradiction between "physical environmental satisfaction and social environmental barriers," which is frequently discussed in the literature of urban morphology and environmental psychology. The fact that the participants find the city's planning, spatial cleanliness, and functional transportation networks highly successful from an aesthetic perspective demonstrates that Konya's objective urban design quality is appreciated by the users. However, the same sample pointing out "the closed-mindedness of the local population" (49.1%) as the least admired feature of the city and emphasizing the "lack of cultural and artistic spaces" (42.7%) demonstrates that spatial perception harbors a double-layered paradox.

***Effects of Demographic Variables on Spatial Preferences and Urban Image Perception***

*Spatial Preferences and Urban Image Perception by Gender*

*Relationship Between Gender and the Region Where Most Time is Spent in the City*

The distribution of the spatial regions where participants spend time most intensively in the city of Konya according to their genders is presented in Table 3; concurrently, the results of the Chi-Square analysis empirically testing the effect of the gender variable on spatial usage practices are provided in Table 4.

Table 3. Distribution of Regions Where Most Time is Spent in the City by Gender

| *Gender* | *Bosna-Hersek Neighbor.* | *City Center (Alaaddin Zafer Square)* | *S.U. Alaeddin Hill, Keykubat Campus* | *The Neighbor./ House/ Dormitory Environment* | *Green Spaces and Parks* | *Total* |
|---|---|---|---|---|---|---|
| Male | 9 (%23,1) | 2 (%5,1) | 17 (%43,6) | 11 (%28,2) | 0 (%0,0) | 39 (%100) |
| Female | 18 (%25,4) | 3 (%4,2) | 27 (%38,0) | 17 (%23,9) | 6 (%8,5) | 71 (%100) |
| Total | 27 | 5 | 44 | 28 | 6 | 110 |

Table 4. Chi-Square Test Results Regarding the Relationship Between Regions Where Most Time is Spent in the City by Gender

| Evaluation Criterion | Value | df | Asymp. Sig. (2-sided) / p |
|---|---|---|---|
| Pearson Chi-Square ($\chi 2$) | 3,768 | 4 | 0,438 |
| Likelihood Ratio | 4,812 | 4 | 0,307 |
| N of Valid Cases | 110 | | |

It is observed that the most dominant focal point for both male (43.6%) and female (38.0%) students is the Selçuk University Alaeddin Keykubat Campus. The campus is followed by housing environments and the Bosna Hersek Neighborhood, respectively. As a result of the Chi-Square analysis performed, no statistically meaningful relationship was found between gender and the region where most time is spent ($\chi 2 = 3.768$; $p > 0.05$). This situation indicates that university youth experience the urban space within the framework of similar functional necessities (education and housing) regardless of gender differences, and the first urban 'district' image in their memories homogenizes along the axis of the S.U. Campus-Bosna Hersek Neighborhood.

*Relationship Between Gender and the First Place to Take a Guest*

The distributions of the participants' first choice of places to take an outside guest in the city of Konya according to their genders are presented in Table 5; concurrently, the results of the Chi-Square analysis empirically testing the effect of the gender variable on mental image and landmark selection are provided in Table 6.

Table 5. Distribution of the First Place to Take a Guest by Gender

| *Gender* | *Alaaddin Hill / Kültürpark* | *Meram Vineyards / Tavusbaba* | *Mevlana Museum and Its Surroundings* | *Sille Village* | *Historical Bazaars* | *Tropical Butterfly Garden* | *Total* |
|---|---|---|---|---|---|---|---|
| Male | 3 (%7,7) | 2 (%5,1) | 21 (%53,8) | 8 (%20,5) | 1 (%2,6) | 4 (%10,3) | 39 |
| Female | 2 (%2,8) | 2 (%2,8) | 43 (%60,6) | 9 (%12,7) | 2 (%2,8) | 13 (%18,3) | 71 |
| Total | 5 | 4 | 64 | 17 | 3 | 17 | 110 |

Table 6. Chi-Square Test Results Regarding the Relationship Between First Places to Take a Guest by Gender

| *Evaluation Criterion* | *Value* | *df* | *Asymp. Sig. (2-sided) / p* |
|---|---|---|---|
| Pearson Chi-Square ($\chi 2$) | 3,944 | 5 | 0,557 |
| Likelihood Ratio | 4,215 | 5 | 0,519 |
| N of Valid Cases | 110 | | |

When the distribution is examined, it is observed that the first choice of 53.8% of males and 60.6% of females is the Mevlana Museum and Its Surroundings. The second strongest image became Sille Village (20.5%) for males and the Tropical Butterfly Garden (18.3%) for females. According to the Chi-Square test result, there is no meaningful relationship between gender and symbolic space preference ($\chi 2 = 3.944$; $p > 0.05$). This finding proves that the counterpart of Kevin Lynch's "landmark" concept in the specific case of Konya is absolute at the collective memory level as the Mevlana Museum, regardless of gender differences.

*The Effect of Temporal Experience on Spatial Preferences and Regional Belonging*

The distribution of the regions where participants spend time most intensively across the city according to their duration of residence in Konya is presented in Table 7; concurrently, the results of the Chi-Square analysis empirically testing the effect of temporal experience on the ways of experiencing and encoding urban space in memory are provided in Table 8.

As a result of the Chi-Square analysis performed, a statistically highly meaningful relationship was detected between the students' duration of residence in the city and the regions where they spend the most time ($p < 0.001$):

- For students who are new to the city (less than 1 year) and those who have been there for a moderate period (1–3 years), the center of gravity in their memory is concentrated in the areas of the Bosna Hersek Neighborhood (42.9%) and the S.U. Campus (55.3%). During this period, the image of the city center is almost entirely absent (0% and 2.6%).

- As the duration of experience in the city increases, and particularly when it reaches 5 years and above, urban space usage practices change radically. While the campus dependency of those residing for 5 years and above regresses to 15%, their own neighborhood environments (45.0%), the city center (10.0%), and green spaces (10.0%) become dominant.

This finding directly aligns with the logic of the "level of environmental experience" in Kevin Lynch's theory. When a student first arrives in the city, their mental space is limited to narrow "districts" such as the campus and its immediate surroundings (Bosna Hersek Neighborhood); however, as the time spent in the city extends, their cognitive map expands, and they develop a more mature urban image by incorporating the macro-scale urban backbone (the city center, other districts, and parks) into their memory.

Table 7. Distribution of Regions Where Most Time is Spent in the City by Duration of Residence

| *Duration of Residence* | *Bosna-Hersek Neighbor.* | *City Center (Alaaddin Hill & Zafer Square)* | *S.U. Alaeddin Keykubat Campus* | *The Neighbor./House/ Dormitory Environment* | *Green Spaces and Parks* | *Total* |
|---|---|---|---|---|---|---|
| Less than 1 Year | 6 (%42,9) | 0 (%0,0) | 5 (%35,7) | 2 (%14,3) | 1 (%7,1) | 14 |
| 1-3 Years | 11 (%28,9) | 1 (%2,6) | 21 (%55,3) | 4 (%10,5) | 1 (%2,6) | 38 |
| 3-5 Years | 2 (%11,1) | 0 (%0,0) | 12 (%66,7) | 4 (%22,2) | 0 (%0,0) | 18 |
| 5 Years and Above | 8 (%20,0) | 4 (%10,0) | 6 (%15,0) | 18 (%45,0) | 4 (%10,0) | 40 |
| Total | 27 | 5 | 44 | 28 | 6 | 110 |

Table 8. Chi-Square Test Results Regarding the Relationship Between Regions Where Most Time is Spent in the City by Duration of Residence

| *Evaluation Criterion* | *Value* | *df* | *Asymp. Sig. (2-sided) / p* |
|---|---|---|---|
| Pearson Chi-Square (χ2) | 33,195 | 12 | 0,0009* |
| Likelihood Ratio | 32,404 | 12 | 0,001 |
| N of Valid Cases | 110 | | |

***Significant level 99.9 % (p<0.001)

*Urban Wayfinding Ability of Local and Outside Participants*

The distributions of the ease of wayfinding within the city according to the participants' locality status are presented in Table 9; concurrently, the results of the Chi-Square analysis empirically testing this relationship are provided in Table 10.

Table 9. Distribution of Ease of Wayfinding According to Being from Konya Status

| *Are you from Konya?* | *Strongly Disagree* | *Disagree* | *Undecided* | *Agree* | *Strongly Agree* | *Total* |
|---|---|---|---|---|---|---|
| Yes | 0 (%0,0) | 1 (%3,6) | 1 (%3,6) | 11 (%39,3) | 15 (%53,6) | 28 (%100) |
| No | 3 (%3,7) | 4 (%4,9) | 15 (%18,3) | 40 (%48,8) | 20 (%24,4) | 82 (%100) |
| Total | 3 | 5 | 16 | 51 | 35 | 110 |

Table 10. Chi-Square Test Results Regarding the Relationship Between Being from Konya Status and Ease of Wayfinding

| *Evaluation Criterion* | *Value* | *df* | *Asymp. Sig. (2-sided) / p* |
|---|---|---|---|
| Pearson Chi-Square (χ2) | 12,862 | 4 | 0,0119* |
| Likelihood Ratio | 14,484 | 4 | 0,006 |
| N of Valid Cases | 110 | | |

*Significant level 95 % (p<0.05)

As a result of the Chi-Square analysis performed, a statistically meaningful relationship was found between whether the students are local (from Konya) or not and their wayfinding skills in the city ($\chi2 = 12.862$; $p < 0.05$):

- While 53.6% of local students from Konya state that they "strongly agree they find their way easily," this rate drops to 24.4% among students coming from outside the city.
- 18.3% of the students coming from outside the city remain "undecided" regarding wayfinding.

This situation indicates that the urban image does not consist of physical objects alone, but is directly related to "spatial familiarity and rootedness." Selçuk University students coming from outside are far from the city center (they are on campus), and since they cannot fully read the city, their mental maps are less distinct compared to local students.

When the effects of demographic variables on the Konya urban image and spatial preferences are examined; it is observed that the Konya urban image in the minds of Selçuk University students harbors a homogeneous structure in terms of "Gender" ($\chi2 = 3.768$ and $\chi2 = 3.944$; $p > 0.05$), whereas it exhibits a highly heterogeneous

structure in terms of "Duration of Residence in the City" ($\chi 2 = 33.195$; $p < 0.001$) and "Locality Status" ($\chi 2 = 12.862$; $p < 0.05$). This situation demonstrates that urban memory and image construction are directly related to urban legibility processes that expand in parallel with the duration of spatial experience and the status of being local.

When the findings are analyzed holistically, a distinct contrast between the tangible urban landscape and the abstract social climate stands out in the university youth's perception of Konya. Although the participants find the city "ordered and livable" on the axis of physical planning, environmental cleanliness, and ease of transportation, they experience difficulties in integrating with the city due to the scarcity of urban activities and the limitations of the socio-cultural fabric. Consequently, the Konya image identified in the mental maps presents a double-layered structure that carries physically rational and legible qualities, yet socially leaves a distance between the user and the space.

**Conclusion and Recommendations**

In this study, the urban images and spatial perceptions of Selçuk University students regarding the city of Konya were evaluated multidimensionally within the framework of Kevin Lynch's (1960) urban morphology theory. Within the scope of the research, the spatial, social, and cultural relationships that university youth establish with the city were analyzed, and the reflections of these interactions on mental maps and collective memory were demonstrated through quantitative data.

The research results indicate that Selçuk University students encode Konya largely through a positive urban image in terms of physical environmental components. A vast majority of the participants find Konya aesthetically successful; they evaluate the city's planned-ordered structure, calm-clean character, and urban transportation opportunities as positive urban attributes. In contrast, it is a striking finding that nearly half of the participants characterize the people of Konya as "closed-minded" in terms of their socio-cultural approach and complain about the insufficiency of social activities in the city. This situation demonstrates that the high level of positive perception developed toward the morphological and physical sustainability of the urban space transforms into an image paradox within the "social environment" component that provides urban vitality and social integration.

The most dominant theoretical conclusion obtained in the study is that the urban image and spatial memory of Konya are absolutely shaped around the focus of Mevlana and Sufi culture, regardless of demographic layers. This cultural heritage was highlighted by the participants as the strongest image promoting the city, and it was declared at a high rate that an outside visitor to the city should first be taken to the Mevlana Museum and its surroundings. This data reveals that the counterparts of the concepts of landmark and node in Kevin Lynch's theory are absolute at the collective memory level with the Mevlana Museum and its immediate surroundings in the specific case of Konya. As a matter of fact, the students taking these symbolic/historical structures as landmarks in their urban wayfinding practices and moments of being lost confirms the orientation success of physical urban elements in cognitive maps.

The statistical cross-analyses of the research revealed that urban image construction is directly related to "spatial familiarity" and "duration of experience." The meaningful difference ($\chi 2 = 12.862$; $p < 0.05$) detected between local students (from Konya) and non-local students (from outside) in terms of ease of wayfinding proves the relationship of urban legibility with the level of taking root in the city. Similarly, the expansion of urban space usage practices from a narrow focus of the campus and Bosna Hersek Neighborhood (districts) toward the macro-scale urban backbone (city center, parks, and residential environments) depending on the prolongation of the residence period ($\chi 2 = 33.195$; $p < 0.001$) shows that urban experience is a dynamic parameter that matures mental maps. This perceptual deepening also manifests itself at the class level; while 1st-grade students newly arriving in the city prioritize tangible natural areas and recreational landscapes (Sille, Meram, etc.) as landmarks, the urban image evolves entirely toward abstract and deep-rooted cultural heritage elements (the perception of Mevlana) as students transition to upper classes and the graduation stage.

Finally, the students' declaration that the linear movement corridors (paths) between the campus and the city center turn into strong spatial routines over time demonstrates that urban consumption becomes uniform along specific transportation axes (the tram line). The Selçuk University Alaeddin Keykubat Campus, located on this routine line, functions not only as an educational area for students but also as the primary focal point (node) where they spend time most intensively and socialize. The fact that cultural-artistic event spaces and quiet/accessible study spaces are at the top of the areas whose absence is felt the most by students in the city offers strategic frameworks for urban planning processes in terms of revealing the expectations and spatial demands of university youth from urban spaces.

In line with these obtained empirical and theoretical findings, the following recommendations have been developed regarding student-oriented urban planning and landscape design strategies that will shape the future of the city of Konya:

- The elements of Mevlana and Sufi culture, which constitute the global and local urban identity of Konya, must be preserved in line with landscape architecture principles, and the urban aesthetics and pedestrianization corridors of the historical city center should be reinforced to support this image.
- In order to break the perception of "social closed-mindedness" in the urban image of outside students and to increase their bonds of belonging to the city in the early period, thematic city tours, interactive awareness programs, and socio-cultural festivals introducing urban morphology and identity should be organized at an institutional level.
- Along the Campus-City Center axis (especially tram and public transport routes), which is most intensively used by university youth and has turned into a routine in their minds, micro-landscape arrangements that increase pedestrian comfort, high-quality urban furniture, continuous bicycle paths, and urban design interventions with high visual legibility should be developed.
- Indoor and outdoor cultural-artistic event spaces, contemporary quiet study libraries, and recreational social meeting points (nodes) most demanded by students should be spatially increased and made accessible, particularly at the campus-city interface (Bosna Hersek Neighborhood and its surroundings).
- Sille, Meram, and other public green spaces/parks, which constitute the first landmarks in the minds of students with a short duration of residence in the city, should be more strongly integrated with urban transportation networks; the landscape aesthetic values of the city should be enriched with next-generation active green space designs that will contribute to the urban image.
- To strengthen the urban legibility of the Mevlana Museum and the Historical City Center Environment, which stand out as the absolute landmark of Konya in collective memory, night lighting designs, urban information-wayfinding signage compatible with the historical fabric, and digital urban guide infrastructures within the scope of smart city applications should be developed.

Furthermore, in future studies, the cognitive maps of students studying at different universities in Konya (e.g., Konya Technical University, Necmettin Erbakan University) could be analyzed comparatively; additionally, the city perceptions of different age, occupation, and socio-economic groups could be deepened through longitudinal research to monitor the change of the urban image over time.

## Declarations

### *Ethical Approval Certificate*

This study was approved by the Scientific Ethics Evaluation Board of the Faculty of Architecture and Design, Selçuk University (Decision No: 04/08, approved on 11 June 2026).

### *Author Contribution Statement*

All contributions belong to authors.

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*ID: 813*

# Allelopathic Effects of Common Weeds on Durum Wheat Germination and Early Growth in Northwest Algeria

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## Abstract

Weeds exert strong allelopathic interactions that can negatively affect crop establishment, making their study essential for sustainable agriculture. This work examined the allelopathic potential of three common species—*Malva sylvestris* L., *Oxalis pes-caprae* L., and *Fumaria capreolata* L.—on the early growth stages of durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.). Plant material was collected from citrus orchards in the Tlemcen region of Northwest Algeria, and aqueous extracts were prepared from dried aerial parts using two methods: shaking (EA1) and maceration (EA2). Extracts were applied at graded concentrations (0%, 25%, 50%, 75%, and 100%) to evaluate their effects on germination, coleoptile elongation, and radicle growth. The study measured germination percentage, coleoptile length, radicle length, and inhibition rates, with statistical analysis performed using two-way ANOVA. Results revealed significant and dose-dependent inhibition of wheat development, with clear differences among species. *M. sylvestris* showed the strongest allelopathic activity, reaching complete inhibition at high concentrations with EA1. *O. pes-caprae* exerted moderate and progressive effects, occasionally stimulating growth at low doses, while *F. capreolata* displayed a subtler but increasingly pronounced impact at higher concentrations, particularly on radicle elongation. Across treatments, EA1 extracts were generally more effective than EA2, emphasizing the importance of extraction protocol in determining bioactivity. These findings demonstrate that *M. sylvestris* and *F. capreolata* possess considerable allelopathic potential and could serve as sources of natural compounds for plant-based weed control. Their valorization may contribute to the development of eco-friendly strategies for managing weeds in cereal production systems.

***Keywords:*** *Allelopathy, weeds, durum wheat, aqueous extracts, biocontrol, germination*

*ID: 815*

# Air Logistics in International Equestrian Sports Organizations: A Comparative Content Analysis on the Operational Roles of Service Providers

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## Abstract

The supply chain processes of organisations involved in international equestrian sports entail multi-stakeholder logistics operations that require intensive coordination and a high degree of precision. Considering that over 200 racehorses are transported by air on a daily basis worldwide, this mode of transport emerges as a specialized research area due to its high volume and operational complexity.

This study aims to examine the operational processes, products and solutions offered, and corporate strategies of horse air transport service providers operating in prestigious international events using a comparative content analysis method. Within the scope of the research, data obtained from the corporate websites, press releases and industry publications of logistics service providers within the supply chains of major organisations were systematically analysed. The scope of services, equipment and infrastructure standards, animal welfare practices, regulatory compliance and organisation-based operational experience dimensions of these businesses were examined.

The findings reveal that service providers are categorised under two distinct structures based on their operational roles. The first comprises air cargo carriers, which stand out for their air transport capacity and robust logistics infrastructure; the second encompasses specialised service providers that manage all logistics coordination and operational planning for the entire organisational process from start to finish. This study aims to provide a unique conceptual and empirical contribution to the fields of logistics for international sporting events and live animal transport.

***Keywords:*** *Air transportation, Supply chain management, Equestrian sports organizations, Logistics service providers, Live animal transport standards, Content analysis*

## Uluslararası Atlı Spor Organizasyonlarında Hava Lojistiği: Hizmet Sağlayıcıların Operasyonel Rolleri Üzerine Karşılaştırmalı Bir İçerik Analizi

## Özet

Uluslararası atlı sporlara ilişkin organizasyonların tedarik zinciri süreçleri, yoğun koordinasyon gerektiren, çok aktörlü ve yüksek hassasiyetli lojistik operasyonları beraberinde getirmektedir. Her gün dünya genelinde 200'den fazla yarış atının havayoluyla taşındığı dikkate alındığında, bu taşımacılık türü, hem işlem hacmi hem de operasyonel karmaşıklığı bakımından özgün ve özel bir araştırma alanı oluşturmaktadır.

Bu çalışma, uluslararası prestijli organizasyonlarda faaliyet gösteren at hava taşımacılığı hizmet sağlayıcılarının operasyonel süreçlerini, sunulan ürün ve çözümleri ve kurumsal stratejilerini karşılaştırmalı içerik analizi yöntemiyle incelemeyi amaçlamaktadır. Araştırma kapsamında, büyük organizasyonların tedarik zincirinde yer alan lojistik hizmet sağlayıcılarının kurumsal web siteleri, basın bültenleri ve sektör yayınlarından elde edilen veriler sistematik biçimde analiz edilmiştir. Söz konusu işletmelerin hizmet kapsamı, ekipman ve altyapı standartları, hayvan refahı uygulamaları, mevzuat uyumu ve organizasyon bazlı operasyonel deneyim boyutları ele alınmıştır.

Elde edilen bulgular, hizmet sağlayıcılarının operasyonel rollerine göre iki farklı yapı altında konumlandığını ortaya koymaktadır. Bunlardan ilki, hava taşımacılığı kapasitesi ve güçlü lojistik altyapısıyla öne çıkan havayolu kargo işletmelerinden oluşurken; ikincisi, organizasyon sürecine ilişkin tüm lojistik koordinasyonu ve operasyonel planlamayı uçtan uca yöneten uzmanlaşmış hizmet sağlayıcılarını kapsamaktadır. Bu çalışma, uluslararası spor organizasyonları lojistiği ile canlı hayvan taşımacılığı alanlarına özgün bir kavramsal ve ampirik katkı sunmayı hedeflemektedir.

***Anahtar Kelimeler:*** *Hava taşımacılığı, Tedarik zinciri yönetimi, Atlı spor organizasyonları, Lojistik hizmet sağlayıcıları, Canlı hayvan taşıma standartları, İçerik analizi*

ID: 817

# Irrigation Automations in Digital Agriculture

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## Abstract

Water is an indispensable natural resource for sustaining all living organisms, yet it is limited in quantity. Rapid population growth, climate change and the associated tendency toward drought observed worldwide are increasing the demand for water resources day by day. This growing demand places serious pressure on water resources and adversely affects agricultural production in particular. The efficient use of water in agricultural production is a decisive factor in productivity. Therefore, the integration of irrigation automation systems into agriculture has become one of the priority issues of the sector. With advancing technology, the use of artificial intelligence based solutions in agricultural irrigation is becoming increasingly widespread; these solutions optimize irrigation processes and make significant contributions to water conservation and sustainable management. In this study, irrigation automation systems implemented in Turkiye and around the world were examined in detail; the functioning of current technologies, their fields of application and the benefits they provide were evaluated from an academic perspective.

***Keywords:*** *Digital agriculture, Irrigation automations*

## Introduction

The deepening global effects of climate change and drought have made water management one of today's most critical agenda items (Boujdi et al., 2024). The sustainable management of water resources and the efficient use of existing water potential are of critical importance in addressing the global water crisis. For this reason, irrigation automation systems which are among the applications of digital agriculture, are among the core technologies that contribute to increasing resource efficiency in agricultural production by ensuring that water is used at the right time, in the right amount and in a controlled manner. Climate induced extreme events such as drought, floods, excessive temperatures and irregular precipitation patterns disrupt ecosystem balances and threaten global food security. Considering that approximately 72% of global freshwater consumption is used by the agricultural sector, agriculture is regarded as both one of the largest users in the water crisis and one of the central areas of the solution process. Current projections indicate that agricultural water demand will increase by approximately 35% by 2050. Therefore, the effective, sustainable and controlled use of agricultural water resources has become imperative.

One of the main problems limiting the effective implementation of irrigation and fertilization practices in agricultural production is the distance between agricultural lands and the living areas of farm owners. In particular when production areas are far from settlement centers, it becomes difficult to monitor irrigation processes regularly and this leads to time losses in practice. In addition, the relatively low cost of irrigation water in Turkiye appears to be one of the major factors encouraging the unconscious and excessive use of water (Süzer, 2014).

To reduce losses arising from agricultural water use and to utilize water resources more efficiently, the use of innovative irrigation technologies is becoming increasingly important. Different methods such as drip irrigation, sprinkler irrigation, pivot systems and smart irrigation technologies are used in agricultural irrigation applications. In particular, automation based irrigation systems integrated with digital agriculture applications provide higher efficiency compared with traditional irrigation methods based on human labor or manual control.

Smart irrigation automation systems can determine the plant's water needs in real time through sensors, remote control systems and data analysis technologies, allowing only the necessary amount of water to be used. This contributes both to reducing water consumption and to lowering energy and labor costs. At the same time, conducting irrigation processes in a more controlled and planned manner supports increased productivity in agricultural production and offers significant advantages for sustainable resource management (Şahin, 2022).

Today, smart irrigation solutions referred to as water management in agriculture have been developed thanks to artificial intelligence and Internet of Things (IoT) technologies. Through these technologies, the management of available resources is optimized and water consumption is significantly reduced. Smart irrigation automation systems continuously read data such as the current amount of water at the source, atmospheric temperature and soil moisture and transfer them to the software layer, enabling real time monitoring of water use. Thanks to this structure, an irrigation regime appropriate to the actual needs of the soil can be applied (Şahin, 2022).

Irrigation automation systems are automatic systems that enable the efficient irrigation of open land and agricultural areas within a specific control mechanism. Through these systems, both water savings are achieved and uniform and balanced water distribution can be provided across the entire area to be irrigated. In addition to

preventing unnecessary water consumption, irrigation automation systems also offer remote control capabilities (Roltek, 2023).

This feature is not limited to contributing to the protection of water resources; it also offers significant advantages in reducing labor costs and increasing agricultural productivity. The remote access and control opportunities provided by technology save farmers time and energy, while contributing to the more planned and sustainable execution of irrigation processes (Şahin, 2022). Although the Netherlands is a country with limited surface area, it has achieved an important position in global agriculture thanks to its advanced technology oriented production policies adopted in the agricultural sector. In particular, the effective use of modern irrigation techniques, digital agriculture applications, data driven management systems and software supported production processes has significantly increased the country's agricultural productivity and export capacity.

The widespread use of sensor technologies, automation systems and digital control mechanisms in agricultural production processes in the Netherlands enables more efficient use of water and energy resources. Thanks to this Technology based approach, production costs are reduced, sustainable resource management is supported and high value-added agricultural production models are developed. Therefore, the example of the Netherlands demonstrates that digital agriculture technologies have strategic importance in terms of increasing efficiency and sustainability in agricultural production (Saygili et al., 2018). Technological transformations implemented in different sectors have also paved the way for digitalization in agriculture, enabling the spread of innovative applications. Digital tools introduced with Industry 4.0 have led to the emergence of smart agriculture applications and new technologies in production have radically transformed the agricultural approach (Gökçe et al., 2024). Industry 4.0 tools applied at all stages of production, from sowing seeds to irrigation, spraying and harvesting, provide significant advantages to the sector. The main advantages are summarized below (Hektaş, 2021):

Thanks to digital irrigation infrastructure, the producer can irrigate the field automatically at any desired time interval and with any desired flow rate. The entire system is monitored through automation software.

Sensors placed in the field measure the instantaneous soil moisture value; the system integrated with weather data prevents unnecessary irrigation and provides significant savings in electricity and water resources.

During periods of high rainfall, the system can be stopped automatically; thus, both energy savings are achieved and the natural moisture balance of the soil is preserved (Hektaş, 2021).

**Basic Components of Smart Irrigation Systems**

Smart irrigation systems are Technology based irrigation solutions developed to make water use in agricultural and landscape areas more efficient, controlled and sustainable. As shown in Figure 1, these systems consist of an integrated structure composed of sensors, control units, communication infrastructure, valves and sprinkler equipment, meteorological stations and data analysis software

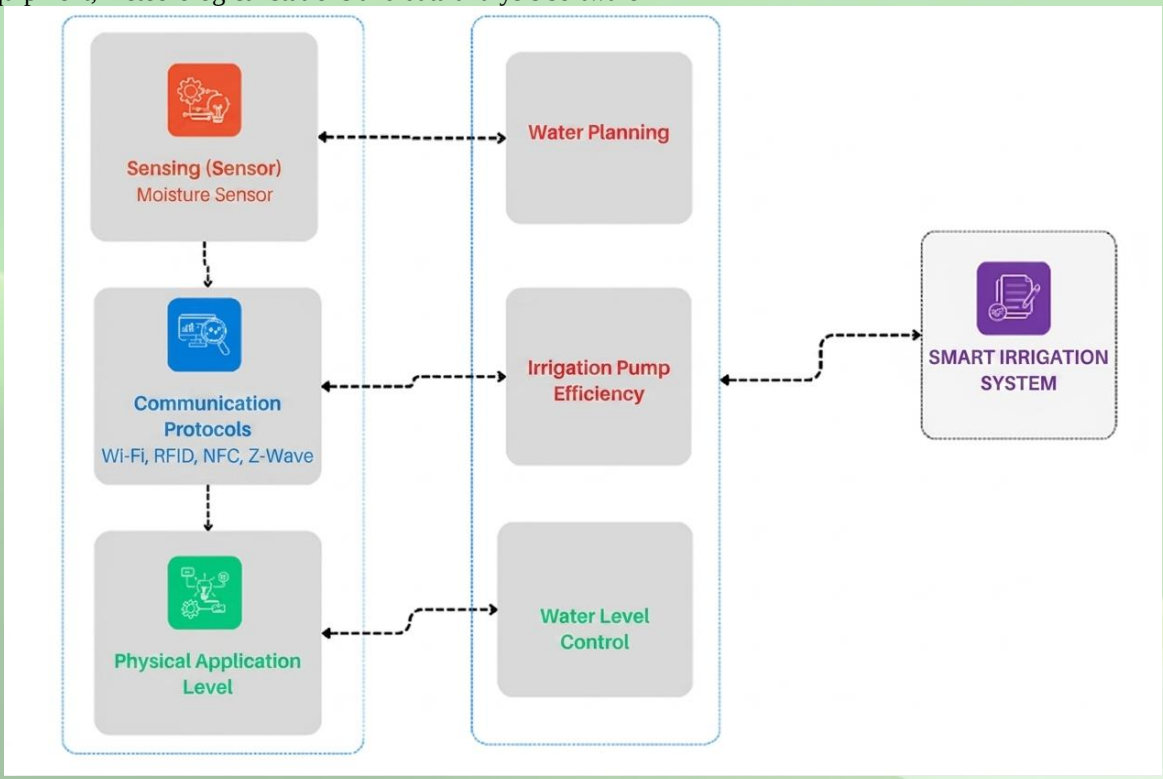



Figure 1. Basic components of smart irrigation systems (Şahin, 2022).

The sensors in the system collect real time data on the soil's moisture status and environmental conditions, while control mechanisms analyze this data to ensure that irrigation operations are carried out automatically. Smart irrigation infrastructures integrated with meteorological data determine the amount of water required by the plant and prevent unnecessary water consumption. Thus, more effective use of water resources is achieved while energy consumption and operating costs are reduced.

The coordinated operation of all these components makes important contributions to increasing water efficiency in agricultural production, protecting natural resources and supporting a sustainable production approach (Şahin, 2022).

The efficiency of irrigation systems holds critical importance in increasing food production. While the excessive and wasteful use of freshwater is usually noticed only in cases of poor management, over-irrigation often leads to the leaching of plant nutrients from the soil and the unnecessary consumption of water resources. Therefore, on-farm irrigation management systems must be precisely designed to provide the right amount of water at the right time, based on the specific water requirements determined for each crop. Consequently, through the integration of the Internet of Things (IoT) and machine learning technologies, high-precision monitoring and control systems supported by mobile and web-based applications can be developed for agricultural irrigation processes; thereby achieving significant savings in water, energy, and labor utilization (Abioye et al., 2022)

## Findings

Smart irrigation systems basically consist of a smart water valve, sensors, controllers, communication systems and valves and sprinkler elements. Within the scope of this study, the functioning, hardware structure and role of each component in irrigation automation were evaluated under separate headings.

### *Smart Water Valve*

Among smart water technologies, smart water valves stand out by enabling the remote monitoring of irrigation status and water consumption through proportional irrigation principles integrated with irrigation methods and by allowing the system to be managed through a central structure. From a hardware perspective, these systems consist of four main groups: a valve controller, a flow meter, a wireless communication module and a power supply. Each component forms a functional integrity for the continuous and reliable operation of the system.

It is observed that, within the reliable operation of the system, the smart valve continuously collects data from measuring devices positioned along the irrigation lines. The obtained data are transferred in real time to cloud based software platforms via wireless communication options; thus, the amount of water consumption and irrigation status can be monitored remotely through smart end devices such as smartphones or computers and necessary interventions can be performed remotely. Continuously supervised by irrigation automation software, the system reinforces operational safety by transmitting alerts in the event of abnormal conditions (Şahin, 2022). When these features are evaluated as a whole, it becomes clear that smart water valves have evolved from being merely irrigation control tools into core devices of a data-driven decision-support mechanism related to the management of water distribution in irrigation (Şahin, 2022).

### *Sensors*

Optimizing water use in smart irrigation systems depends on monitoring dynamic environmental parameters such as soil moisture, ambient temperature, humidity and rainfall. Real-time data obtained through strategically placed field sensors prevent both over-irrigation and water stress caused by insufficient irrigation (Şahin, 2022). Data collected from these sensors are transmitted to a cloud server using wireless communication infrastructures such as Wi-Fi, Bluetooth, or cellular networks. The central cloud server processes the raw data through specialized software and provides the main input for the system's decision-support mechanisms (Erdal & Ergüzen, 2020). In this context, the DHT22 sensor is widely preferred because it offers high reliability in temperature and humidity measurements, calibrated digital output and stable operating performance (Liu, 2013). The physical structure of the sensor is presented in Figure 2.

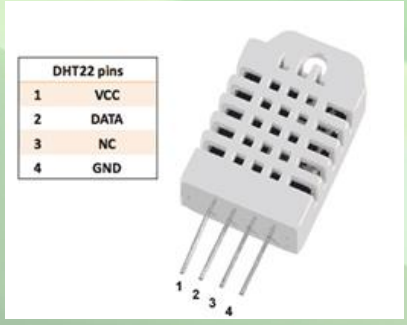



Figure 2. DHT22 Temperature and Humidity Sensor (Taştan, 2019).

Digital signals obtained from the DHT22 can be read directly by microcontrollers. For soil moisture measurements, the HL-69 hygrometer moisture sensor is also widely used. The resistance value of this sensor, which is placed into the soil and contains copper conductive paths, changes depending on the soil's moisture level. The circuit converts this resistance change into a voltage change, making it possible to obtain digital data regarding soil moisture (Taştan, 2019).

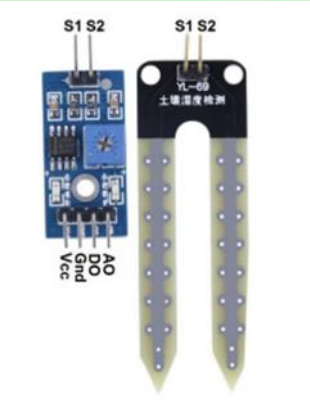



Figure 3. HL-69 Soil Hygrometer Moisture Sensor (Taştan, 2019).

***Controllers***

In smart irrigation systems, controllers are the components that act as the brain of the system and manage its overall operation. By analyzing data received from sensors through the communication infrastructure, they ensure the optimization of the irrigation program. Microcontrollers widely used in the market appear in many different application areas in the agricultural sector (Şahin, 2022).

A microcontroller board is used to evaluate sensor data and the most frequently preferred platform is Arduino. Thanks to its ease of programming, minimal additional hardware requirements and adaptable structure for different projects, Arduino stands out among microcontroller boards. An image of the Arduino Uno board is shown in Figure 4 (Bilgin, 2024).

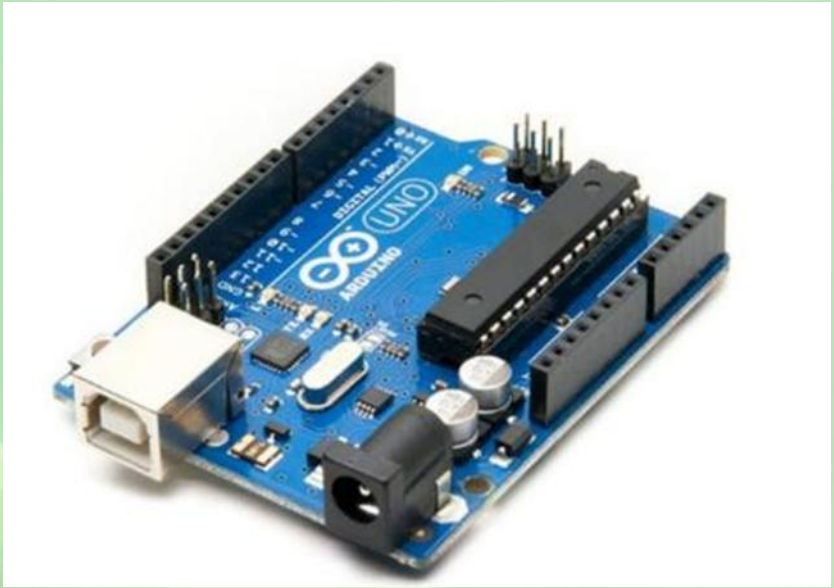



Figure 4. Arduino Uno microcontroller board (Bilgin, 2024)

Arduino can be used to create independently operating interactive systems and can also be integrated with software on a computer. Ready-made boards may be preferred, or development with original hardware design is also possible. The microprocessor (AtmegaXX) on the development board is coded in the Arduino programming language and the prepared program is uploaded to the board via the Processing-based Arduino Integrated Development Environment (IDE). The pin structure of the Arduino board is shown in Figure 5.

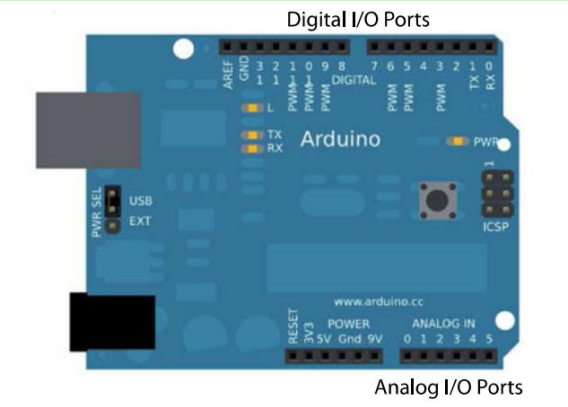



Figure 5. Arduino board pin structure (D'Ausilio, 2012).

In addition to Arduino, NodeMCU is also widely preferred as an IoT controller with Wi-Fi module support. NodeMCU contains a 32-bit RISC Tensilica LX106 microcontroller operating at 160 MHz and C++ is used as the programming language. The pin structure of NodeMCU, which can be programmed directly thanks to its mini-USB port, is shown in Figure 6, while its technical specifications are given in Table 1. Since the controller's ADC input pin can perform 10-bit conversion for 1V, the sensor output (maximum 5V) is reduced to 1V by means of a voltage divider circuit (Taştan, 2019).

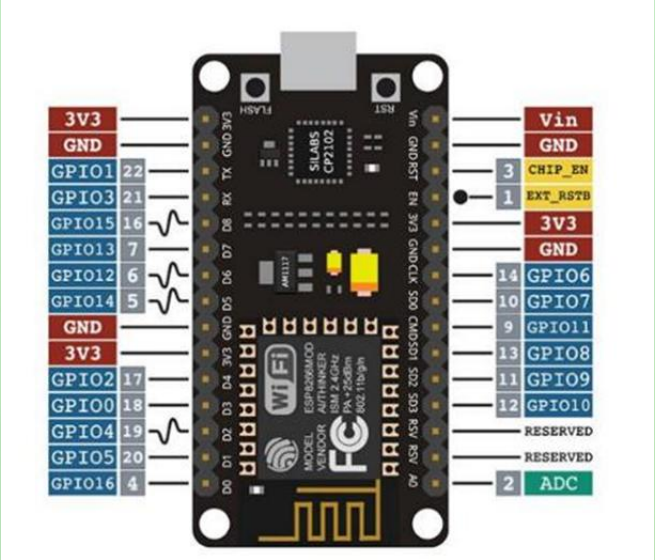



Figure 6. NodeMCU pin structure (Taştan, 2019).

***Communication Systems***

The communication infrastructure used in smart irrigation systems enables uninterrupted information flow by providing data exchange among sensors, controllers and other components of the system. This communication, carried out through wired or wireless networks, enables the remote management of the irrigation system and makes it easier for users to monitor the process via mobile devices, smartphones, or computers. In this way, irrigation planning suitable for field conditions can be carried out and water resources can be used more efficiently (Şahin, 2022). Technological developments have made the planning tools used in smart irrigation systems more accessible.

Especially in regions of the United States where crops requiring intensive irrigation, such as cotton, are grown, irrigation processes can be successfully managed through smartphone applications (Vellidis et al., 2016).

These mobile applications allow users to manage irrigation processes easily; thanks to notifications that optimize irrigation needs and user-friendly interfaces, critical events can be responded to quickly. Thus, sustainability in agricultural production is supported while water resources can be assessed more comprehensively (Vellidis et al., 2016).

The SIM800L module, which equips Arduino and many similar microcontroller boards with GSM communication and GPRS capabilities, is also widely used. Thanks to SIM800L, it is possible to make and receive calls, send and receive SMS messages, track location and control devices remotely. When Arduino-based system designs are combined with SMS integration, irrigation system control can be carried out as shown in Figure 7 (Alpay, 2021).

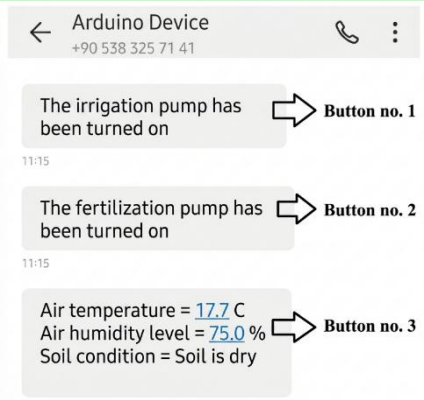



Figure 7. Warning the receiver via SMS (Alpay, 2021).

There are iOS- and Android-compatible IoT applications that enable controllers such as Arduino, Raspberry Pi, ESP32 and NodeMCU to be controlled over the internet. Among these, the Blynk application stands out by making it possible to remotely monitor and control the irrigation system.

Using the widgets on this platform, a user interface can be designed for IoT projects. Figure 8 presents the application windows of the "Smart Irrigation" user interface. In the window in Figure 8a, data received from the soil moisture sensor, time schedule settings, manual start button, lower soil moisture threshold, date-time information, Wi-Fi signal level and the moisture-time graph are shown. On the screen in Figure 8b, Twitter and e-mail notification widgets, air temperature and humidity and information on water and electricity consumption are presented (Taştan, 2019).

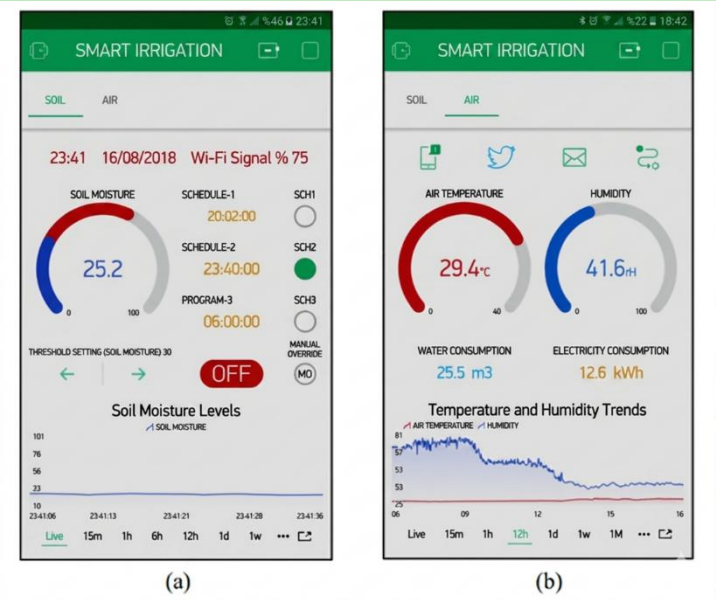



Figure 8. "Smart Irrigation" interface: a) Soil measurement b) Weather measurement (Taştan, 2019).

***Valves and Sprinkler Elements***

Valves and sprinkler system elements are the components directly responsible for irrigation processes in agricultural production. In a smart irrigation system, these components are equipped with flow control, pressure regulation and zone-based control features. In this way, water distribution can be carried out precisely and the amount of water required by agricultural crops can be supplied adequately (Şahin, 2022).

For example, in the United States, a soil water budget model was developed for apple trees grown under drip irrigation and seven different irrigation scheduling models based on feedforward and feedback approaches were proposed (Osroosh et al., 2016). Through these studies, the aim was to develop reliable irrigation methods that are cost-effective and not overly complex for apple cultivation in the arid regions of Washington (Osroosh et al., 2016).

A solenoid valve is used to activate the irrigation system depending on the moisture level and temperature in the soil.

**Operation of the Smart Irrigation System**

In the smart irrigation system, the NodeMCU controller works in an integrated manner with temperature and humidity sensors and controls the solenoid valve through the designed software, thereby activating the system. During this process, the collected data are stored in cloud infrastructure via the Blynk application. The system can be monitored and controlled through iOS and Android devices, thus enabling users to manage it remotely (Taştan, 2019). The overall structure of the system developed for smart irrigation is shown in Figure 9.

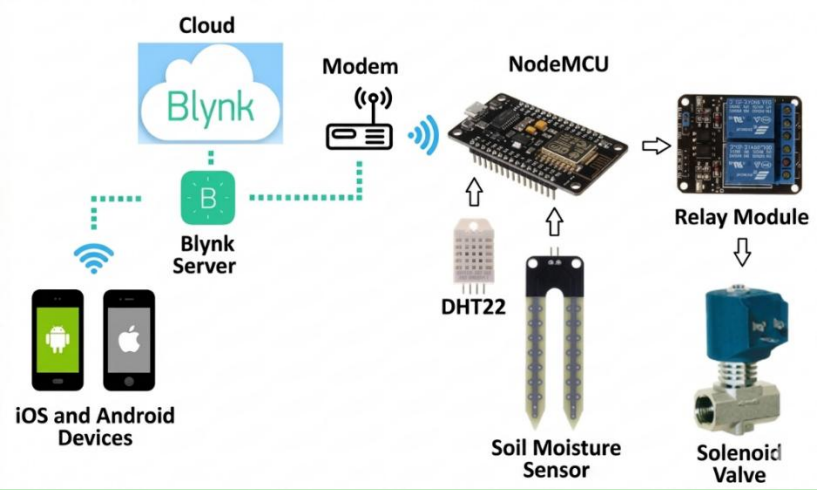



Figure 9. Structure of the Smart Irrigation System (Taştan, 2019).

Figure 10 presents the flow diagram of the "Smart Irrigation" system. In this diagram, a sample operating scenario of the system was designed. According to data received from the sensors, the "Smart Irrigation" interface obtains numerical information related to the system from the server at certain intervals. The system is managed through software and sensor control and when necessary, the user can directly intervene and direct the irrigation flow (Taştan, 2019).

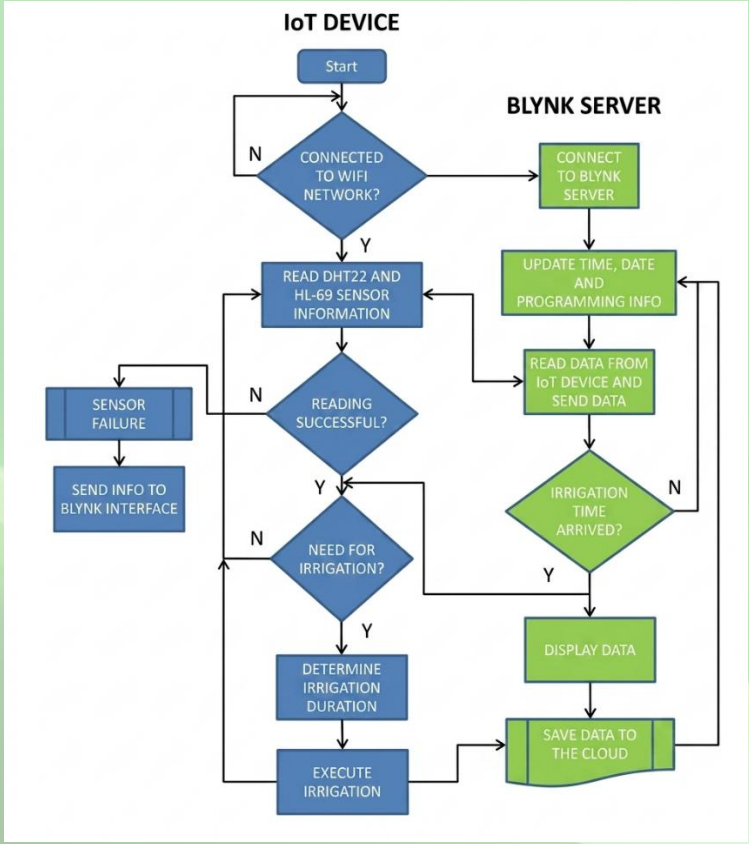



Figure 10. Flow diagram of the "Smart Irrigation" system (Taştan, 2019).

All data related to agricultural activities carried out together with the smart irrigation system are collected in cloud systems and processed through artificial intelligence technology; in this way, agricultural production processes can be improved and productivity gains can be achieved. Many parameters, from the amount of water to be delivered to the field to the amounts of fertilizer and pesticides, can be monitored within this structure.

## Conclusion

The foundation of smart irrigation systems is data analysis and software infrastructure. Data obtained from sensors and other sources are processed through advanced algorithms to generate recommendations for irrigation management. In this way, users can make more informed decisions and ensure the efficient and sustainable use of water.

With the acceleration of digitalization in agriculture, irrigation automation systems have emerged as one of the most important application areas of the innovations offered by IoT technologies. In addition to the efficient use of water resources, these systems make remarkable contributions to the agricultural sector in matters such as optimizing plant health and saving labor. These automation solutions, which can be accessed and controlled remotely over the internet, provide significant economic and environmental gains for agricultural production.

Within the framework of the opportunities offered by the Internet of Things (IoT), irrigation automation systems are expected to become smarter and fully automatic in the future. This development raises productivity and efficiency standards in agriculture (Taştan, 2019). However, smart irrigation systems, like other advanced technologies, have certain limitations. While some of these limitations stem directly from the system itself, factors such as user skills, field size, land structure, crop type, access to water sources and environmental conditions can often make the process more difficult. Continuous research, development and training programs are required to improve the design and functionality of these systems. In addition, it is of great importance for policy makers and stakeholders to act in cooperation in order to expand sustainable irrigation practices (Şahin, 2022).

While the use of smart agricultural technologies is rapidly becoming widespread around the world, it is inevitable that Turkiye will also benefit from this technological transformation. However, the small-scale and fragmented structure of lands in Turkiye, the large number of enterprises, high investment costs and limited water resources make the dissemination of smart agricultural technologies more difficult. Therefore, with the participation of the Ministry of Agriculture and Forestry, the relevant institutions of the Ministry of Industry and Technology, universities, non-governmental organizations and private sector representatives, an “Agriculture 4.0 Action Plan” should be prepared to increase the use of smart agricultural technologies (Ağızan et al., 2022).

In conclusion, smart irrigation systems enable users to access remotely located agricultural areas or regions that require continuous irrigation. In this way, water and energy resources are used more efficiently and irrigation operations are carried out only when the conditions required by the soil are met. In addition, because farmers do not need to travel to the field for irrigation purposes, workload is reduced and operational efficiency increases. The system reduces the amount of fuel spent reaching the field, labor requirements, time loss and unnecessary water consumption, thereby achieving a noticeable decrease in input costs. Moreover, optimization of irrigation processes also leads to increased crop yield. Considering this reduction in input costs, it can be said that the installation costs of smart irrigation systems can be amortized in a relatively short time.

## Declarations

### *Ethics Approval*

This study does not require ethics committee approval because it does not involve any experimental applications on human or animal subjects.

### *Author Contributions*

The authors jointly contributed to the study design, literature review, content analysis and writing of the manuscript and they together reviewed and approved the final version of the text.

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Automation Systems Used in Animal Shelters

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## Abstract

Global population growth necessitates not only an increase in food production but also the adoption of more efficient, sustainable and advanced livestock production systems. In this context, the development of automation systems used in animal shelters has emerged as a process requiring technical expertise and professional competence. These processes require the accurate analysis of data and sound decision making by taking into account critical factors such as animal health and welfare and the use of human labor. Consequently, the use of automation systems in livestock enterprises is gaining increasing importance both worldwide and in Türkiye. This study discusses the functions and benefits of advanced technologies, automation, sensors and farm applications that have been developed and implemented in livestock enterprises from past to present.

***Keywords:*** *Animal shelters, shelter automation, technologies used in animal shelters*

## Introduction

As the course of life continues, the human population of the world is also increasing. Depending on this population growth, changes are taking place in the techniques used in agricultural and livestock production in order to meet humanity's food requirements (EFSA, 2009). From this perspective, animal shelters have evolved from small scale enterprise environments into facilities with higher capacity and more advanced technology. As the number of animals has increased, the importance of larger capacity and more technologically sophisticated shelters has also grown within this sector. Shelters are constructed as closed, semi open and open systems. In Türkiye and Europe, the number of enclosed shelters is quite high and one study reported that 60% of barns in Sweden are enclosed tie stall barns (Bilgin, 2024).

Recent technological developments and the increasing number of animals have provided the necessary platform for the advancement of animal automation systems. Automation systems used in animal shelters are intended to support animals' daily lives while reducing the need for direct intervention by farmers (Buller et al., 2020). Sensors are employed within these systems to help predict and prevent diseases, increase productivity, reduce human error and improve both production performance and animal welfare. The data obtained from sensors provide significant convenience and efficiency to personnel working in animal shelters across all operational processes (Neethirajan, 2020).

Today, various automation systems and sensor technologies are widely used to improve animal welfare, monitor health and increase productivity. For example, they provide the option of real time monitoring of animal movements, feed consumption rates, sleep patterns and environmental conditions within shelters. In particular, monitoring parameters such as temperature, humidity, air quality and ammonia levels is essential for animal health. Owing to the monitoring and management of this information, farms can achieve savings in input costs (Himu & Raihan, 2024).

The widespread adoption of digital technologies in agriculture has enabled the development of technology based devices and communication tools to support farmers' work processes. In this context, innovative solutions such as ear tags, robots, sensors, global positioning systems (GPS), animal tracking systems and virtual fences are being used effectively in modern agricultural practices (Aquilani et al., 2022).

## Feeding Technologies

The main technologies used in this field are automatic calf feeding systems, precision feeding stations, feed pushing robots and feed mixing and distribution robots. Feeding occupies an important place in the daily routine. Even in modern enterprises, feeding is a highly time consuming operation. Because filling the mixing unit, transporting it to the shelters and distributing the feed are often carried out manually, the outcome largely depends on the operator's attention and care. With the use of these robots, labor is required only for preparing and cleaning the feed storage area, referred to as the "kitchen." At the same time, feeds are prepared through software assisted systems using feed mixing machines and are distributed in accordance with predetermined programs. Following feed distribution, feed pushing robots are activated to ensure that feed is distributed evenly along all rows (Figures 1–2). This process enables animals to access feed continuously and easily, thereby increasing feeding efficiency (Kaya & Örs, 2015).



Figure 1. Automated feed pushing robot (Haotun et al., 2018).



Figure 2. Feed Mixing and Distribution Machine (Erzurumlu & Şen, 2020).

**Automatic Milking Robots**

An automatic milking robot can perform the milking process more rapidly than manual milking machines and without requiring personnel; in doing so, it also eliminates the problems that arise when animals are forced to the milking area. The milking process begins when cows are trained to enter the stall structure. After the cow enters the stall, a camera is used to determine the positions of the teats. Following this procedure, the robotic arm takes the milking unit and moves it to the coordinates determined by the camera and milking is carried out under computer program control (Özüduruk, 2014). One such system is the automatic Lely Astronaut milking machine, which was introduced in the Netherlands, one of the leading countries in the agricultural sector.

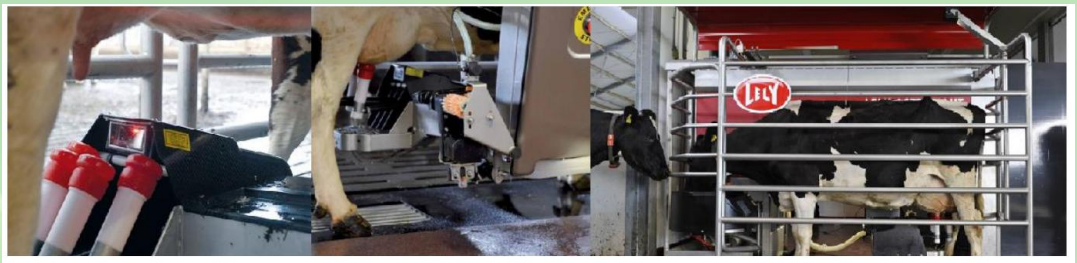



Figure 3. Automatic Milking Robots (Kaya & Örs, 2015).

**Use of Sensors in Animal Shelters**

In animal shelters, numerous sensor technologies are used both to monitor animal health and to support practices aimed at increasing productivity. Information regarding these sensors is presented below.

**Animal Health Monitoring Sensors**

Sensors used in livestock farming are devices that transmit important data related to animals such as body temperature, step count and location to farm systems instantaneously and wirelessly (Figure 4). These sensors make individual animal monitoring more efficient while simultaneously facilitating management processes. The relevant sensors offer the possibility of performing measurement-based operations rapidly and in real time. Data obtained from sensors are processed quickly, thereby enabling the detection and assessment of animal health status (Özcan, 2022).

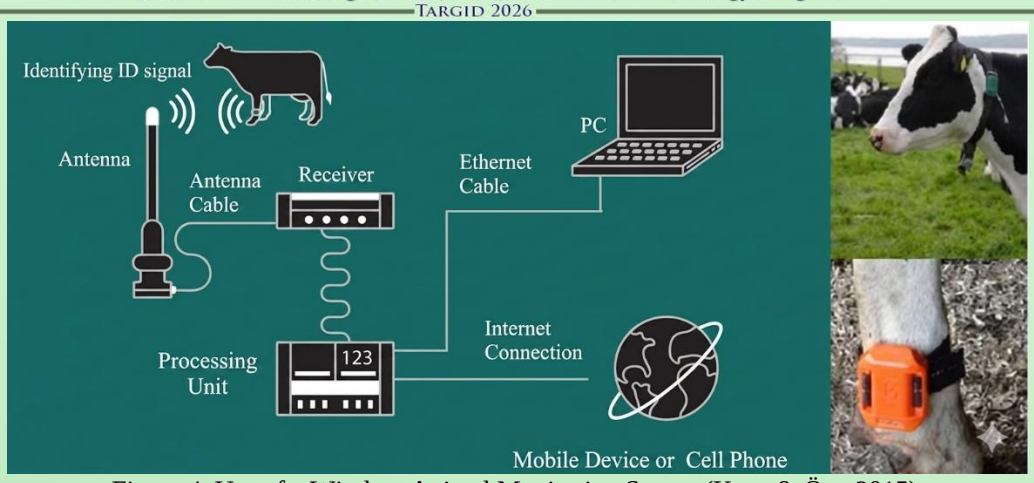


Figure 4. Use of a Wireless Animal Monitoring Sensor (Kaya & Örs, 2015).

**Ear Tags**

Monitoring animal behavior is of paramount importance for livestock health and surveillance. Accurate information regarding behavior, associated short and long term trends and variations can provide valuable insights into animal productivity, health and welfare. However, manual monitoring of animal behavior is time consuming, labor intensive, prone to human error and potentially disruptive to the animals. Consequently, with recent technological advancements, the utilization of electronic ear tags has emerged as a viable solution (Wang et al., 2023).

This approach represents the most widely adopted methodology within livestock housing. Beyond enabling individual tracking of animals within the herd, it facilitates real time, continuous monitoring of critical parameters such as estrus, pregnancy and health status directly through the system. Driven by its operational ease of use and recent technological advancements, the utilization of ear tags has emerged as a prominent choice for livestock producers (Çakmakçı et al., 2024)

In particular, the utilization of RFID and IoT based electronic ear tags stands out as a pivotal component of smart agriculture applications. These technologies enable the instantaneous monitoring of individual body temperatures and the real time localization of cattle within both pasture and shelter environments. This continuous surveillance mechanism plays a crucial role in preventing the spread of diseases across the herd by facilitating early detection (Peng et al., 2025)


Figure 5. Ear Tags (Wang et al., 2023).

When tracking location via GPS, the concurrent use of accelerometers enables the measurement of livestock's daily activities such as walking, ruminating and lying down mostly at various times of the day. This approach allows for the accurate detection of estrus and calving. Furthermore, abrupt behavioral changes can also signal the onset of parturition. Consequently, this helps farmers identify various parameters related to animal welfare, reproductive performance, health and the nutritional status of both individual animals and whole groups (Hlimi et al., 2024).

**Use of Indoor $NH_3$ Sensors in Shelters**

Since ammonia originating from livestock production can significantly contribute to environmental pollution, emissions must be mitigated across all potential sources, including housing systems, manure storage and outdoor grazing. In recent years, along with the increase in the livestock population, the atmospheric concentration of

ammonia ($NH_3$) has risen. For this reason The measurement of $NH_3$ gas one of the gases such as methane, sulfur compounds and nitrogen oxides that are released in indoor shelter environments and are undesirable above certain levels is carried out through sensors (Monteny & Erisman, 1998). In particular, $NH_3$ is a hazardous compound and is known to pose a health risk to humans and animals due to its toxic properties (Algers et al., 2009). Different types of sensors, such as metal oxide, polymer, diode laser, electrochemical, surface acoustic and field effect transistor sensors are used to measure $NH_3$ gas (Kwak et al., 2019). In shelter design, the MQ 137 gas sensor, which can measure ammonia, has been used to determine the $NH_3$ concentration of indoor air (Figure 6).

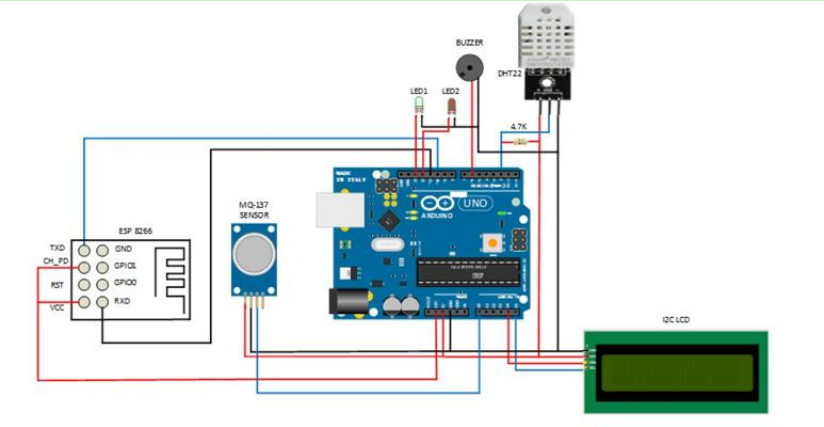



Figure 6. Circuit Diagram Used for Ammonia Measurement (Bilgin, 2024).

**Use of Virtual Fences in Animal Shelters**

Fences used in animal shelters are barrier systems designed to prevent animals from exceeding designated areas (Çakmakçı et al., 2024). These systems can also be created virtually by taking advantage of the capabilities offered by fixed or adjustable technologies. In particular, in virtual fencing systems, audible and electrical warning mechanisms integrated into sensors attached to the animal help redirect the animal when it violates designated boundaries. Operating through a two stage warning system, the technology first provides an audible warning when the animal crosses the fence boundary. If the animal does not heed this warning and continues to cross the boundary, a mild electrical stimulus is applied at a non harmful level to enable the animal to change direction (Jachowski et al., 2014; Lomax et al., 2019; McSweeney et al., 2020).

**Microcontroller Board**

A microcontroller board is used to evaluate $NH_3$ and sensor data measurements. The Arduino board is preferred for this purpose. Its ease of use and the fact that it does not require specialized hardware for programming distinguish it from other platforms, while also making it a popular microcontroller board compatible with different types of projects (Bilgin, 2024).

**Temperature and Humidity Sensor**

Temperature and humidity are among the most important parameters that must be monitored in animal shelters. Because they directly affect animal health, they should be tracked continuously. From this perspective, sensors are used to measure ambient temperature and humidity. One of the most widely used sensors for this purpose is the DHT22 temperature and humidity sensor. The DHT22 is an advanced sensing unit that provides a calibrated digital signal output. It is highly reliable and exhibits stable operating characteristics (Liu, 2013).

**Body Weight and Condition Measurement Devices**

***Condition Scoring***

Periodic assessment of body condition in dairy cattle makes it possible to identify body energy reserves, which are directly associated with health, reproductive performance and productivity and thereby enables effective herd management. Camera systems, usually positioned above the corridor leading to the milking system, capture top view images of animals' bodies, which can then be analyzed through specialized software on a computer, as shown in Figure 7 (Kaya & Örs, 2015).




Figure 7. Body Condition Measurement (Kaya & Örs, 2015).

Scoring is performed according to the fullness between the spinal ridge and the transverse processes along the back. On 5 point scale, one body condition score (BCS) corresponds to approximately 40 kg live weight for Holstein cattle and 25 kg for Jersey cattle (Ministry of Agriculture and Forestry, 2017; Erdal and Ergüzen, 2020). In this context, the DHT22 sensor is widely preferred for temperature and humidity measurements because it offers high reliability, calibrated digital output and stable operating performance; the physical structure of the sensor is presented (Liu, 2013).

***Weight Measurement***

Together with condition scoring, the weight of an animal is an important indicator of its health status and value. However, only a small number of breeders weigh their animals regularly because the process requires substantial labor and appropriate weighing equipment is often unavailable (Frost et al., 1997).

The daily body weight measurement system functions as an important indicator in the evaluation of body condition score and energy balance. It generally performs the weighing of animals automatically through an electronic scale system placed on the path leading to the milking area (Figure 8). When the animal passes over the scale, the weight information is transmitted to and recorded in the computer together with the animal's identification number (Kaya & Örs, 2015).

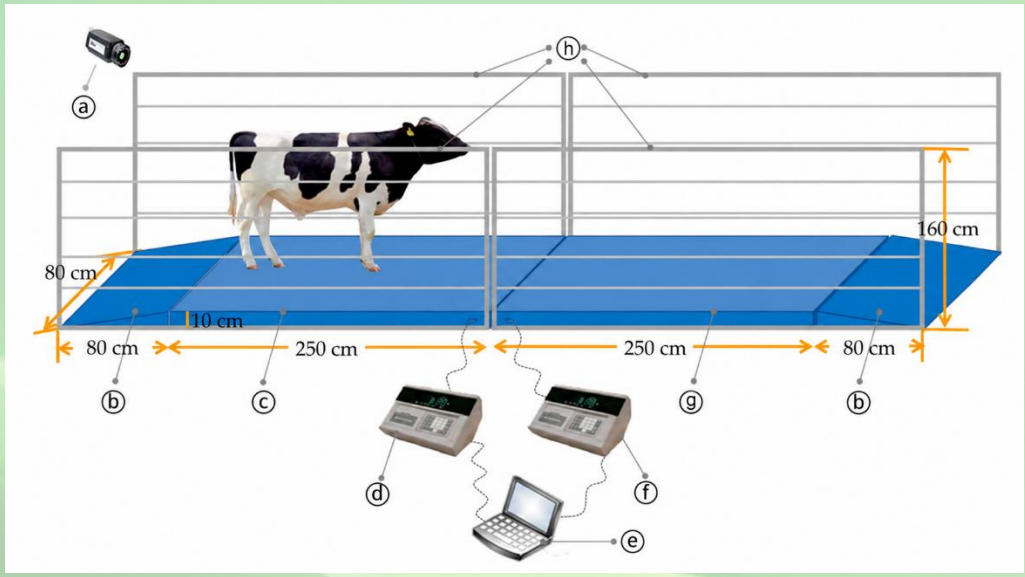



Figure 8. Cow weighing diagram: (a) camera, (b) slope, (c) dynamic weighing platform, (d) dynamic weighing indicator, (e) computer, (f) static weighing indicator, (g) static weighing platform and (h) guardrail (Yan et al., 2020).

**Farm Management by Computer**

Real time monitoring of animals has become possible thanks to advances in computer technologies and increasing data storage capacities. Today, computer programming technology has begun to be used extensively in animal shelters for data collection, analysis and the development of decision support systems. Applications of this technology in the livestock sector are also increasing rapidly (Li et al., 2021).

Monitoring of farm animals has become possible through Computer based programming systems integrated with fixed and mobile devices. Among fixed systems, cameras placed on shelter ceilings, passage points, or specific areas are prominent whereas mobile systems can monitor and analyze animal movement through technologies such as rail mechanisms, robots and drones (Çakmakçı et al., 2024). Through recording devices, images or videos in various forms and formats are captured and transmitted to the system software. Computers or cloud computing servers are used to process the collected data. Analyses carried out on these data enable operations such as identification, classification and modeling with the aid of machine learning algorithms (Li et al., 2021; Narendra & Hareesh, 2010).

***Internet of Things (IoT)***

These systems are also described as integrated sensor object and smart device based systems supported by artificial intelligence, in which components can communicate and operate with one another without the need for direct human supervision (Ambrosin et al., 2016). The use of IoT in livestock farming is a precision system employed to perform analytical measurements in order to optimize enterprise decisions (Abdullahi et al., 2020). IoT use in livestock production can be further expanded particularly in rural areas where herd grazing is practiced and animals are more likely to become lost, as it enables effective animal monitoring (Dlodlo & Kalezhi, 2015). In summary, IoT based devices are used in the management of data collection, analysis and decision-making processes related to farm animals.

***Smart Dairy Farms***

Smart dairy farm applications are emerging as an important solution to meet the increasing global demand for high quality dairy products (Akbar et al., 2020). The use of advanced data analytics and sensor technologies helps dairy farms become less affected by environmental factors while also enabling improvements in animal health. Especially since 2015, milk has become one of the most demanded products in the global market and one of the animal based food sources most affected by price fluctuations (Akbar et al., 2020).

Across the world, many dairy farms use these technologies not only to increase productivity but also to transition more easily to sustainable agricultural production methods. For example, smart dairy farms equipped with IoT based sensors and automation systems in countries such as the Netherlands and Germany both reduce production costs and enhance quality (Akbar et al., 2020).

The table 1 below presents the status of milk production and milk demand in nine different countries. When these data are evaluated, global milk consumption can be interpreted in relation to market significance as well as regional differences between production volume (tons) and milk demand (million tons).

Table 1. Milk Production and Demand Amounts of Countries (Akbar et al., 2020).

| *Country* | *Milk Production (Million Tons)* | *Milk Demand (Million Tons)* |
|---|---|---|
| United States | 3,4 | 14,6 |
| Germany | 1,6 | 4,9 |
| France | 2 | 3,9 |
| New Zealand | 1,8 | 2,8 |
| China | 4,7 | 6 |
| India | 16,5 | 8,4 |
| Brazil | 8,7 | 5,3 |
| Pakistan | 3,8 | 2,1 |
| Bangladesh | 1,5 | 2 |

**Conclusion**

The objectives of automation system applications used in animal shelters can generally be summarized as follows (Kopuzlu, 2023; Bewley, 2008):

- Reduction of the breeder's physical and psychological workload,
- Improvement of the enterprise's level of success and reduction of risk,
- The most efficient use of enterprise resources,
- Ensuring the highest possible compatibility of input factors with animals' requirements,
- Providing support to humans in herd management tasks and the early diagnosis of diseases,
- Minimizing drug use through early diagnosis and preventive measures,
- Maximizing the utilization of each animal's individual potential.

The benefits obtained from automation systems used in animal shelters can be stated as increased productivity, reduced costs, improved animal health and welfare, minimization of the effects of adverse environmental factors and more efficient management of production processes (Aydin, 2021).

These systems bring about substantial changes in health, reproduction and quality control. Especially in shelters where large groups of animals are managed, it may be difficult to examine each animal individually; however,

through the data collection and analysis processes provided by automation systems, exceptions can be detected rapidly and managed more easily (Aydin, 2021). For this reason, while the care of animals and the monitoring of their health status can be followed more easily, the utilization of products obtained from animals can also be carried out more efficiently (Aydin, 2021).

Automation systems used in animal shelters make it possible to collect a wide range of data, including individual feeding, regular health monitoring, movement tracking, analysis of lying behavior, body temperature, respiratory rate and heart rate. These systems collect data at the individual animal level through devices such as pedometers, pressure plates and temperature and humidity sensors. In addition, important indicators of health and performance, such as rumination duration, locomotion ability and animal behaviors, can be monitored through image analysis. The data provided by automation systems enable the development of a proactive care strategy focused on animal health, thereby supporting the early detection of diseases and more effective herd management.

A well planned and properly maintained lighting system can also yield rapid benefits in animal shelters, both in terms of animal performance and for shelter personnel (Demir et al., 2020).

## Declarations

### *Ethics Approval*

This study does not require ethics committee approval, as it does not involve any experimental application on human or animal subjects.

### *Author Contributions*

The authors jointly contributed to the study design, literature review, content analysis and manuscript preparation and they reviewed and approved the final version of the text together.

### *Conflict of Interest*

There is no conflict of interest among the authors.

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*ID: 820*

# Impact of various plant protein isolates (lupin, chickpea, pea, and soy) on the quality parameters of vegan mayonnaise

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## Abstract

This study evaluated the effects of various plant protein isolates (including lupin, chickpea, pea, and soy) used as egg yolk substitutes on the physicochemical, rheological, and textural properties of vegan mayonnaise and compared these formulations with conventional mayonnaise. Various methods were used to assess emulsion quality, including pH, stability, colorimetric analysis (L*, a*, b*), apparent viscosity, and textural profile analysis. The findings indicated that the control group exhibited the most stable emulsion, with 96.74% stability and a maximum apparent viscosity of 4.85 Pa·s. Among plant isolates, soy protein nearly matched egg yolk thanks to its strong network of hydrophobic amino acids, with 92.49% emulsion stability and 81.39 g textural behavior. Lupin protein showed a color profile (b* = 25.61) similar to egg yolk because of its pigments and effectively preserved structural integrity with 90.96% emulsion stability. Chickpea and pea proteins showed emulsion stabilities of 86.21% and 78.53%, respectively, but formed a more fluid emulsion matrix with lower viscosities of 1.32 Pa.s and 1.27 Pa.s. The pH values of all analyzed formulations ranged from 3.63 to 4.04, remaining within an acceptable acidic range designed to ensure microbial stability. In conclusion, soy and lupin proteins provide innovative structural and aesthetic alternatives for the production of vegan mayonnaise.

***Keywords:*** *Vegan Mayonnaise, Plant Protein Isolates, Emulsion Stability, Rheological Properties, Texture Profile Analysis.*

**Çeşitli bitkisel protein izolatlarının (acı bakla, nohut, bezelye ve soya) vegan mayonezin kalite parametreleri üzerindeki etkisi**

## Özet

Bu çalışma, yumurta sarısı yerine kullanılan çeşitli bitki proteini izolatlarının (acı bakla, nohut, bezelye ve soya dahil) vegan mayonez formülasyonlarının fizikokimyasal, reolojik ve dokusal özellikleri üzerindeki etkilerini değerlendirdi ve bu formülasyonları geleneksel mayonezle karşılaştırdı. Emülsiyon kalitesini değerlendirmek için pH, stabilite, kolorimetrik analiz (L*, a*, b*), görünür viskozite ve dokusal profil analizi dahil olmak üzere çeşitli yöntemler kullanıldı. Bulgular, kontrol grubunun %96.74 stabilite ve 4.85 Pa·s'lik maksimum görünür viskozite ile en stabil emülsiyonu sergilediğini gösterdi. Bitki izolatları arasında, soya proteini, güçlü hidrofobik amino asit ağı sayesinde %92.49 emülsiyon stabilitesi ve 81.39 g dokusal davranış ile yumurta sarısına neredeyse eşdeğerdi. Acı bakla proteini, pigmentleri nedeniyle yumurta sarısına benzer bir renk profili (b* = 25.61) gösterdi ve %90.96 emülsiyon stabilitesi ile yapısal bütünlüğünü etkili bir şekilde korudu. Nohut ve bezelye proteinleri sırasıyla %86.21 ve %78.53 oranında emülsiyon stabilitesi göstermiş, ancak 1.32 Pa.s ve 1.27 Pa.s daha düşük viskoziteye sahip daha akışkan bir emülsiyon matrisi oluşturmuştur. Analiz edilen tüm formülasyonların pH değerleri 3.63 ile 4.04 arasında değişmiş ve mikrobiyal stabiliteyi sağlamak için tasarlanmış kabul edilebilir asidik aralıkta kalmıştır. Sonuç olarak, soya ve acı bakla proteinleri, vegan mayonez üretimi için yenilikçi yapısal ve estetik alternatifler sunmaktadır.

***Anahtar Kelimeler:*** *Vegan Mayonez, Bitkisel Protein İzolatları, Emülsiyon Stabilitesi, Reolojik Özellikler, Doku Profili Analizi.*

*ID: 821*

# Artificial intelligence and machine learning for texture and sensory optimization in plant-based meat analogs: A review

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## Abstract

In the production of plant-based meat analogs, mimicking the characteristic texture (toughness, elasticity, fibrous structure) and sensory properties of real meat is a critical technological challenge in food science. Traditional formulation development and extrusion processes are often based on time-consuming, resource-intensive, and costly trial-and-error methods. The aim of this systematic review is to evaluate the optimization potential of artificial intelligence and machine learning algorithms in plant-based meat design and to highlight the advantages they offer compared to traditional methods (e.g., Response Surface Methodology). A review of the current literature shows that machine learning models successfully model the complex and nonlinear relationships between input components and the textural quality of the final product. Neural networks such as decision tree-based algorithms (Random Forest, XGBoost) and Multilayer Perceptrons have been found to provide very high accuracy in texture and rheology prediction. In particular, Multi-Objective Bayesian Optimization has proven to significantly reduce the number of experimental trials required to achieve the target tissue profile compared to traditional methods, maximizing process efficiency. Furthermore, the integration of these algorithms with non-destructive "Green Analytics" tools such as hyperspectral imaging and electronic nose (e-nose) has been shown to support environmental sustainability by reducing sample waste and chemical waste by up to 90%. Consequently, the integration of artificial intelligence and machine learning goes beyond traditional input-to-output approaches in plant-based meat production, enabling the "Inverse Design" vision where the targeted tissue is rationally constructed directly using algorithms. This allows for the much faster development of innovative food formulations that increase resource efficiency and meet consumer expectations.

***Keywords:*** *Plant-Based Meat Analogs, Artificial Intelligence, Machine Learning, Tissue Optimization, Reverse Design.*

**Bitkisel bazlı et analoglarında doku ve duyusal optimizasyon için yapay zeka ve makine öğrenimi: Bir inceleme**

## Özet

Bitkisel bazlı et analoglarının üretiminde, gerçek etin karakteristik dokusunu (sertlik, esneklik, lifli yapı) ve duyusal özelliklerini taklit etmek, gıda biliminde kritik bir teknolojik zorluktur. Geleneksel formülasyon geliştirme ve ekstrüzyon süreçleri genellikle zaman alıcı, kaynak yoğun ve maliyetli deneme-yanılma yöntemlerine dayanmaktadır. Bu incelemenin amacı, bitkisel bazlı et tasarımında yapay zeka ve makine öğrenmesi algoritmalarının optimizasyon potansiyelini değerlendirmek ve geleneksel yöntemlere (örneğin, Yanıt Yüzey Metodolojisi) kıyasla sundukları avantajları vurgulamaktır. Mevcut literatürün incelenmesi, makine öğrenmesi modellerinin girdi bileşenleri ile nihai ürünün dokusal kalitesi arasındaki karmaşık ve doğrusal olmayan ilişkileri başarıyla modellediğini göstermektedir. Karar ağacı tabanlı algoritmalar (Random Forest, XGBoost) ve Çok Katmanlı Algılayıcılar gibi sinir ağlarının, doku ve reoloji tahmininde çok yüksek doğruluk sağladığı bulunmuştur. Özellikle, Çok Amaçlı Bayes Optimizasyonu, geleneksel yöntemlere kıyasla hedef doku profilini elde etmek için gereken deneysel deneme sayısını önemli ölçüde azaltarak süreç verimliliğini en üst düzeye çıkarmıştır. Dahası, bu algoritmaların hiperspektral görüntüleme ve elektronik burun (e-burun) gibi tahribatsız "Yeşil Analiz" araçlarıyla entegrasyonunun, numune atığını ve kimyasal atığı %90'a kadar azaltarak çevresel sürdürülebilirliği desteklediği gösterilmiştir. Sonuç olarak, yapay zeka ve makine öğreniminin entegrasyonu, bitki bazlı et üretiminde geleneksel girdi-çıktı yaklaşımlarının ötesine geçerek, hedef dokunun algoritmalar kullanılarak doğrudan rasyonel bir şekilde oluşturulduğu "Ters Tasarım" vizyonunu mümkün kılmaktadır. Bu, kaynak verimliliğini artıran ve tüketici beklentilerini karşılayan yenilikçi gıda formülasyonlarının çok daha hızlı geliştirilmesine olanak tanımaktadır.

***Anahtar Kelimeler:*** *Bitki Bazlı Et Analogları, Yapay Zeka, Makine Öğrenimi, Doku Optimizasyonu, Ters Tasarım.*

*ID: 822*

# Preliminary Findings on Nest Ecology, Reproductive Parameters, and Anthropogenic Waste in Yellow-legged Gulls (*Larus michahellis*) Breeding on Akliman Gull Island, Sinop

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## Abstract

The yellow-legged gull (*Larus michahellis*) is an opportunistic seabird that exhibits high ecological flexibility in its feeding and nest-building behaviours. Owing to these characteristics, it frequently comes into contact with anthropogenic waste in coastal and aquatic habitats, landfill sites, harbour areas, and urban feeding grounds where human influence is intense. In particular, materials such as plastic, paper, string, fishing line, fabric fragments, packaging waste, wet wipes, and similar debris are known to be directly ingested by gulls or used as nesting material. These materials may pose several risks to chicks, including entanglement, injury, restricted mobility, infection, ingestion, impaired development, and, in severe cases, mortality. In addition, the fragmentation of plastics into macroplastics and microplastics under environmental conditions may increase the risk of physical, chemical, and microbial exposure for both adult individuals and chicks. Therefore, the study conducted on Akliman Gull Island aims to evaluate, at the level of preliminary findings, the possible effects of anthropogenic materials found in nests on chick health and nest ecology, together with basic reproductive parameters. Within the scope of the project, observational studies began in mid-February, while systematic field studies started at the end of March, and the study is still ongoing. Based on its habitat characteristics, Gull Island was divided into three main habitat types: wooded, grassy, and rocky areas. By the field survey conducted at the end of May, a total of 154 nests, 368 eggs, and 146 gull chicks had been recorded on the island. In addition, 52 dead eggs were documented. During field observations, cannibalistic behaviour was commonly observed on the island, particularly attacks by neighbouring adult individuals on chicks located in other nests or near nest sites. In this process, the number of detectable dead chicks was determined as 19. As the study continues, these data will be evaluated together in relation to habitat type, nest characteristics including length, width, and depth, egg parameters including width, length, and weight, chick parameters including body weight, and the presence of anthropogenic waste. This project is supported by the Scientific Research Projects Coordination Unit of Sinop University under project code EĞTF-1901-25-010.

***Keywords:*** *Gull, Anthoropogenic waste, Pollution, Macroplastic, Microplastic, Bird Health*

*ID: 823*

# Sustainable Livestock Production in the Context of the European Green Deal

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## Abstract

Climate change is one of the major environmental challenges affecting natural resources, ecosystems, and food production systems worldwide. Livestock production plays a strategic role in food security and rural development; however, it also contributes to greenhouse gas emissions through enteric fermentation, manure management, feed production, energy consumption, and waste generation. This review evaluates the contribution of the livestock sector to greenhouse gas emissions within the framework of the European Green Deal and assesses the potential of sustainable livestock production practices to reduce emissions and enhance environmental sustainability. To this end, climate change, livestock-related greenhouse gas emissions, and sustainable production approaches were examined, with particular emphasis on sustainable pasture management, feeding strategies, manure management, renewable energy use, and resource-use efficiency. The available evidence indicates that methane emissions, declining pasture productivity, drought, rising temperatures, and increasing pressure on water resources pose significant threats to the sustainability of livestock production systems. Given its substantially higher global warming potential than carbon dioxide, methane has become a major focus of climate mitigation efforts. The European Green Deal and the Farm to Fork Strategy promote the transition toward more sustainable and resource-efficient livestock production systems. Within this framework, sustainable pasture management, high-quality forage production, efficient feed utilization, improved animal nutrition strategies, manure and waste management, renewable energy use, and enhanced resource-use efficiency have emerged as important practices for reducing environmental impacts. The review highlights that reducing greenhouse gas emissions while maintaining production efficiency is essential for the long-term sustainability of livestock systems. Consequently, sustainability-oriented livestock policies and production practices are critical for environmental protection, conservation of natural resources, and future food security.

***Keywords:*** *European Green Deal; sustainable livestock production; greenhouse gas emissions; methane emissions; climate change; livestock production systems*

## Introduction

Climate change is currently recognized as one of the most significant global challenges, with profound environmental, economic, and social implications for humanity. Since the Industrial Revolution, increased fossil fuel consumption, rapid population growth, urbanization, industrialization, deforestation, and the unsustainable use of natural resources have led to a rise in the concentration of greenhouse gases in the atmosphere. Greenhouse gases such as carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) contribute to global warming, resulting in rising temperatures, drought, extreme weather events, biodiversity loss, and reductions in water resources (IPCC, 2023). These developments have significantly affected the agricultural sector, which is highly dependent on natural resources, particularly livestock production systems.

Agriculture is considered not only one of the sectors most affected by climate change but also one of the major sources of global greenhouse gas emissions. Agricultural and food systems accounted for approximately 32% of global greenhouse gas emissions in 2023 (FAO, 2025). Livestock production, an important component of the agricultural sector, contributes substantially to greenhouse gas emissions through processes such as enteric fermentation, manure management, feed production, fertilizer use, and energy consumption (Bellarby et al., 2012; Nugrahaeningtyas et al., 2024). In particular, methane emissions from ruminants contribute substantially to climate change because of methane's high global warming potential (Gerber et al., 2013). Despite these environmental impacts, the livestock sector is of strategic importance for ensuring food security, sustaining rural livelihoods, and supporting agricultural economies (Steinfeld et al., 2006).

In this context, sustainable livestock production is defined as a holistic approach that integrates environmental, economic, and social dimensions while aiming to reduce environmental impacts, conserve natural resources, and maintain production efficiency (Dumont et al., 2013).

The European Green Deal, introduced by the European Union (EU) as part of its efforts to combat climate change, represents a comprehensive transformation policy aimed at achieving net-zero greenhouse gas emissions by 2050 (European Commission, 2019). The Farm to Fork Strategy, developed within the framework of the European Green Deal, aims to establish sustainable food systems, reduce environmental impacts, conserve natural resources, and promote environmentally sustainable production practices (European Commission, 2020a).

Accordingly, the development and implementation of sustainable production strategies in livestock farming are becoming increasingly important for environmental sustainability. This study aims to assess the contribution of the livestock sector to greenhouse gas emissions within the framework of the European Green Deal and to examine the potential contributions of sustainable livestock practices to emission reduction and environmental sustainability. In this regard, the roles of practices such as sustainable pasture management, feeding strategies, manure management, renewable energy use, and resource use efficiency in the livestock sector are reviewed.

## Climate Change and Greenhouse Gas Emissions

### *Global Climate Change*

Global climate change refers to long-term changes in temperature, precipitation patterns, and climatic events associated with increased concentrations of greenhouse gases in the atmosphere. In particular, the increased use of fossil fuels following the Industrial Revolution, together with industrialization, urbanization, deforestation, and the intensive use of natural resources, has led to increased concentrations of greenhouse gases in the atmosphere and accelerated global warming (IPCC, 2023). While global warming refers to the increase in the Earth's average temperature, climate change encompasses the environmental, ecological, and socio-economic changes resulting from this temperature increase (Diffenbaugh & Burke, 2019; IPCC, 2023).

The greenhouse effect, one of the fundamental components of the climate system, occurs when certain gases in the atmosphere allow incoming shortwave solar radiation to pass through while retaining part of the longwave radiation emitted from the Earth's surface. Although the natural greenhouse effect is essential for sustaining life on Earth, the increase in greenhouse gas concentrations resulting from human activities disrupts the natural balance of this process and accelerates global warming. The major greenhouse gases in the atmosphere are carbon dioxide, methane, nitrous oxide, and fluorinated gases (IPCC, 2023). The mechanism of the greenhouse effect and the major radiation processes in the atmosphere are schematically illustrated in Figure 1.

Climate change leads to major environmental problems, including rising temperatures, drought, extreme weather events, floods, reductions in water resources, biodiversity loss, and ecosystem degradation. These environmental changes have significant impacts on natural resources, ecosystems, and human life. Therefore, reducing greenhouse gas emissions and promoting sustainable production models have become increasingly important for mitigating climate change.

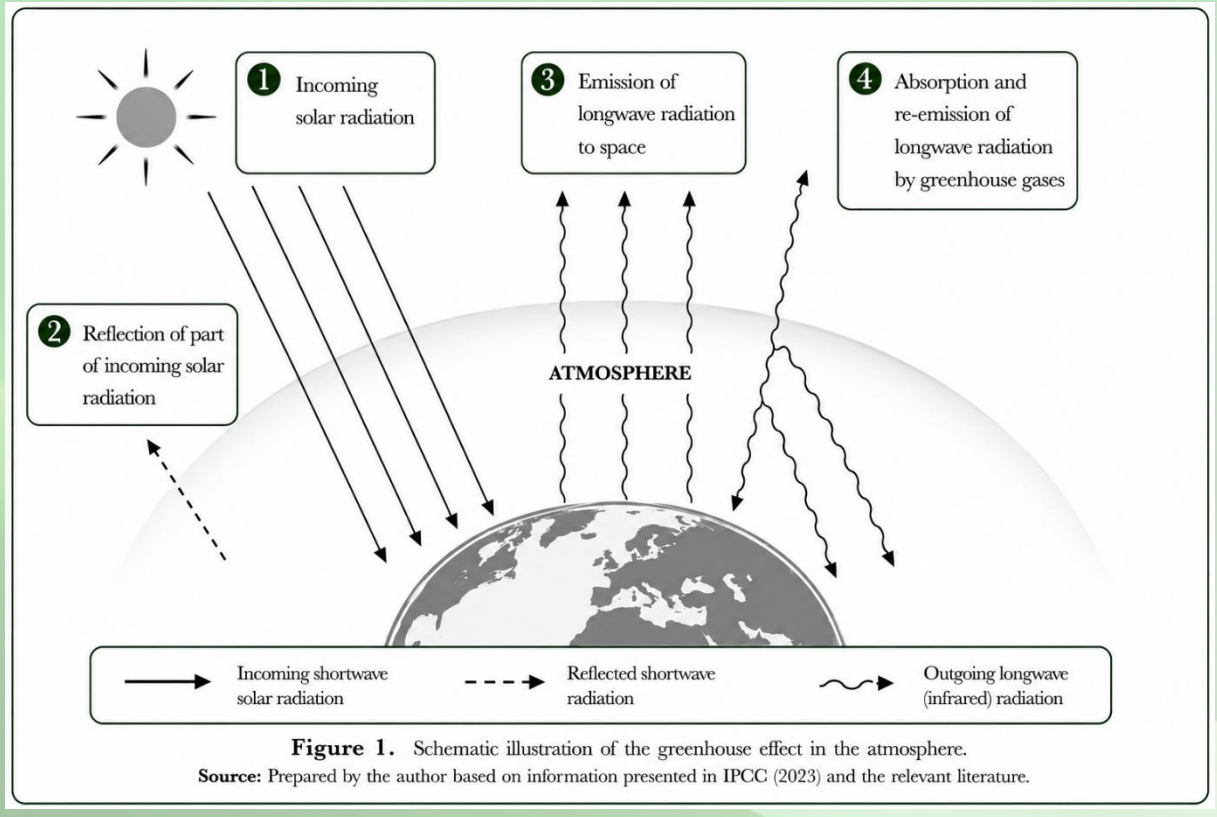



Figure 1. Schematic illustration of the greenhouse effect in the atmosphere.

***Greenhouse Gases and Their Sources***

Greenhouse gases differ in terms of their global warming potential, atmospheric lifetimes, and emission sources. Most greenhouse gas emissions originate from anthropogenic activities, including energy production, industrial processes, transportation, agriculture, and land-use change (FAO, 2025).

Carbon dioxide is the greenhouse gas that accounts for the largest share of global greenhouse gas emissions. The major sources of carbon dioxide emissions include fossil fuel combustion, energy production, industrial activities, and deforestation (IPCC, 2023). In particular, the increasing use of coal, oil, and natural gas in the energy sector has led to higher atmospheric carbon dioxide concentrations. In addition, land-use changes and the reduction of forest areas weaken carbon sinks.

Although its atmospheric concentration is lower than that of carbon dioxide, methane is one of the major greenhouse gases because of its high global warming potential. The main sources of methane emissions include fossil fuel production, waste management, rice cultivation, and livestock production. In the livestock sector, most methane emissions originate from enteric fermentation and manure management (Nugrahaeningtyas et al., 2024).

Nitrous oxide is one of the major greenhouse gases because of its high global warming potential and long atmospheric lifetime. A large proportion of nitrous oxide emissions originates from agricultural activities. In particular, chemical fertilizer use, manure application, and soil management practices contribute to increased nitrous oxide emissions (IPCC, 2023). Excessive nitrogen use in agriculture further increases soil-derived nitrous oxide emissions.

***Contribution of Agriculture to Greenhouse Gas Emissions***

Agricultural activities contribute significantly to global greenhouse gas emissions through energy use, chemical fertilizer application, irrigation, land-use change, and livestock production (IPCC, 2023). In recent years, agricultural and food systems have accounted for an increasing share of global greenhouse gas emissions. According to FAO (2025), greenhouse gas emissions from agricultural and food systems account for approximately 32% of global emissions.

Carbon dioxide emissions mainly originate from agricultural mechanization, energy use, and land-use changes. In addition, rice cultivation, livestock production, and waste management contribute to increased methane emissions, whereas chemical fertilizer use and soil management practices lead to increased nitrous oxide emissions (IPCC, 2023).

Livestock production is considered one of the major contributors to agricultural greenhouse gas emissions. In particular, livestock production accounts for a substantial share of agricultural emissions, and its environmental impacts are mainly associated with processes such as enteric fermentation, manure management, feed production, and energy use (Bellarby et al., 2012; Nugrahaeningtyas et al., 2024).

In line with global trends, the agricultural sector is an important source of greenhouse gas emissions in Türkiye. As of 2024, the agricultural sector accounted for 12.6% of the country's total greenhouse gas emissions (TURKSTAT, 2026). Among agricultural emissions, enteric fermentation and manure management, particularly those associated with cattle production, are the major sources of emissions. This situation highlights the importance of promoting sustainable livestock practices in Türkiye.

**Environmental Impacts of the Livestock Sector**

***Greenhouse Gas Emissions from Livestock Production***

Livestock production is considered one of the major drivers of climate change because of its substantial contribution to global greenhouse gas emissions. In particular, enteric fermentation, manure management, feed production, and land-use change are among the major sources of emissions from livestock production systems (Gerber et al., 2013; Herrero et al., 2016; Nugrahaeningtyas et al., 2024). These processes result in different levels of greenhouse gas emissions depending on the characteristics of the production system and management practices.

Methane accounts for a substantial proportion of greenhouse gas emissions from livestock production. Most methane emissions from livestock originate from enteric fermentation. Methanogenic microorganisms in the rumen produce methane by utilizing hydrogen and carbon dioxide generated during carbohydrate fermentation (Janssen & Kirs, 2008). Most of the methane produced is released into the atmosphere primarily through eructation. Most enteric methane is produced in the rumen (Getabalew et al., 2019). In addition, enteric fermentation accounts for a considerable proportion of global anthropogenic methane emissions (FAO, 2023).

Enteric methane production is influenced by several factors, including animal species, feed intake level, diet composition, feed digestibility, and production system (Thornton, 2010; Beauchemin et al., 2022). In particular, prolonged ruminal fermentation associated with low-quality forages may increase methane production. In contrast, highly digestible feeds, appropriate feed processing methods, and balanced diets can contribute to reducing enteric methane emissions (Beauchemin et al., 2022).

Globally, a substantial proportion of livestock-related greenhouse gas emissions originates from cattle production systems. In particular, dairy and beef cattle production systems occupy a dominant position within the sector because of methane emissions associated with enteric fermentation (FAO, 2017; Getabalew et al., 2019).

The relative contributions of ruminant species to methane emissions from enteric fermentation and manure management are presented in Figure 2. As shown in the figure, methane emissions from ruminants are predominantly derived from enteric fermentation. Total methane emissions from enteric fermentation are approximately 101.6 Mt $CH_4$ $year^{-1}$, of which 78% is attributed to cattle, 11% to buffaloes, 7.2% to sheep, and 3.8% to goats. In contrast, total methane emissions from manure management are approximately 17.5 Mt $CH_4$ $year^{-1}$, with cattle accounting for 57%, buffaloes for 18%, sheep for 15%, and goats for 10% of total emissions.

These findings indicate that approximately 85% of methane emissions from ruminants originate from enteric fermentation, whereas the remaining 15% is associated with manure management. The dominant contribution of cattle production to both emission sources suggests that methane mitigation strategies implemented in cattle production systems may provide greater emission reduction potential. The higher enteric methane production observed in cattle is associated with their larger body size, higher feed intake, and greater rumen volume (Broucek, 2014).

**A. Methane Emissions from Enteric Fermentation**

| | Cattle | Buffalo | Sheep | Goat |
|---|---|---|---|---|
| Contribution (%) | 78% | 11% | 7.2% | 3.8% |
| Annual methane emissions (Mt $CH_4$ $year^{-1}$) | 79.0 | 11.5 | 7.3 | 3.8 |

**B. Methane Emissions from Manure Management**

| | Cattle | Buffalo | Sheep | Goat |
|---|---|---|---|---|
| Contribution (%) | 57% | 18% | 15% | 10% |
| Annual methane emissions (Mt $CH_4$ $year^{-1}$) | 10.0 | 3.2 | 2.6 | 1.7 |

**Figure 2.** Methane emissions from enteric fermentation and manure management in ruminants.
(Relative contributions and absolute values in the global total)
**Note:** Mt $CH_4$ $year^{-1}$ = million tonnes of methane per year.
**Source:** FAO (2023). Global Livestock Environmental Assessment Model (GLEAM 3.0).

Figure 2. Methane ($CH_4$) emissions from enteric fermentation and manure management in ruminants.

These results indicate that priority should be given to reducing enteric fermentation-related emissions in greenhouse gas mitigation strategies for the livestock sector. In this context, improving feed quality, optimizing diet composition, and using methane-reducing feed additives are among the most promising approaches for reducing enteric methane emissions. In addition, biogas technologies, composting practices, and advanced manure management systems can contribute to reducing manure-related methane emissions.

Another important source of emissions from livestock production is manure management. Methane is produced as a result of the anaerobic decomposition of organic matter during the storage of animal feces and urine. In addition, nitrification and denitrification processes occurring after manure application contribute significantly to nitrous oxide emissions (IPCC, 2023). In particular, liquid manure storage systems and high nitrogen contents contribute to increased greenhouse gas emissions, especially nitrous oxide emissions.

The environmental impacts of livestock production systems are not limited to direct emissions but are also influenced by indirect processes, including feed production, energy use, and land-use change (Bellarby et al., 2012; Herrero et al., 2016).

***Feed Production and Resource Use***

Livestock production systems exert significant environmental pressures on natural resources because of activities such as feed production, energy use, water consumption, and land use. Globally, approximately 26% of the ice-free terrestrial surface is used for grazing, while nearly one-third of cropland is devoted to feed production (Steinfeld et al., 2006). Increasing demand for animal products, increased feed production, and the widespread adoption of intensive production systems have increased pressure on natural resources (Herrero et al., 2016).

Feed production is considered one of the key factors determining the environmental impacts of livestock production systems. Chemical fertilizers, irrigation practices, and agricultural mechanization used in feed crop production result in substantial energy consumption and greenhouse gas emissions (de Vries & de Boer, 2010). In addition, the expansion of agricultural land for feed production may lead to land-use changes, resulting in ecosystem degradation, reductions in carbon sinks, and increased greenhouse gas emissions (Leip et al., 2015).

Energy use in livestock production is associated with processes related to feed production, mechanization, housing systems, and production infrastructure. In particular, increased energy use in intensive production systems contributes to greater fossil fuel consumption and associated carbon dioxide emissions. Energy requirements vary depending on the production system, farm size, and technologies used (de Vries & de Boer, 2010). Therefore, improving energy efficiency and promoting the use of renewable energy sources are considered effective strategies for reducing environmental impacts.

Water use is also one of the key factors determining the environmental sustainability of livestock production. Water use in livestock systems is not limited to drinking water requirements but also includes feed production, farm operations, and product processing, all of which contribute substantially to total water use (FAO, 2019). In particular, irrigation practices used in feed crop production account for a large proportion of total water consumption. Increasing pressure on water resources may negatively affect the sustainability of livestock production systems, especially in regions with a high risk of drought.

Land use and natural resource consumption are among the major factors affecting the environmental performance of livestock production systems. The need for extensive areas for feed production and grazing may increase pressure on land resources, thereby increasing the risk of soil degradation, erosion, and biodiversity loss (Leip et al., 2015). The intensive use of natural resources poses various environmental risks to the long-term sustainability of production systems.

***Impacts of Climate Change on Livestock Production***

Climate change has both direct and indirect effects on livestock production systems, with important consequences for milk and meat production, animal health, feed resources, and water availability (Cheng et al., 2022). Rising temperatures, changes in precipitation patterns, and the increasing frequency of extreme climatic events pose significant risks to the sustainability of the livestock sector (Godde et al., 2021). These effects may occur directly through impacts on animal thermoregulation, metabolism, immune function, and production performance, or indirectly through changes in feed production, water availability, and disease and parasite pressure (Cheng et al., 2022). The direct and indirect effects of climate change on livestock production systems and their consequences for production performance and sustainability are schematically illustrated in Figure 3.

One of the most evident effects of climate change on livestock production systems is heat stress. High ambient temperatures challenge the thermoregulatory mechanisms of animals, resulting in reduced feed intake. Particularly in ruminants, increasing temperatures may lead to declines in feed intake, milk yield, body weight gain, and reproductive performance. Prolonged heat stress may result in adverse consequences, including metabolic disorders, impaired immune function, and increased mortality. In addition, high-producing animals have been reported to be more susceptible to heat stress (Das et al., 2016).

Changes in precipitation patterns and drought conditions also significantly affect livestock production systems. In particular, reduced forage yield in pasture areas, declines in forage availability, and insufficient water resources may increase production costs (Thornton, 2010). Climate change may alter pasture composition, plant growth, and feed quality, while increasing temperatures and water stress may negatively affect biomass production and the digestibility of forage crops (Chapman et al., 2017). Consequently, changes in feed supply and feed quality may adversely affect livestock production performance. In addition, drought conditions have been reported to reduce pasture productivity and compromise the sustainability of grazing systems (Boone et al., 2018).

Climate change also exerts various adverse effects on animal health. High temperature and humidity conditions may weaken the immune system of animals and increase their susceptibility to disease (Naqvi & Sejian, 2011). In addition, changing environmental conditions may facilitate the spread of parasites, pathogens, and vector-borne diseases. Changes in temperature and precipitation patterns have been reported to increase disease and parasite pressure (Godde et al., 2021). Increased disease incidence and health problems may lead to production and economic losses in livestock systems.

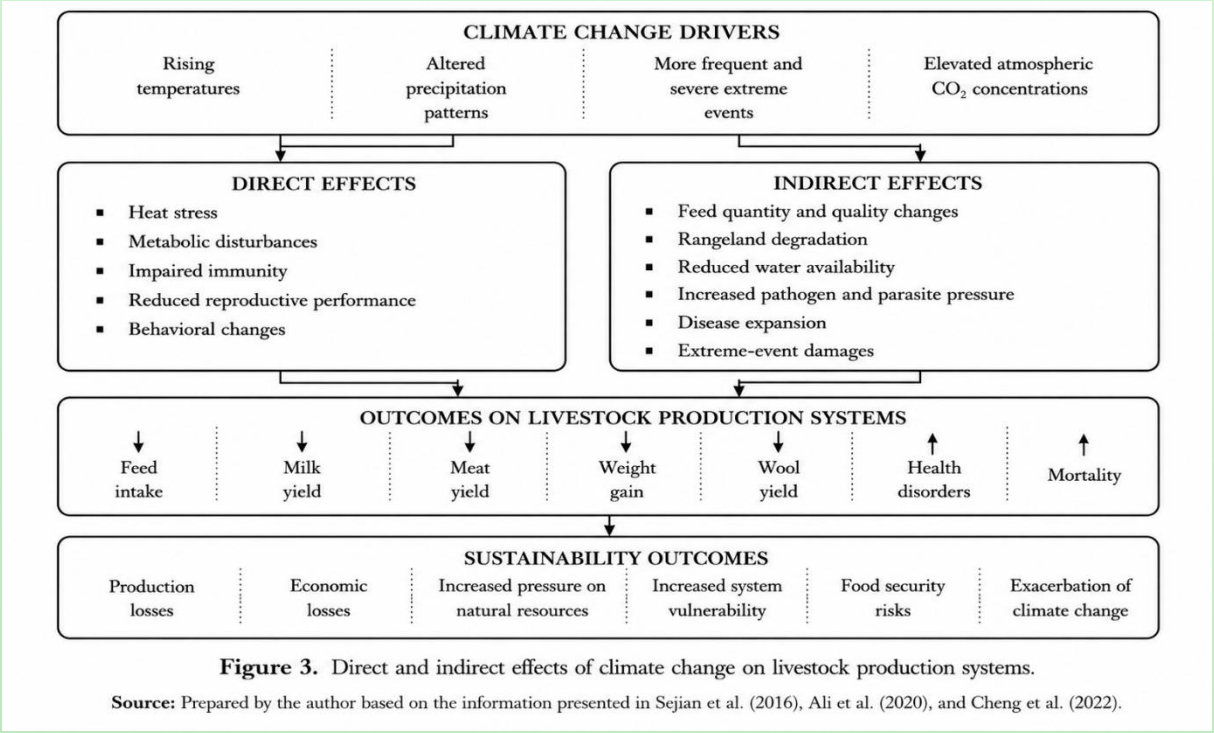



**Figure 3.** Direct and indirect effects of climate change on livestock production systems.

**Source:** Prepared by the author based on the information presented in Sejian et al. (2016), Ali et al. (2020), and Cheng et al. (2022).

Figure 3. Direct and indirect effects of climate change on livestock production.

The increasing frequency of extreme climatic events also negatively affects livestock production systems. Events such as floods, heat waves, droughts, and wildfires may damage pasture areas, disrupt feed supply, and cause animal losses. In particular, extreme temperature conditions in intensive production systems may adversely affect animal welfare and increase productivity losses (Gaughan & Cawdell-Smith, 2015). In addition, extreme climatic events have been reported to exert significant adverse effects on feed supply chains and livestock production activities (Dellar et al., 2018).

Overall, climate change threatens the sustainability of livestock production systems by exerting multifaceted effects on production performance, feed resources, water availability, and animal health (Sejian et al., 2016; Ali et al., 2020; Godde et al., 2020). As a consequence, reductions in feed intake, milk and meat production, body weight gain, and reproductive performance may occur, whereas disease incidence, health problems, and mortality rates may increase (Figure 3). Therefore, the development of sustainable production models adapted to climate change, the use of heat-tolerant genotypes, the sustainable management of pasture and water resources, and the adoption of production systems resilient to environmental stress are considered key adaptation strategies.

**European Green Deal and Livestock**

The European Union (EU) has implemented the European Green Deal as a comprehensive transformation framework to address environmental challenges such as climate change, biodiversity loss, and the unsustainable use of natural resources. The main objective of the European Green Deal is to make the EU climate-neutral by 2050 and to transform it into a sustainable economy in which economic growth is decoupled from resource use (European Commission, 2019). In this context, livestock production systems are considered one of the priority areas for transformation under the European Green Deal because of their greenhouse gas emissions and pressures on land and water resources (Guyomard et al., 2020).

The Farm to Fork Strategy and the Biodiversity Strategy for 2030, developed within the framework of the European Green Deal, aim to strengthen environmental sustainability in food production systems (European Commission, 2020a; European Commission, 2020b). These strategies aim to achieve a 50% reduction in pesticide use and associated risks, a reduction in chemical fertilizer use, a 50% reduction in antimicrobial use in livestock production, and an increase in the share of organic agricultural land to 25% of total agricultural land (Jongeneel et al., 2021). In addition, the conservation of biodiversity, the reduction of natural resource use, and the promotion of sustainable production practices are among the key priorities of the European Green Deal (European Commission, 2020b).

Within the framework of the European Green Deal, the livestock sector is regarded both as a source of environmental impacts that need to be reduced and as one of the key components of the transition toward sustainability. According to the European Environment Agency, the agricultural sector accounts for approximately

10% of total greenhouse gas emissions in the EU, and a substantial proportion of these emissions originates from livestock production (EEA, 2020). According to the European Commission, 80.7% of methane emissions from livestock production originate from enteric fermentation, whereas 17.4% are associated with manure management (European Commission, 2020c). However, the environmental impacts of livestock production are not limited to methane emissions but also result from processes such as feed production, land-use change, and manure management (Gerber et al., 2013; Herrero et al., 2016).

Low-emission production practices, sustainable feeding strategies, pasture-based livestock systems, and environmentally friendly production models are among the approaches promoted under the European Green Deal. In this context, the use of alternative protein sources, the development of feeding strategies aimed at reducing enteric methane emissions, and the increased use of local feed resources are encouraged. In addition, the European Green Deal aims to reduce dependence on imported critical feed ingredients by supporting plant proteins produced within the EU and alternative feed resources such as insects, algae, and by-products derived from the processing of biological resources (European Commission, 2020a).

Improving animal welfare standards is also considered one of the priority policy areas under the European Green Deal. In particular, reducing antibiotic use, supporting pasture-based production systems, and further integrating animal welfare criteria into production systems are among the major objectives (European Commission, 2020a). In this regard, sustainable production models are emphasized not only as a means of reducing environmental impacts but also as an approach to improving animal health and welfare (Peyraud & MacLeod, 2020).

Extensive production systems based on permanent grasslands are considered production models that support environmental sustainability under the European Green Deal. These systems have been reported to contribute to soil carbon sequestration and support biodiversity conservation (EESC, 2021). Organic livestock production systems promoted under the European Green Deal are also closely associated with these production models and may contribute to reducing environmental pressures (Horrillo et al., 2020).

Jongeneel et al. (2021) reported that the implementation of the European Green Deal targets could result in an approximately 10–15% reduction in livestock production in the EU. In particular, regulations aimed at reducing greenhouse gas emissions, restrictions on fertilizer use, and stricter environmental standards may have significant effects on production levels, production costs, feed costs, farm income, and market competitiveness (Guyomard et al., 2020; Jongeneel et al., 2021). These effects are expected to be more pronounced in intensive production systems.

Within the framework of the European Green Deal, restructuring the Common Agricultural Policy (CAP) in line with environmental objectives is considered one of the key components of livestock policies. It has been suggested that the current CAP regulations are only partially aligned with the goals of the European Green Deal and that the climate and environmental dimensions of the policy need to be strengthened to achieve environmental objectives. In this regard, the expansion of performance-based support mechanisms, the promotion of practices that enhance animal welfare, and the development of policy instruments that contribute to climate and biodiversity targets have been recommended (Guyomard et al., 2020).

Nevertheless, the effects of this transition on production costs, farm income, and competitiveness should be carefully evaluated. Therefore, the development of balanced policy instruments that ensure both environmental sustainability and the economic viability of livestock production is of great importance.

**Sustainable Livestock Production Approaches**

Practices aimed at reducing the environmental impacts of livestock production systems include improved feeding strategies, the use of feed additives, efficient manure management, sustainable pasture management, biogas technologies, precision livestock farming applications, and the use of renewable energy sources. These practices contribute to maintaining production efficiency while also reducing greenhouse gas emissions and improving resource-use efficiency.

One of the key issues in sustainable livestock production is reducing greenhouse gas emissions. In particular, methane emissions resulting from enteric fermentation in ruminants account for a substantial proportion of emissions associated with livestock production (Herrero et al., 2016; Nugrahaeningtyas et al., 2024). Therefore, considerable research efforts have been directed in recent years toward developing nutritional and management strategies to reduce enteric methane emissions.

One of the principal approaches for reducing methane emissions is improving feed quality and optimizing diet composition. The use of highly digestible feeds, an increased proportion of high-quality forages, and the implementation of balanced diets can contribute to reducing enteric methane emissions. In addition, improving feed efficiency helps reduce greenhouse gas emissions per unit of product (Herrero et al., 2016; Beauchemin et al., 2022).

Feed additives are among the most extensively studied approaches for reducing enteric methane emissions. In particular, tannins, saponins, essential oils, and certain plant extracts can suppress methane formation by altering ruminal microbial activity (Nugrahaeningtyas et al., 2024; Türkmen, 2024). Tannins have been reported to exert

inhibitory effects on methanogenic microorganisms and protozoa, whereas saponins may indirectly reduce methane production by decreasing ruminal protozoal populations. In addition, lipid supplementation has been shown to limit methane formation by altering hydrogen utilization (Beauchemin et al., 2022). However, the effectiveness of these approaches may vary depending on animal species, diet composition, and production systems.

In recent years, next-generation feed additives such as 3-nitrooxypropanol (3-NOP) and red algae have emerged as promising approaches for reducing enteric methane emissions. It has been reported that 3-NOP suppresses methane synthesis by inhibiting certain enzymes involved in methanogenesis (Türkmen, 2024). Similarly, bioactive compounds present in certain species of red algae have been shown to substantially reduce methanogenic activity in the rumen (Beauchemin et al., 2022; Nugrahaeningtyas et al., 2024). Nevertheless, the economic feasibility, long-term impacts, and efficacy of these approaches under different production systems are still being investigated.

Strategies aimed at reducing methane emissions are not limited to feed additives. Precision feeding practices, improved animal management, and increased productivity per animal can also contribute to reducing emission intensity (Beauchemin et al., 2022). In addition, studies focusing on the selection of animals with low methane production potential are considered valuable for sustainable livestock production. Genetic selection has been suggested to provide long-term and cumulative mitigation potential (Türkmen, 2024).

Manure management is one of the fundamental components of sustainable livestock production. Inadequate storage and management of animal waste may increase methane and nitrous oxide emissions. Accordingly, improved manure management systems, composting practices, and the widespread adoption of biogas technologies are important sustainable production approaches (Herrero et al., 2016; Nugrahaeningtyas et al., 2024). In particular, biogas systems contribute to renewable energy production while reducing greenhouse gas emissions associated with animal waste. Furthermore, recycling animal waste as compost and organic fertilizer supports circular production systems by enabling nutrient reuse.

Pasture management and pasture-based production systems are among the principal approaches supporting the environmental dimension of sustainable livestock production. Sustainable management of pasture resources can contribute to reducing erosion, conserving biodiversity, and enhancing soil carbon sequestration (Bellarby et al., 2012). In addition, appropriate grazing management practices have been reported to improve pasture productivity and help preserve soil carbon. Moreover, pasture-based production systems may provide several environmental advantages compared with systems relying on intensive feed inputs.

Organic livestock production systems are considered important components of sustainable production approaches. These systems may reduce environmental impacts because of their greater reliance on pasture resources, limited use of synthetic inputs, and stronger emphasis on animal welfare (Horrillo et al., 2020). Furthermore, this production approach may contribute to biodiversity conservation and environmental sustainability. However, their economic sustainability should be carefully evaluated, as productivity levels may be lower under certain conditions.

Technological developments play a critical role in the advancement of sustainable livestock production systems. Precision livestock farming technologies enable real-time monitoring of feed intake, animal behavior, health status, and environmental conditions. Sensor systems, automated monitoring technologies, and data-driven management practices have been reported to improve productivity, optimize resource use, and help reduce environmental impacts (Herrero et al., 2016). In addition, the use of renewable energy systems in livestock farms may contribute to reducing energy costs and greenhouse gas emissions associated with fossil fuel use.

Climate change adaptation strategies are also important components of sustainable livestock production systems. Practices such as the use of heat-tolerant genotypes, appropriate housing designs, improved ventilation systems, and efficient water management can help mitigate the adverse effects of climate change on animals. In particular, the development of production systems resilient to environmental stress conditions is important for maintaining production continuity and animal health (Türkmen, 2024). In addition, practices aimed at improving animal welfare have been reported to have positive effects on productivity and animal health.

## Conclusions

Climate change poses increasing environmental, economic, and social challenges to livestock production systems. Methane and nitrous oxide emissions associated with livestock production, together with natural resource use, pressures on land resources, and water consumption, represent some of the major challenges to the environmental sustainability of the sector. Nevertheless, livestock production systems remain strategically important because of their contributions to food security, support for rural livelihoods, and their role in human nutrition.

The European Green Deal and related policy frameworks aim to promote the adoption of more sustainable livestock production models. Achieving these objectives requires stronger integration of environmental criteria

into agricultural support policies, along with effective policy instruments that encourage sustainable production practices.

Practices aimed at reducing the environmental impacts of livestock production offer considerable mitigation and adaptation potential. However, drought, rising temperatures, declining pasture productivity, and increasing pressure on water resources are expected to pose greater risks to the sustainability of livestock production systems in the future.

The transition to sustainable production systems should be implemented not only in accordance with environmental objectives but also while taking into account economic feasibility, farm income, production costs, and food security. Measures aimed at reducing greenhouse gas emissions and changes in production systems may have significant effects on production costs and market competitiveness. Therefore, sustainability policies should be supported through producer incentives, investments in technical infrastructure, educational activities, and scientific research.

In conclusion, the widespread adoption of practices aimed at reducing greenhouse gas emissions and conserving natural resources in livestock production systems is important for mitigating the environmental impacts of the sector. At the same time, considering the importance of livestock production for food security, rural development, and economic productivity, a balanced approach should be adopted to align environmental objectives with production continuity. In this context, holistic policies that simultaneously address environmental, economic, and social dimensions will play a decisive role in ensuring the long-term sustainability of livestock production systems.

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# Adoption of Improved Technologies amongst Plantain Farmers in Bayelsa State

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## Abstract

Despite the economic significance of plantain and the availability of yield-enhancing technologies, productivity remains low due to limited adoption of technologies. This study examined the factors affecting plantain farmers' use of improved technologies in Bayelsa State, Nigeria. The study specifically described farmers' socio-economic characteristics, identified technologies available and used, examined the level of adoption, analyzed factors influencing the use of technologies, and identified constraints. A multi-stage sampling technique was used to select 100 plantain farmers, from whom primary data were collected using a structured questionnaire. Data were analyzed using descriptive statistics and a logistic regression model. The results revealed a predominantly female (70%), middle-aged (mean=45 years), and reasonably educated farming population (mean =14 years), operating small-scale farms (mean=2.1 ha) with limited access to credit (27%) and extension services (25%). While awareness of technologies (92%) was high, adoption was highest for low-cost practices (mulching: 81%) and lowest for capital-intensive ones (tissue culture: 12%). The regression analysis identified education, farm size, access to credit, and extension services as significant positive drivers of adoption, while older age, high perceived cost, and complexity were significant negative barriers. The major constraints were high input cost (85%), inadequate extension support (75%), and lack of credit access (73%). The study concludes that technology adoption in Bayelsa State is influenced by a complex mix of socioeconomic, institutional, and perceptual factors. It is recommended that policy interventions focus on providing input subsidies, improving access to credit, and revitalizing agricultural extension services. Extension agencies should promote farmer-centric low-cost technologies and leverage farmer cooperatives for effective dissemination. Addressing these barriers is crucial for enhancing technology adoption, improving plantain productivity, and ensuring sustainable agricultural development in the region.

***Keywords:*** *adoption; improved technologies; plantain farmers; logistic regression; Bayelsa State*

## Introduction

Agricultural transformation remains a critical pathway for economic growth, food security, and poverty reduction in Nigeria. Among the staple crops that contribute significantly to livelihoods and nutritional security in Southern Nigeria is plantain (*Musa paradisiaca*). Plantain is a vital food crop and income generator for rural households, particularly in the humid forest zones of Nigeria, including Bayelsa State. It serves both as a staple food and a cash crop, with its demand increasing in urban and peri-urban markets (Nwaobiala & Chikaire, 2020). Despite its importance, the productivity of plantain remains below potential due to a combination of socio-economic, institutional, and technical constraints.

According to Jimmy et al., (2026), although plantain has a huge export potential, Nigeria has not met the demand for local consumption, making domestic demand for plantain very high, with supply struggling to meet demand thus making the crop a highly sort after commodity in our markets. They further opined that even though Nigeria has vase land for the cultivation of plantain, depending on land area alone for higher production is not sustainable because of other competing crops, and needs such as urbanization, hence the need for the use of improved technologies. Although Nigeria still ranks top in terms of plantain production in Africa, the yield per hectare of plantain is low compared to that of other neighbouring countries like Ghana and Cameroun (Jimmy et al., 2026)

In response to low productivity and post-harvest losses, agricultural researchers and development organizations have promoted a variety of improved technologies, including hybrid cultivars, sucker treatment techniques, mulch application, improved spacing, organic and inorganic fertilization, and post-harvest handling technologies (Okoro et al., 2021). These technologies aim to enhance plant health, increase yields, and boost market competitiveness. However, the adoption and sustained use of these technologies among plantain farmers have been relatively limited and inconsistent in many parts of Nigeria, especially in the Niger Delta region.

Bayelsa State is blessed with fertile alluvial soils and suitable climatic conditions for plantain cultivation. The area plays a significant role in local food systems and provides employment opportunities through smallholder plantain farming. Yet, the full benefits of improved technologies have not been realized due to varying levels of adoption among farmers. Factors such as age, gender, education, farm size, extension contact, credit access, and market access influence technology choice and adoption (Onu & Madukwe, 2020; Nwachukwu et al., 2022).

Proper knowledge of the factors affecting farmers' decision to adopt improved technologies is therefore essential for designing appropriate interventions that are context-specific and farmer-friendly. A better understanding of these determinants will not only enhance the adoption process but also contribute to sustainable agricultural productivity and food security in the region (Akinbile et al., 2021). This research will focus on the socio-economic, institutional, and technological factors influencing plantain farmers' choices and adoption of improved agricultural technologies in Bayelsa State.

Despite the recognized potential of plantain as a staple crop and its economic significance, farmers continue to experience low yields, high post-harvest losses, and market constraints. Several improved agricultural technologies have been introduced to address these challenges, but their adoption remains limited and inconsistent among smallholder plantain farmers. This disparity raises critical questions regarding what influences farmers' choices and the uptake of these innovations.

In Bayelsa State, many farmers rely on traditional practices which often result in low productivity, increased vulnerability to pests and diseases, and inefficient resource utilization (Ugbomeh & Modebelu, 2023). Although technologies such as improved cultivars, sucker treatment, composting, and modern spacing techniques have proven to be effective, their low usage suggests the presence of barriers to adoption.

Previous studies like that of Okoye et al., (2020); Ibeawuchi et al., (2022) have suggested that variables such as limited access to credit, inadequate extension services, low literacy levels, land tenure issues, and cultural practices play vital roles in influencing technology adoption. Jimmy et al., (2026), compared the efficiencies between adopters and non-adopters of improved technologies by plantain farmers in South- South, Nigeria. However, this study differs significantly from the earlier study by Jimmy et al; 2026 because this study examined only 9 technologies, and was carried out solely in Bayelsa State. In reference to the above, there is a paucity of localized data specifically targeting plantain farmers in Bayelsa State, necessitating empirical investigation.

The broad objective of this study was to examine the factors affecting plantain farmers' use of improved agricultural technologies in Bayelsa State, Nigeria.

Specifically, the study seeks to identify the types of improved agricultural technologies available and used by plantain farmers; examine the level of adoption of improved agricultural technologies among plantain farmers; analyze the factors influencing farmers' adoption of improved technologies; and identify the constraints hindering the adoption of improved plantain production technologies in the study area. The study was guided by the null hypothesis: socioeconomic characteristics do not significantly influence plantain farmers' use of improved agricultural technologies in Bayelsa State

**Materials and Method**

This study was conducted in Bayelsa State, situated in the South-South region of Nigeria, geographically located within the tropical rainforest zone and is characterized by a humid climate, high rainfall (2,500–4,000 mm annually), and average temperatures ranging from 24°C to 32°C, which provide favourable agro ecological conditions for plantain cultivation (NiMet, 2023). Bayelsa is located at Latitude $4^0$ 15' North and $5^0$ 15' South and Longitude $5^0$ 22' West and $6^0$ 45' East, bounded in the West and South by the Atlantic Ocean, bounded by the East by River State and the North by Delta State.

The people are predominantly of Ijaw ethnic origin and depend heavily on farming and fishing for livelihood. Plantain is a major crop cultivated across the State. Serving both as a staple food and income-generating commodity.

This study adopted a multi-stage random sampling technique. In the first stage 4 Local Government Areas (LGAs) were randomly selected from the 8 LGAs of the State. The selected Local government area were Yenagoa, Southern Ijaw, Ogbia and Sagbama LGAs. The second stage involved the random selection of 5 communities from each of the LGAs making a total of 20 communities. The final stage involved the random selection of 5 plantain farmers from each of the selected communities making a total of 100 plantain farmers for the study. The sample frame for this study was gotten from registered plantain farmers list from Bayelsa Agricultural Development Programme (BSADP). Random selection was done using the lottery method to ensure that all LGAs, communities and farmers have equal chances of being sampled. The study relied on primary data, which were collected through the use of a structured questionnaire designed to capture the key variables in the study objectives and hypotheses. The questionnaire included both closed and open-ended questions and was organized into sections.

Table 1 Summary of Sampling Procedure

| *LGAs in Bayelsa State* | *Randomly selected LGAs* | *Randomly selected communities* | *Randomly selected farmers (5 per Comm)* |
|---|---|---|---|
| Yenagoa | Yenagoa | Beseni, Akaibiri, Akaba Ikolo Agudama- Ekpetiama | 25 |
| Sagbama | Sagbama | Ofoni, Ebedebiri, Trofani, Adagbabiri Bulu-Orua | 25 |
| Southern Ijaw | Southern Ijaw | Korokosei, Otuan, Peremabiri, Amassoma Ayama | 25 |
| Brass | Brass | Odioma, Sangana, Okpoama, Twon-Brass, Olodiama | 25 |
| Nembe | | | |
| Kolokuma/Opokuma | | | |
| Ekeremor | | | |
| Ogbia | | | |
| 8 LGAs | 4 LGAs | 20 communities | 100 farmers |

Data collected were analyzed using both descriptive and inferential statistical techniques. Frequencies, percentages, means, and standard deviations were used to summarize socio-economic characteristics of plantain farmers, identify the types of improved plantain technologies available and used, assessed the levels of adoption and identify constraints to adoption.

Binary logistic regression analysis was used to examine the determinants of farmers' adoption of improved technologies. Logistic regression is appropriate when the dependent variable is binary, i.e., whether or not a farmer adopted improved technologies.

**Logistic Model Equation:**

$$Logit\ (P) = In\ \left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k + \varepsilon \quad \text{---------- (1)}$$

Where:

- $P$ = Probability that a farmer adopts improved plantain technologies
- $\left(\frac{P}{1-P}\right)$ = Odds ratio of adoption
- $ln$= Natural logarithm

**Dependent Variable (Y):**

- Adoption of improved technology
  - 1 = Adopter
  - 0 = Non-Adopter
- $\beta_0$ = Intercept
- $\beta_1 \ldots \beta_k$ = Coefficients of the independent variables
- $X_1 \ldots X_k$ = Independent variables
- ε = Error term

Table 2. Independent Variables (X):

| *Variable* | *Code* | *Measurement* |
|---|---|---|
| Age of farmer | $X_1$ | Years |
| Gender | $X_2$ | 1 = Male, 0 = Female |
| Marital status | $X_3$ | 1 = Married, 0 = Otherwise |
| Educational level | $X_4$ | Years of formal education |
| Farming experience | $X_5$ | Years |
| Household size | $X_6$ | Number of persons |
| Farm size | $X_7$ | Hectares |
| Access to credit | $X_8$ | 1 = Yes, 0 = No |
| Access to extension services | $X_9$ | 1 = Yes, 0 = No |
| Membership of cooperative | $X_{10}$ | 1 = Yes, 0 = No |
| Perceived cost of technology | $X_{11}$ | 1 = High, 0 = Low |
| Compatibility with current practices | $X_{12}$ | 1 = Compatible, 0 = Not Compatible |
| Relative advantage | $X_{13}$ | 1 = Perceived as better, 0 = Not better |
| Complexity | $X_{14}$ | 1 = Complex, 0 = Simple |

## Results and Discussion

### *Socio-economic characteristics of Plantain Farmers*

The results on Table 3 reveals that the typical plantain farmer in Bayelsa State is a middle-aged female, married with secondary education, over 20 years of farming experience, and manages a small-scale farm of about 2 hectares. There is limited access to cooperatives, credit, and extension services. Specifically, 70% of respondents were female, with a mean age of 44.8 years. A large majority (75%) were married and had an average household size of 7 members. Educationally, most farmers were literate, with 52% having secondary education and 34% possessing tertiary education. The farmers were highly experienced, averaging 21.5 years in plantain cultivation. The average farm size was 2.1 hectares, classifying the majority (64%) as smallholders. Critically, access to institutional support was low: only 22% were in cooperatives, 27% had access to credit, and a mere 25% had contact with extension services.

The dominance of women in plantain farming aligns with their significant role in food production and post-harvest activities in Southern Nigeria, where they are often central to subsistence and small-scale commercial agriculture (Okello et al., 2020). The reasonably high literacy level is a positive finding, as education enhances the ability to understand and apply technical information related to new technologies (Sheahan & Barrett, 2017). However, the limited access to cooperatives, credit, and extension services represents a significant bottleneck. This institutional weakness has been identified as a major impediment to agricultural development in the Niger Delta region, as it limits farmers' exposure to innovations and their capacity to finance them (Ogunlade et al., 2020; Okoroafor & Akpan, 2021).

Table 3: Socio-economic Characteristics of Respondents (n=100)

| *Variable* | *Category* | *Frequency* | *Percentage (%)* | *Mean* | *Std. Deviation* |
|---|---|---|---|---|---|
| Sex | Male | 30 | 30.0 | - | - |
| | Female | 70 | 70.0 | - | - |
| Age (Years) | Youth (18-35) | 23 | 23.0 | 44.8 | 15.7 |
| | Middle-aged (36-55) | 52 | 52.0 | | |
| | Elderly (>55) | 25 | 25.0 | | |
| Marital Status | Single | 17 | 17.0 | - | - |
| | Married | 75 | 75.0 | - | - |
| | Widowed/Divorced | 8 | 8.0 | - | - |
| Level of Education | No Formal Education | 6 | 6.0 | - | - |
| | Primary Education | 8 | 8.0 | - | - |
| | Secondary Education | 52 | 52.0 | - | - |
| | Tertiary Education | 34 | 34.0 | - | - |
| Household Size | 1-5 members | 37 | 37.0 | 7.2 | 3.1 |
| | 6-10 members | 52 | 52.0 | | |
| | >10 members | 11 | 11.0 | | |
| Farming Experience (Years) | 1-10 years | 30 | 30.0 | 21.5 | 13.4 |
| | 11-20 years | 24 | 24.0 | | |
| | >20 years | 46 | 46.0 | | |
| Farm Size (Hectares) | Small (<2 Ha) | 64 | 64.0 | 2.1 | 1.3 |
| | Medium (2-5 Ha) | 33 | 33.0 | | |
| | Large (>5 Ha) | 3 | 3.0 | | |
| Membership in Cooperative | Yes | 22 | 22.0 | - | - |
| | No | 78 | 78.0 | - | - |
| Access to Credit | Yes | 27 | 27.0 | - | - |
| | No | 73 | 73.0 | - | - |
| Access to Extension | Yes | 25 | 25.0 | - | - |
| | No | 75 | 75.0 | - | - |

Source: Field Survey Data, 2025

***Awareness and Adoption of Improved Plantain Technologies Used by Plantain Farmers***

Awareness of various improved technologies was generally high. Awareness of technologies like mulching, spacing, and fertilizer use is high (>85%). However, adoption is highest for low-cost, low-risk practices like mulching (81%) and spacing (76%), and lowest for capital or skill-intensive technologies like tissue culture (12%) and post-harvest processing (32%).

Over 85% of farmers were aware of mulching, proper spacing, and the use of organic and inorganic fertilizers. However, awareness dropped for more modern technologies like tissue culture plantlets (45%). A clear disparity between awareness and adoption was observed. Low-cost, less complex practices like mulching (81% adoption) and proper spacing (76% adoption) saw the highest uptake. In contrast, technologies requiring significant capital or technical knowledge, such as tissue culture plantlets (12%) and post-harvest processing techniques (32%), had the lowest adoption rates.

The high awareness levels for certain technologies suggest that previous extension efforts and farmer-to-farmer knowledge transfer have been somewhat successful. However, the stark gap between awareness and adoption, particularly for capital-intensive technologies, underscores the influence of economic and technical barriers. This pattern is consistent with the findings of Mwangi and Kariuki (2019), who noted that in sub-Saharan Africa, the adoption of complex or expensive innovations often lags behind awareness due to financial constraints and perceived risks. The high adoption of mulching and spacing reflects their compatibility with existing practices and low initial cost, making them more accessible to resource-constrained smallholders.

Table 4 Awareness and Adoption of Improved Plantain Technologies (n=100)

| *Technology* | *Awareness* Frequency (%)* | *Adoption* Frequency (%)* |
|---|---|---|
| Improved plantain varieties (e.g., PITA) | 68 | 45 |
| Treated suckers | 75 | 38 |
| Organic manure/compost | 88 | 72 |
| NPK/Inorganic fertilizers | 85 | 55 |
| Mulching techniques | 92 | 81 |
| Proper spacing | 89 | 76 |
| Pest and disease control measures | 79 | 49 |
| Post-harvest processing techniques | 71 | 32 |
| Tissue culture plantlets | 45 | 12 |

Source: Field Survey Data, 2025 *multiple responses observed

***Examined levels of adoption of improved agricultural technologies among Plantain farmers***

According to the results presented in Table 5, the study classified farmers into four adoption categories. Only 11% were non-adopters, while 29% were low adopters (using 1-2 technologies). The largest group was medium adopters (42%), who used 3-5 technologies. A notable 18% of farmers were high adopters, having integrated more than five improved technologies into their farming systems.

This distribution indicates a promising trend where the majority of farmers (59%) are medium to high adopters. This suggests a general willingness to innovate within the farming community. However, the fact that 40% of farmers are low or non-adopters highlights a significant segment that remains underserved or faces persistent barriers. This heterogeneity in adoption levels necessitates targeted, rather than blanket, intervention strategies to move low adopters up the ladder and support high adopters as potential community champions (Rogers, 2003).

Table 5 Level of Adoption of Improved Technologies

| *Adoption Category* | *Frequency* | *Percentage (%)* |
|---|---|---|
| Non-Adopter (Adopted none) | 11 | 11.0 |
| Low Adopter (Adopted 1-2 technologies) | 29 | 29.0 |
| Medium Adopter (Adopted 3-5 technologies) | 42 | 42.0 |
| High Adopter (Adopted >5 technologies) | 18 | 18.0 |
| Total | 100 | 100.0 |

Source: Field Survey Data, 2025

***Factors Influencing the Adoption of Improved Technologies***

A logistic regression model was used to determine the factors influencing adoption. The regression model is highly significant. Education, farm Size, access to credit, and access to extension services are strong positive drivers of adoption. Perceived high cost and complexity are significant negative barriers. Age has a small but significant negative effect, suggesting younger farmers are slightly more likely to adopt. The model was statistically significant ($\chi^2 = 68.32$, $p < 0.001$), correctly classifying 82% of cases and explaining 52.4% (Nagelkerke $R^2$) of the variance. The results identified several significant factors:

**Positive Drivers:** Education level, farm size, access to credit, and access to extension services significantly increased the likelihood of adoption. Farmers with extension access were over 8 times more likely to adopt. **Negative Barriers:** Older age, high perceived cost, and perceived complexity significantly reduced adoption. Farmers who perceived technologies as costly were 79% less likely to adopt them.

These findings robustly refute the null hypothesis that socioeconomic characteristics do not significantly influence adoption. The positive role of education and farm size is well-documented, as they enhance financial capacity and risk-bearing ability (Ayinde et al., 2021). The profound impact of access to extension and credit underscores the critical role of institutional support in facilitating adoption, a finding consistent with the results of Jimmy et al., 2026; Kumari & Adekunle, 2022; Okoroafor & Akpan, 2021, that access to credit and extension service play a critical role in the adoption of technologies among crop farmers in Nigeria. The negative influence of age suggests that younger farmers are more receptive to change, a trend noted by Jimmy Furthermore, the significance of perceptual factors (cost, complexity, relative advantage) validates the relevance of Rogers' (2003) Diffusion of Innovations theory, confirming that a technology's characteristics are as crucial as the farmer's profile in determining its uptake.

Table 6: Logistic Regression Analysis of Factors Influencing Adoption of Improved Technologies

| *Independent Variable* | *Coefficient (β)* | *Std. Error* | *p-value* | *Odds Ratio [Exp(β)]* |
|---|---|---|---|---|
| Constant | -4.225 | 1.812 | 0.020** | 0.015 |
| Age ($X_1$) | -0.045 | 0.022 | 0.041** | 0.956 |
| Gender ($X_2$: 1=Male) | 0.512 | 0.589 | 0.384 | 1.668 |
| Education Level ($X_4$) | 0.321 | 0.151 | 0.033** | 1.379 |
| Farming Experience ($X_5$) | 0.018 | 0.025 | 0.470 | 1.018 |
| Farm Size ($X_7$) | 0.654 | 0.245 | 0.008*** | 1.923 |
| Access to Credit ($X_8$: 1=Yes) | 1.842 | 0.701 | 0.009*** | 6.312 |
| Access to Extension ($X_9$: 1=Yes) | 2.105 | 0.744 | 0.005*** | 8.205 |
| Membership in Cooperative ($X_{10}$: 1=Yes) | 1.224 | 0.662 | 0.065 | 3.400 |
| Perceived Cost ($X_{11}$: 1=High) | -1.551 | 0.612 | 0.011** | 0.212 |
| Compatibility ($X_{12}$: 1=Compatible) | 1.887 | 0.688 | 0.006*** | 6.598 |
| Relative Advantage ($X_{13}$: 1=Better) | 2.431 | 0.795 | 0.002*** | 11.370 |
| Complexity ($X_{14}$: 1=Complex) | -1.298 | 0.599 | 0.030** | 0.273 |

Log-Likelihood: -42.15; Chi-Square ($\chi^2$): 68.32 ($p < 0.001$); Pseudo $R^2$ (Nagelkerke): 0.524; Percentage Correct Prediction: 82.0%; ***Significant at 1% (p<0.01), ***Significant at 5% (p<0.05)*

***Constraints to Adoption of Improved Technologies***

Results displayed in Table 7 shows that farmers faced multiple, overlapping constraints. The top three barriers are economic and institutional. High cost of inputs, inadequate extension support, and lack of access to credit. The most severe was the high cost of inputs (85%), followed by institutional failures such as inadequate extension support (75%) and lack of access to credit (73%). Other significant barriers included the unavailability of inputs (62%), a lack of awareness or training (58%), and the perceived complexity of technologies (45%).

The constraints identified are both economic and institutional, forming a vicious cycle that hinders technology adoption. The high cost of inputs, consistently reported as the primary barrier, reflects the financial vulnerability of smallholder farmers and aligns with the findings of Adeyeye et al. (2022). The prominence of inadequate extension and credit access directly mirrors the institutional determinants found significant in the regression analysis, highlighting a critical area for policy intervention. These barriers have been previously reported in the region, suggesting that past interventions have not sufficiently addressed these core challenges (Chukwu et al., 2020). Addressing these foundational issues is a prerequisite for widespread and sustainable adoption of improved plantain technologies in Bayelsa State.

Table 7 Constraints Hindering the Adoption of Improved Technologies (n=100)

| *Constraint* | *Frequency* | *Percentage (%)* | *Rank* |
|---|---|---|---|
| High cost of inputs | 85 | 85.0 | 1st |
| Inadequate extension support | 75 | 75.0 | 2nd |
| Lack of access to credit | 73 | 73.0 | 3rd |
| Unavailability of inputs | 62 | 62.0 | 4th |
| Lack of awareness/training | 58 | 58.0 | 5th |
| Complexity of technologies | 45 | 45.0 | 6th |
| Poor market for produce | 40 | 40.0 | 7th |
| Cultural/Traditional beliefs | 18 | 18.0 | 8th |

Source: Field Survey Data, 2025; *Note: Multiple Responses Recorded constraints.*

## Conclusion

This study provides critical empirical evidence on the determinants of technology adoption among plantain farmers in the Niger Delta region. It conclusively demonstrates that the socio-economic characteristics of farmers significantly influence their decision to adopt improved technologies, thereby rejecting the stated null hypothesis. The findings underscore that while farmers are generally aware of innovations, the transition to sustained adoption is hampered by a triad of critical barriers: economic limitations, institutional failures, and negative perceptions.

The prominence of financial constraints as the primary barrier highlights the economic vulnerability of smallholder plantain farmers in Bayelsa State, who lack the capital to invest in improved inputs. Furthermore, the crippling lack of access to extension services and credit facilities points to a systemic institutional failure that stifles agricultural innovation and productivity. The significant influence of farmers' perceptions regarding cost, complexity, and relative advantage emphasizes that technology design and promotion must be farmer-centric and context-specific.

Ultimately, the potential of improved agricultural technologies to enhance plantain productivity, increase farmers' incomes, and contribute to food security in Bayelsa State remains largely untapped. Achieving widespread adoption requires moving beyond mere dissemination of technologies to addressing the underlying economic and institutional bottlenecks that disempower farmers. A holistic approach that simultaneously strengthens support systems, improves financial access, and builds farmers' capacity through targeted training is essential to unlock this potential and foster a sustainable agricultural transformation in the region.

## Recommendations and Policy Implication

Based on the findings of this study, the following recommendations were proposed:

a) **Implement Input Subsidy Programs:** Develop and faithfully execute targeted subsidy programs to reduce the upfront cost of critical inputs like hybrid plantain suckers, fertilizers, and simple post-harvest equipment for genuine smallholder farmers.
b) **Strengthen Rural Financial Systems:** Partner with microfinance banks and develop innovative, low-collateral credit schemes specifically tailored for smallholder farmers to improve their access to finance for technological investments.
c) **Revitalize Extension Services:** Increase the number, funding, and mobility of extension agents to ensure regular and effective contact with farmers in remote communities. The focus should be on practical, hands-on training and demonstrations.
d) **Promote Farmer-Centric Technologies:** Prioritize the promotion of technologies that are low-cost, less complex, and easily compatible with existing farming systems, such as mulching and improved spacing, to ensure higher uptake.
e) **Utilize Lead Farmers:** Identify and train high-adopter farmers to serve as demonstration hosts and peer educators within their communities, leveraging social networks for technology diffusion.
f) **Strengthen Farmer Groups:** Farmers should be encouraged to form or join active cooperatives to benefit from economies of scale, collective bargaining for inputs, and better access to government support and training programs

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# Assessing the Lethal Concentration ($LC_{50}$) of Cadmium Chloride on Mediterranean Mussel (*Mytilus galloprovincialis*)

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## Abstract

(1) Background: Heavy metal pollution of industrial and domestic origin in aquatic ecosystems is a critical environmental problem that threatens human health through the food chain. This study aimed to determine the acute toxicity levels of cadmium chloride and early-stage ecotoxicological responses in the Mediterranean mussel (*Mytilus galloprovincialis*). (2) Methods: Mussels were obtained from the Sinop University Mussel and Oyster Cultivation Facility, with an average length of 40.48±0.82 mm and width of 20.58±0.42 mm. Triplicate 96-hour static bioassays were conducted in filtered seawater maintained at 17.78±0.03°C and pH 7.93±0.05. Organisms were exposed to cadmium concentrations of 0.5, 1, 5, 10, and 15 ppm, alongside a control group. (3) Results: During the first 24 hours of exposure, distinct physiological stress and avoidance behaviours-such as valve closure, immobility, and increased faecal production-were observed, particularly at high doses of 10 ppm and above. Based on mortality data at the end of the 96-hour observation period, a Probit analysis determined the median lethal concentration ($LC_{50}$) value for *M. galloprovincialis* to be 3.03326 ppm. (4) Conclusions: The findings indicate that *M. galloprovincialis* possesses a relatively high tolerance threshold to acute cadmium exposure. The numerical data obtained serve as an early warning criterion for food safety risk analysis and monitoring programs in regional bivalve aquaculture. Furthermore, this study provides a foundational baseline dataset for future sublethal chronic evaluations at lower concentrations and for elucidating the cellular mechanisms of cadmium toxicity in bioindicator species.

***Keywords:*** *Mytilus galloprovincialis, Cadmium Chloride, Acute Toxicity, Median Lethal Concentration ($LC_{50}$), Bioindicator*

## Introduction

Along with technological advancements, rapid transformations in economic, social, and cultural fields continuously shape human life. The continuity of these changes, combined with a growing global population, drives a quantitative and qualitative increase in the use of chemicals for industrial, agricultural, and domestic purposes. This widespread chemical utilization escalates air, soil, and water pollution through anthropogenic activities, leading to a rapid elevation of metal concentrations in aquatic ecosystems (Vutukuru, 2005; Karthikeyan, 2014). Agricultural runoff, sewage discharges, and the indiscriminate disposal of industrial effluents into water bodies constitute the primary causes of aquatic pollution (Cook et al., 1995). Sedentary aquatic organisms, such as the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819), are directly affected by this negative trajectory, facing severe exposure to potentially toxic metal pollution resulting from both anthropogenic and geogenic (natural/crustal) activities in marine ecosystems (Bat and Arıcı, 2018).

The introduction of chemical substances into aquatic ecosystems renders toxic exposure inevitable across a broad biological spectrum, ranging from unicellular microorganisms like algae and bacteria to complex vertebrates such as fish and marine mammals. Toxicology, which investigates the damaging and destructive effects of physical or chemical factors on living organisms, plays a critical role at this juncture for environmental and public health. Accordingly, aquatic toxicology tests aim to determine the specific concentrations at which pollutants induce toxicity in the aquatic biota and the extent to which they disrupt the vital functions of these organisms (Bouldin et al., 2008).

As the leading species in bivalve aquaculture in Türkiye, *M. galloprovincialis* stands out as a robust model organism characterized by its lack of need for external feed supplementation, suitability for low-cost production systems, and eco-friendly cultivation features. Its protein-rich content and high nutritional quality render the aquaculture of these organisms highly advantageous compared to both other seafood and terrestrial protein sources. Offering a strategic alternative to meeting the increasing global demand for animal protein since the 1990s, this species is heavily preferred in coastal regions as a commercial and gastronomic seafood asset due to its high protein ratio and healthy unsaturated fatty acid profile (Akdemir, 2025).

In semi-closed basins like the Black Sea, where coastal inputs from industrial and agricultural activities are intense, metal accumulation levels in mussel tissues are recognized as sensitive biological indicators (bioindicators) for assessing environmental pollution and ecosystem health. As filter-feeding organisms, these bivalves accumulate potentially toxic metals and other persistent pollutants from the surrounding environment, serving as a biological bridge that transfers these contaminants directly to humans through the food chain.

Therefore, analysing the pollution loads of the stations where mussels are gathered and regularly monitoring bioaccumulation processes prior to the harvesting and consumption stages are essential to determining the current state of the aquatic ecosystem (Bat et al., 1999; Yarsan et al., 2007; Küçüksezgin et al., 2010). Such monitoring studies constitute a legal and practical imperative for protecting public health, particularly by preventing products harvested from high-risk contaminated areas from entering the market (Jović et al., 2011).

Considering all these ecological, economic, and epidemiological factors, alongside its strategic importance for the region, *M. galloprovincialis* was selected as the live material for this study. Within the scope of this preliminary research, acute toxicology tests using cadmium chloride ($CdCl_2$) were performed on specimens obtained from the Mussel and Oyster Cultivation Facility. Through this preliminary investigation, it is aimed to contribute to the infrastructure for regular health monitoring of local production areas and to reveal potential metal pollution levels using updated, scientific data.

## Materials and Methods

### *Biological Material*

The Mediterranean mussels (*Mytilus galloprovincialis*), which constitute the live material of this study, were obtained from the Mussel and Oyster Cultivation Research Facility operating under the Faculty of Fisheries at Sinop University. The specimens were collected manually from the main longlines located within the production area of the facility.

### *Transportation and Acclimatization*

To minimize transport-induced stress factors, the collected live specimens were immediately placed into plastic containers filled with seawater from the sampling site. During transportation to the faculty laboratory, the samples were protected from direct sunlight and maintained under appropriate temperature and dark conditions.

Upon arrival at the laboratory, the mussels were transferred to aquariums filled with natural seawater supported by a continuous aeration and filtration system. Prior to placement in the stock tanks, the outer shell surfaces of each individual were mechanically cleaned to eliminate potential microorganisms, epibionts, and foreign debris. Following the cleaning process, the organisms were transferred to a pre-prepared and conditioned seawater system, where they underwent a 2-day depuration (starvation) period to clear metabolic wastes and evacuate their digestive tract contents.

### *Acute Toxicity Assays ($LC_{50}$)*

Acute lethal concentration ($LC_{50}$) tests were performed to determine the heavy metal resistance threshold of the laboratory-acclimatized *M. galloprovincialis* individuals. Prior to the definitive experiment, range-finding preliminary tests were conducted to establish the sensitivity limits of the species. Accordingly, a definitive experimental design was established in triplicate, comprising a control group and five cadmium chloride ($CdCl_2$) exposure concentrations: 0.5, 1.0, 5.0, 10.0, and 15.0 ppm (Table 1).

Table 1. Experimental design and exposure concentrations established for cadmium (ppm).

| *Replicates* | *Control* | *$Cd_1$* | *$Cd_2$* | *$Cd_3$* | *$Cd_4$* | *$Cd_5$* |
|---|---|---|---|---|---|---|
| I | 0.0 | 0.5 | 1.0 | 5.0 | 10.0 | 15.0 |
| II | 0.0 | 0.5 | 1.0 | 5.0 | 10.0 | 15.0 |
| III | 0.0 | 0.5 | 1.0 | 5.0 | 10.0 | 15.0 |

Before commencing the exposure phase, biometric parameters, including shell length (mm) and width (mm), were precisely measured for each individual. Following these measurements, the mussels were randomly and gradually distributed into the experimental chambers across the respective concentration groups. Throughout the experiment, continuous low-flow aeration was applied to each setup to maintain dissolved oxygen levels within ideal physiological limits.

To ensure that toxicity results were not compromised by degrading water quality, water parameters (pH, temperature, and dissolved oxygen) were monitored daily. Dissolved oxygen levels were maintained above 60% saturation throughout the 96-hour period via continuous gentle aeration. The 2-day depuration period also ensured that no pseudo-faces or excessive metabolic ammonia accumulated in the test chambers prior to chemical dosing.

Organism mortality and behavioural responses were monitored and recorded at regular intervals during the observation period. The acute toxicity tests were conducted via a 96-hour static bioassay method. The exposure concentration ranges, spanning the spectrum between the "no observed mortality rate" and "complete mortality rate," were determined in strict compliance with the standardized guidelines of the USEPA (1998, 2016). Dead individuals were counted and recorded every 24 hours and promptly removed from the experimental chambers to prevent any deterioration of water quality. In accordance with the nature of acute static bioassays, no feeding was provided to the organisms during the 96-hour test period.

## Results

Throughout the 96-hour static bioassays conducted in filtered seawater, the average water temperature was maintained at 17.78±0.03°C, and the pH remained stable at 7.93±0.05. The biometric measurements of the experimental mussel specimens revealed an average shell length of 40.48±0.82 mm and an average shell width of 20.58±0.42 mm.

***Behavioural and Physiological Alterations (Valve Activity and Stress Responses)***

During the acute toxicity tests, prominent dose- and time-dependent behavioural avoidance mechanisms and physiological stress responses were recorded in the mussels due to cadmium exposure.

During the first hour of the adaptation period, individuals in the control group exhibited complete adaptation to the environment. In contrast, mussels exposed to the 10-ppm concentration remained immobile, exhibiting a distinct avoidance behaviour where the foot became trapped between the two valves. By the 6th hour of exposure, valve closure behaviour became dominant in the high-dosage groups (≥10 ppm) as a protective mechanism against toxic stress, accompanied by a noticeable increase in faecal production.

In the early phase of toxicity exposure (first 24 hours), acute avoidance behaviours and physiological stress indices-such as prolonged valve closure, complete immobility, and excessive faecal production-were explicitly recorded, particularly at high concentrations of 10 ppm and above.

***Lethal Effects and Alterations in Environmental Parameters***

The physiological differentiation between the control and experimental groups became more pronounced from the 24th hour of exposure onward. The control group individuals exhibited a 100% survival rate and maintained normal filtration activities until the end of the experiment.

Conversely, in the cadmium-exposed groups, a complete loss of mobility, an increase in mortality cases, and intensive foaming in the experimental water were observed starting from the 24th hour.

***Estimation of the Cadmium Chloride $LC_{50}$ Value***

Based on the data obtained from the exposure groups, which included the control and five concentration levels (0, 0.5, 1.0, 5.0, 10.0, and 15.0 ppm), the median lethal concentration ($LC_{50}$) value of cadmium chloride for *M. galloprovincialis* was determined to be 3.03326 ppm (Table 2). The Probit Analysis method developed by Finney (1952) was utilized to determine the lethal concentration ($LC_{50}$) values, and calculations were executed via a specialized algorithm software tailored for this methodology (Mekapogu, 2021).

Table 2. Probit analysis results and the 96-hour median lethal concentration ($LC_{50}$) of cadmium (Cd) for *Mytilus galloprovincialis*.

| *Lethal Concentration* | *Value (ppm)* | *95% Confidence Interval (Lower Limit - Upper Limit) (ppm)* |
|---|---|---|
| $LC_{50}$ | 3.03326 | 1.97708 - 4.65367 |

The relationship between cadmium exposure concentrations and the mortality percentage of *M. galloprovincialis* was further evaluated using a polynomial regression model (Figure 1). The generated regression equation was determined as $y = -0.7002x^2 + 17.157x$, exhibiting an exceptionally strong coefficient of determination ($R^2 = 0.9918$), which demonstrates a highly predictable and dose-dependent lethal effect.

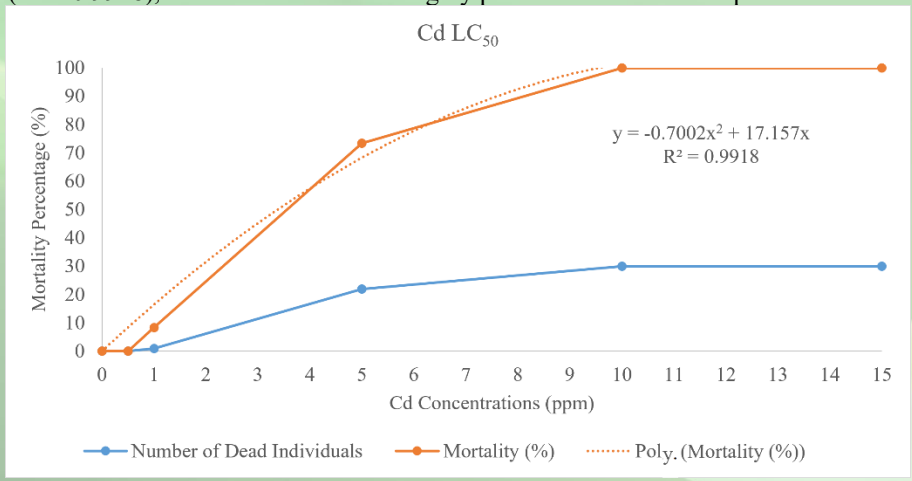



Figure 1. Dose-response curve and polynomial regression model showing the mortality percentage of *Mytilus galloprovincialis* exposed to various cadmium (Cd) concentrations over 96 hours.

**Discussion**

The immediate behavioural and physiological responses-specifically valve closure (isolation) and foot restriction-observed within the first 1 to 6 hours in mussels exposed to cadmium in this preliminary study confirm the acute physiological disruption induced by this heavy metal. This behavioural transition from protective valve closure to neuromuscular paralysis is strongly supported by Yasmeen and Pathan (2021), who reported that while freshwater bivalves (*Lamellidens marginalis*) tightly close their shells during the initial hours of cadmium exposure as an avoidance mechanism, prolonged stress leads to an irreversible failure of adductor muscles, forcing the valves to remain widely open until death. Furthermore, their observations regarding heavy mucus extrusion and the exacerbating role of elevated temperatures on cadmium toxicity align perfectly with the intense foaming and physiological stress recorded in our experimental groups, confirming that exogenous environmental factors and time-dependent adductor muscle dysfunction collectively dictate bivalve survival thresholds under acute metal stress.

In toxicity assays conducted with aquatic organisms, the parameters $LC_{50}$ (Median Lethal Concentration), representing the toxicant concentration that kills 50% of the test population, and $EC_{50}$ (Median Effect Concentration), representing the concentration that induces a specific target response in half of the population, are widely utilized as standard benchmarks for toxicity assessment.

The relatively high acute tolerance threshold ($LC_{50}$ = 3.03326 ppm) exhibited by *M. galloprovincialis* in this study aligns with the well-known physiological resilience of bivalves. This resistance is often attributed to internal detoxification mechanisms, such as the synthesis of metallothioneins—low-molecular-weight, cysteine-rich proteins that safely bind and immobilize free cadmium ions within intracellular compartments (e.g., lysosomes), thereby preventing systemic enzyme inhibition. Additionally, the low-salinity nature of the Black Sea may influence ionoregulation and the bioavailability of free metal ions ($Cd^{2+}$), which warrants further physicochemical investigation.

The incorporation of a post-exposure monitoring phase in toxicological bioassays is crucial for capturing delayed lethality and long-term recovery dynamics, which are often masked during standard observation windows (Alonso, 2023). Evaluating post-exposure outcomes reveals a heightened sensitivity in aquatic organisms, as physiological injuries sustained during initial contact can progress over time, even after the toxicant is removed. Conversely, the behavioural and metabolic recovery observed in surviving individuals highlights the activation of efficient internal detoxification pathways among highly tolerant phenotypes. This latent toxicity framework suggests that the 96-h $LC_{50}$ threshold determined for *M. galloprovincialis* in the current study could trigger progressive, non-immediate stress, emphasizing that environmental risk assessments must account for both post-exposure latency and individual detoxification capacities to accurately predict bivalve population survival.

Estimating ecotoxicological thresholds such as the $LC_{50}$ value for *M. galloprovincialis*, a species with a high potential for transferring metals to humans via the food chain, plays a fundamental role in evaluating both the pollution pressure exerted on marine ecosystems and the indirect risks posed to public health. The adverse physiological reactions and toxicity rates detected in these bivalves, even under short-term exposure, function effectively as an ecological early warning system capable of identifying potential environmental hazards prior to severe ecological degradation.

In a similar toxicological context, Bat et al. (2013) calculated $LC_{50}$ values during acute (96-hour) and chronic (28-day) bioassays investigating the toxicity of copper, lead, and zinc in Mediterranean mussels. They emphasized that such ecotoxicological monitoring parameters are crucial for shedding light on legal regulations, ensuring the safe consumption of seafood, and managing environmental pollution. The high tolerance threshold ($LC_{50}$= 3.03326 ppm) determined for cadmium in the present study further contributes to understanding the bioindicator capacity of *M. galloprovincialis* harvested from regional aquaculture structures under acute chemical stress.

The observed variations in acute tolerance and the subsequent failure of neuromuscular control in larger bivalves can be closely linked to the complex toxicokinetic feedback between body size, age, and weight-specific filtration rates. Although smaller or younger individuals possess a higher surface-area-to-volume ratio, they process a structurally lower absolute volume of contaminated water during acute exposure. Conversely, larger individuals subject their expansive gill surfaces to continuous, high-volume water currents, rapidly accelerating the influx of free $Cd^{2+}$ ions. Consistent with these size-dependent dynamics, younger bivalves often exhibit higher baseline metallothionein levels than their older counterparts, providing a greater physiological buffer against rapid heavy metal influx (Wallace et al., 2003). This enhanced cellular buffering capacity in smaller individuals is frequently accompanied by an accelerated growth dilution effect, wherein the rapid increase in soft tissue mass prevents internal concentrations of contaminants from reaching lethal thresholds (Otchere, 2022).

On the other hand, larger bivalves face increased physiological stress due to their reduced metabolic activity, which leads to longer equilibration times and slower depuration of accumulated metals (Bates et al., 2021). In these larger organisms, the massive volumetric water exposure leads to early tissue saturation, triggering a 'spillover' effect where unbound cadmium overwhelms the calcium-regulated pathways essential for adductor muscle contraction (Wallace et al., 2003; Geffard et al., 2007).

Interestingly, while Wallace and Luoma (2003) also hypothesized that size-dependent uptake rates could concurrently increase the sensitivity of smaller bivalves under specific scenarios, our findings with adult *M. galloprovincialis* clearly demonstrate that the physical advantage of a lower absolute filtration volume combined with enhanced cellular buffering decisively overcomes such risks during acute cadmium pulses. This outcome underscores that variations in efflux rates and age-dependent physiological constraints are not strictly uniform across different life stages and species (Cadmus et al., 2019; Alonso, 2023). This allometric complexity is further illuminated by Cao et al. (2025), who revealed that age exerts a more pronounced influence on trace metal accumulation dynamics than organismal size alone. Their findings indicate that older bivalves exhibit a significantly diminished bioaccumulation capacity, fundamentally driving negative correlations between body size and metal concentration. In the context of our study, this age-dependent decline in metabolic efficiency explains why larger, older *M. galloprovincialis* present a lower threshold of tolerance during acute shocks; their compromised internal machinery is less capable of dynamically partitioning and binding influxing contaminants compared to younger cohorts. Consequently, incorporating age-driven physiological shifts alongside pure physical dimensions is paramount for refining the interpretation of marine biomonitoring data and accurately evaluating environmental risk factors.

## Conclusion

Unregistered activities within the bivalve aquaculture sector in Türkiye complicate the precise determination of consumption rates, while potential toxic metal pollution in mussels harvested from unmonitored areas poses a severe gap in public health protection (Aytekin et al., 2019; Bat et al., 2023). The bioaccumulation and biomagnification characteristics of *M. galloprovincialis* present a chronic threat to aquatic ecosystems, and the consumption of contaminated organisms serves as a direct source of heavy metal exposure for humans (Aytekin et al., 2019; Bat et al., 2023). Because *M. galloprovincialis* possesses a high potential to transfer metals to humans via the food chain, an in-depth investigation of this risk is a critical imperative for food safety (Bat et al., 2023). Accordingly, static acute and chronic toxicity tests play a fundamental role in evaluating the pollution pressure exerted on mussels and their potential for food chain transfer by establishing baseline toxicity benchmarks such as the $LC_{50}$ value (Bat et al., 2023).

In comparative risk assessments, the wet weight-based limits established by FAO/WHO (1986) standards (upper limits of 0.1–0.5 mg $kg^{-1}$ for Cd, 20 mg $kg^{-1}$ for Cu, and 1 mg $kg^{-1}$ for other trace elements) and current national/international regulations (EC, 2023; TGK, 2023; setting a maximum limit of 1.0 mg $kg^{-1}$ for Cd) serve as foundational guidelines for determining acceptable risk thresholds for human health (Bat et al., 2021). To ensure the sustainability of public health and food safety, enforcing legal prohibitions on the human consumption of mussels harvested from high-risk, contaminated regions that exceed these established limits is mandatory.

This preliminary study calculating the median lethal concentration provides a concrete ecotoxicological foundation for the effective enforcement of legal thresholds and the preservation of the Black Sea marine ecosystem. The generated data establish a fundamental and referential baseline dataset for future sublethal chronic bioassays designed at lower concentrations, which are required to elucidate the cellular-level toxic mechanisms of cadmium in bioindicator species.

In conclusion, the numerical benchmarks generated in this preliminary study do not only provide a reference baseline for future sublethal and cellular-level chronic bioassays, but they also serve as vital mathematical inputs for localized human health risk models, such as Target Hazard Quotient (THQ) and Estimated Daily Intake (EDI) assessments, ensuring the long-term economic and biological safety of regional bivalve aquaculture.

## Declarations

### *Ethical Approval*

The authors declare that the experimental procedures conducted in this study do not require Ethical Committee approval.

### *Author Contribution Statement*

- Zeynep HASANÇAVUŞOĞLU: Data curation, Investigation, Writing – original draft.
- Levent BAT: Formal analysis, Supervision, Conceptualization, Methodology, Writing – review & editing.

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The authors declare that no financial support or funding was received from any institution or organization for the conduct of this study.

### *Conflict of Interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Biofilm Formation by Foodborne Pathogens in Meat Processing Facilities: Challenges and Control Strategies

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## Abstract

Biofilm formation by foodborne pathogens in meat-processing facilities represents a major challenge to food safety, product quality, and public health worldwide. In these environments, microorganisms attach to food-contact surfaces and develop structured microbial communities embedded within extracellular polymeric substances (EPS), which enhance resistance to disinfectants, environmental stress, and conventional sanitation procedures. This review examines the mechanisms of biofilm formation, including microbial attachment, colonization, maturation, and dispersion, as well as the environmental and operational factors that promote biofilm persistence in meat-processing systems. The review further discusses major biofilm-forming foodborne pathogens associated with meat-processing environments, including Salmonella enterica, Listeria monocytogenes, Escherichia coli O157:H7, Campylobacter spp., and Pseudomonas spp., alongside critical contamination sites such as slaughter lines, cutting equipment, storage areas, and drainage systems. The public health significance of biofilms is highlighted through their contribution to pathogen persistence, foodborne disease outbreaks, antimicrobial resistance, and reduced meat shelf life. Additionally, current detection and characterization techniques, including microbiological, molecular, and imaging-based approaches, are evaluated.

Finally, the review explores conventional and emerging biofilm control strategies, with particular focus on natural antimicrobials, bacteriophage applications, enzymatic treatments, surface modification technologies, and integrated HACCP-based hygiene systems. Understanding the ecology and persistence of biofilms within meat-processing facilities is essential for developing effective prevention and control strategies capable of improving meat safety, reducing contamination risks, and supporting sustainable food production practices.

***Keywords:*** *Biofilms; Meat processing facilities; Foodborne pathogens; Antimicrobial resistance; Food safety management*

## Introduction

The meat production sector stands out as one of the major and most economically important segments of the agri-food production industry (Suychinov et al., 2024). Meats provide a rich source of proteins, amino acids, and various essential nutrients; at the same time, they are highly vulnerable to microbial colonization and subsequent spoilage processes due to the presence of moisture, abundant nitrogen-containing compounds, neutral pH levels, and nutrient-rich body tissues (Rathod et al., 2025). The growing number of contacts between animals, their body parts and the processing equipment creates ideal conditions (High moisture content, abundant nitrogenous compounds, neutral pH, and nutrient-rich tissues ) for pathogen multiplication in the meat processing facilities (Sokra et al., 2026). The industrialization process involved in the meat processing has brought about great improvements in processing efficiency and product availability; however, increased contamination risks have also been created in a complex and highly interlinked chain of meat processing stages (Zwirzitz et al., 2020). Modern slaughterhouse plants and meat production companies operate through intensive contacts of the carcass with industrial equipment, water lines, transport belts, cutting devices, people, packaging containers, etc. (Zwirzitz et al., 2020).

All these processes increase contamination risks and contribute to the need for microbial protection of meat production and processing facilities, which has become the key challenge worldwide (Belk, 2025). Every year, millions of Americans suffer from foodborne illnesses with meat products serving as a primary source of deadly infections related primarily to Salmonella and Listeria monocytogenes infections (Megahed et al., 2020). In addition to these issues, the high incidence of zoonotic agents in animal hides and intestines requires regular development of anti-microbial strategies in slaughterhouses (Yang et al., 2024). Microbial contamination during meat processing is especially dangerous due to the ability of the pathogens to persist not only as planktonic microorganisms but also in the form of biofilms adhered to the equipment surface and providing resistance against cleaning agents and disinfection procedures as well as desiccation (Bhusal et al., 2026). Such biofilms enable survival of the pathogens within meat processing facilities for long periods of time.

## Biofilms as an Emerging Challenge in Meat Processing Environments

Biofilm formation is one of the many microbial threats in meat processing settings that have proved to be extremely difficult to manage technologically. Biofilms refer to organized microorganisms encased in self-secreted EPS, firmly adhering to both biotic and abiotic surfaces (Lynch & Robertson, 2008). Unlike free-floating planktonic bacteria, biofilm-associated microbes demonstrate greater resistance to sanitizers, stress, lack of nutrients, and antibiotics (Lynch & Robertson, 2008). Biofilms can form on a variety of surfaces in meat processing plants, such as stainless steel walls, conveyor belts, cutting boards, drain pipes, refrigeration units, rubber gaskets, knives, and pipelines supplying water for washing purposes. Moisture, organic debris, fat, protein, and temperature variations provide ideal conditions for bacterial attachment and biofilm development. These microbial films act as sources of contamination, sporadically releasing pathogenic cells into the processing area and infecting meat products during subsequent production. Such biofilms provide a protective shield against disinfectants while allowing microbial interactions via quorum sensing (Melander et al., 2020).

Thus, the aim of this paper is to presents an analysis of the role of food pathogen biofilms on meat processing facilities and the risk of health hazards, concentrating on mechanisms of biofilm formation, sustainability of pathogens, and related health hazards. This work further considers the role played by processing methods in creating contamination that is resistant to sanitation and causes shortened shelf life.

## Biofilm Biology and Development Mechanisms

Biofilms are organized microbial communities irreversibly attached to biotic or abiotic surfaces and embedded within a self-produced extracellular polymeric substance (EPS) matrix. Unlike planktonic cells, biofilm-associated microorganisms function collectively within coordinated communities that exhibit enhanced adaptability and environmental persistence (Flemming et al., 2016). Biofilm formation is recognized as a major microbial survival strategy in meat-processing environments. Biofilms commonly develop on stainless steel equipment, conveyor belts, cutting tools, drains, refrigeration systems, plastics, rubber seals, and water pipelines, where pathogenic bacteria, spoilage organisms, yeasts, and fungi coexist within a shared matrix, thereby increasing resistance to cleaning and sanitation procedures (Bridier et al., 2015).

Biofilms possess heterogeneous three-dimensional structures in which variations in oxygen, pH, nutrient availability, and metabolic activity promote physiological diversification, including the formation of dormant persister cells capable of surviving antimicrobial treatments (Hall et al., 2004). The EPS matrix, composed mainly of polysaccharides, proteins, extracellular DNA (eDNA), and lipids, anchors cells to surfaces while protecting them from environmental stressors (Flemming & Wingender, 2010). The matrix also acts as a diffusion barrier that limits disinfectant penetration and enhances biofilm resistance. Biofilm formation occurs through sequential stages of attachment, colonization, maturation, and dispersion regulated by microbial genetics, environmental conditions, surface properties, and quorum sensing mechanisms.

## Biofilms in Meat Processing Facilities: Major Foodborne Pathogens and Contamination Ecology

Biofilms in meat-processing facilities are typically composed of diverse microbial communities containing both pathogenic and spoilage-associated organisms embedded within extracellular polymeric substances (EPS). Microorganisms originating from animal hides, intestinal contents, contaminated water, aerosols, equipment surfaces, and worker handling can become persistent colonizers capable of surviving routine sanitation procedures (Giaouris et al., 2014). Major biofilm-associated microorganisms in meat industries include Salmonella spp., Listeria monocytogenes, Escherichia coli O157:H7, Pseudomonas spp., and Campylobacter spp., all of which possess adaptive mechanisms that enhance persistence and foodborne disease transmission (Muhammad et al., 2020).

Salmonella spp. are among the most common pathogens associated with meat-related foodborne outbreaks worldwide. Contamination frequently originates from animal feces, intestinal leakage during slaughter, contaminated feed, transport equipment, and poorly sanitized processing surfaces (Carrasco et al., 2012). In meat and poultry-processing environments, Salmonella enterica adheres effectively to stainless steel, polyethylene, rubber, and glass surfaces through fimbriae, flagella, cellulose, and curli-mediated attachment mechanisms that promote biofilm formation (Vestby et al., 2009). Once incorporated into mature biofilms, Salmonella becomes highly tolerant to desiccation, disinfectants, and nutrient limitation, enabling long-term persistence in slaughter lines, conveyor systems, drainage areas, and cutting equipment. Persistent strains have repeatedly been isolated from meat-processing facilities despite routine sanitation, demonstrating the resistance of biofilms to conventional cleaning methods (Giaouris et al., 2015). Infections caused by Salmonella may result in gastroenteritis, septicemia, and severe systemic complications, particularly in vulnerable individuals.

Listeria monocytogenes is especially problematic because of its psychrotrophic nature and ability to survive under refrigeration conditions. The organism can persist and proliferate at temperatures below 4°C, allowing colonization of refrigeration units, cold rooms, storage areas, and chilled processing equipment (Carpentier & Cerf, 2011). Biofilm formation increases resistance to sanitizers and promotes long-term persistence in ready-to-eat (RTE) meat-processing environments, where contamination risks are particularly serious because products are consumed without additional cooking (Ferreira et al., 2014). Recurrent contamination by genetically identical Listeria strains has been documented in many facilities, indicating that biofilm-associated populations may survive for months or years. Clinically, L. monocytogenes causes listeriosis, a severe disease associated with septicemia, meningitis, fetal loss, and high hospitalization rates, particularly among pregnant women, neonates, elderly individuals, and immunocompromised patients (Finn et al., 2023).

Escherichia coli O157:H7, Pseudomonas spp., and Campylobacter spp. also contribute significantly to biofilm-associated contamination in meat industries. E. coli O157:H7 is particularly important in beef-processing systems because cattle are major reservoirs of Shiga toxin-producing E. coli (STEC). The pathogen adheres effectively to food-contact surfaces through curli fibers, fimbriae, and extracellular polysaccharides, enabling stable biofilm formation on industrial equipment (Wang et al., 2013). Biofilm-associated populations exhibit increased resistance to disinfectants and environmental stress, increasing the risk of carcass contamination and severe outbreaks involving hemorrhagic colitis and hemolytic uremic syndrome (HUS). In contrast, Pseudomonas spp. are mainly spoilage-associated psychrotrophic organisms that dominate refrigerated meat environments. Their strong EPS production and surface adhesion capabilities accelerate meat spoilage through slime formation, discoloration, off-odors, and shelf-life reduction (Doulgeraki et al., 2012). Although not always pathogenic, Pseudomonas biofilms may facilitate pathogen persistence within mixed-species communities. Campylobacter spp., particularly Campylobacter jejuni, are strongly associated with poultry-processing environments where they survive through biofilm formation and viable-but-nonculturable (VBNC) states. Although relatively sensitive under laboratory conditions, Campylobacter can persist in water systems, equipment surfaces, and poultry-processing lines when incorporated into multispecies biofilms (Teh et al., 2014).

**Detection and Characterization of Biofilms in Meat Processing Systems**

There are two factors which are considered most important when dealing with Effective control of biofilm-associated contamination in meat-processing facilities, and these are accurate detection and monitoring of microbial communities. This is because unlike planktonic microorganisms, biofilm-associated cells are embedded within extracellular polymeric substance (EPS) matrices and often exist in heterogeneous physiological states, making detection more difficult (Flemming et al., 2016). Conventional culture-based methods, including swab sampling and microbial enumeration, remain widely used for monitoring pathogens on food-contact surfaces (Chmielewski & Frank, 2003). The problem though is that these techniques are not only limited by long incubation periods and reduced sensitivity for stressed or injured cells, but they often underestimate contamination because attached microorganisms can evade routine sampling, while viable but non-culturable (VBNC) cells may persist undetected (Oliver, 2010).

To address these limitations, advanced microscopic and molecular techniques are now being used. Scanning electron microscopy (SEM) enables detailed visualization of biofilm architecture and microbial attachment on industrial surfaces, while confocal laser scanning microscopy (CLSM) provides three-dimensional imaging of intact biofilms and differentiates viable from damaged cells (Shi & Zhu, 2009; Neu & Lawrence, 2015). Then there are the molecular approaches such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) improve detection sensitivity by targeting species-specific genes and biofilm-associated markers (Law et al., 2015). More recently, metagenomic and transcriptomic analyses have enhanced understanding of biofilm ecology by identifying microbial diversity, gene expression, and interspecies interactions without reliance on culture-based isolation.

Rapid detection systems and biosensor technologies are also gaining importance for real-time industrial monitoring. ATP bioluminescence assays are commonly used for hygiene verification because of their speed and simplicity, although they cannot distinguish microbial contamination from residual organic matter (Velusamy et al., 2010). Electrochemical and optical biosensors show strong potential for detecting biofilm-associated pathogens through identification of microbial metabolites, nucleic acids, and surface antigens (Dwevedi et al., 2016). Nevertheless, biofilm detection remains challenging due to surface heterogeneity, inaccessible equipment areas, intermittent microbial shedding, and the presence of VBNC populations. Consequently, integrated monitoring approaches combining microbiological, molecular, imaging, and biosensor-based techniques are increasingly recommended for effective surveillance in meat-processing environments.

**Control and Prevention Strategies Against Biofilms in Meat Processing Facilities**

Because mature biofilms exhibit substantial resistance to disinfectants, environmental stress, and mechanical removal, effective control requires integrated interventions targeting microbial attachment, EPS stability, metabolic activity, and recolonization processes (Bridier et al., 2011). Conventional cleaning-in-place (CIP) systems remain central components of industrial hygiene programs and involve automated circulation of detergents, alkaline solutions, acids, and disinfectants through processing equipment without disassembly. These systems are designed to remove organic residues and reduce microbial loads while minimizing production downtime (Simões et al., 2010). Chemical sanitizers such as chlorine compounds, quaternary ammonium compounds, peracetic acid, and hydrogen peroxide are widely employed due to their broad-spectrum antimicrobial activity; however, their effectiveness against mature biofilms is often limited by inadequate penetration through EPS matrices and the presence of metabolically dormant persister cells (Bridier et al., 2011). Repeated exposure to sub-lethal sanitizer concentrations may additionally select for increasingly tolerant microbial populations and contribute to adaptive resistance mechanisms. These limitations have intensified interest in alternative and sustainable antimicrobial approaches capable of disrupting biofilm integrity while reducing chemical dependence within food-processing systems.

Among emerging interventions, natural antimicrobials have attracted substantial scientific and industrial attention due to their biodegradability, consumer acceptance, and broad-spectrum anti-biofilm properties. Plant-derived essential oils obtained from thyme, oregano, clove, cinnamon, rosemary, and garlic contain bioactive compounds such as thymol, carvacrol, eugenol, cinnamaldehyde, and allicin that disrupt microbial membranes, interfere with quorum sensing pathways, and inhibit EPS synthesis. These compounds have demonstrated inhibitory activity against biofilm-forming pathogens including Listeria monocytogenes, Salmonella enterica, and E. coli O157:H7 under meat-processing conditions. Organic acids such as lactic, acetic, and citric acid additionally contribute to microbial control by reducing intracellular pH and destabilizing membrane integrity, while bacteriocins such as nisin and pediocin exhibit targeted antimicrobial activity against Gram-positive pathogens, particularly Listeria monocytogenes (Gálvez et al., 2007). Phytochemicals including flavonoids, tannins, and phenolic acids further inhibit microbial adhesion and quorum sensing, thereby reducing biofilm maturation and structural stability. Importantly, synergistic combinations involving essential oils, bacteriocins, organic acids, and conventional sanitizers frequently demonstrate enhanced anti-biofilm efficacy compared with single-agent applications. Nanoencapsulation and controlled-release delivery systems have also emerged as promising technologies for improving the stability, solubility, and sustained antimicrobial activity of natural compounds within industrial environments (López-Romero et al., 2015). Nevertheless, industrial implementation remains constrained by factors including sensory alterations in meat products, volatility of essential oils, regulatory considerations, and variability in antimicrobial performance under complex processing conditions.

Additional strategies for controlling industrial biofilms include bacteriophage therapy, surface engineering technologies, enzymatic treatments, and integrated hygiene management systems. Bacteriophages are highly specific bacterial viruses capable of penetrating biofilm matrices, lysing target pathogens, and disrupting microbial communities without significantly affecting beneficial microbiota (Sillankorva et al., 2012). Phage cocktails targeting Salmonella, Listeria, and E. coli have shown promising efficacy in food-processing environments, although regulatory limitations, host specificity, and resistance development remain challenges. Surface engineering approaches, including antimicrobial coatings, nanoparticle-functionalized materials, superhydrophobic surfaces, and photocatalytic systems, are also being developed to reduce microbial adhesion and improve self-cleaning properties (Hasan & Chatterjee, 2015).

Enzymatic treatments targeting extracellular polymeric substance (EPS) components such as polysaccharides, proteins, and extracellular DNA can weaken biofilm structure and improve sanitizer penetration. Competitive exclusion strategies using beneficial microorganisms may also suppress pathogen colonization through nutrient competition and antimicrobial metabolite production. Despite advances in these technologies, long-term biofilm control still depends on effective implementation of Hazard Analysis and Critical Control Point (HACCP) systems, environmental monitoring, sanitation verification, and staff training. Strong hygiene management remains essential because advanced antimicrobial technologies alone cannot compensate for poor sanitation practices or inadequate equipment design.

**Conclusion**

Biofilms formed by foodborne pathogens in meat-processing facilities represent a major challenge to both food safety and public health. Their ability to adhere to industrial surfaces, resist sanitation procedures, and persist under harsh environmental conditions enables continuous contamination of meat products and increases the risk of foodborne outbreaks. Pathogens such as Listeria monocytogenes, Salmonella enterica, and Escherichia coli O157:H7 are particularly concerning due to their strong biofilm-forming capacity and clinical significance. Environmental factors including moisture, organic residues, poor sanitation, and surface irregularities strongly

promote biofilm development within processing systems. Consequently, conventional cleaning procedures alone are often insufficient for complete eradication. Effective control therefore requires integrated strategies involving advanced detection methods, improved hygienic design, natural antimicrobials, bacteriophage applications, and strict HACCP-based sanitation management. A deeper understanding of biofilm ecology and microbial persistence will remain essential for improving meat safety, reducing industrial contamination, and protecting public health in modern food-processing environments.

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# Current Status of Goose Breeding in the World and Türkiye

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## Abstract

Goose production represents a relatively small but economically and culturally important branch of the global poultry sector. This review aimed to provide a comprehensive overview of the current status, production characteristics, and international trade structure of goose breeding worldwide and in Türkiye using recent statistical data and relevant literature sources. Although geese are utilized for meat, fatty liver, feathers/down, egg production, and alternative production systems, global goose production remains highly concentrated geographically. According to recent FAO statistics, China overwhelmingly dominates both world goose population and goose meat production. In 2024, Asia accounted for approximately 89.66% of the global goose population, while China alone represented 88.68% of the world goose population and 98.05% of global goose meat production. In contrast, geese represented only 1.29% of the total world poultry population. In Türkiye, goose breeding is predominantly based on indigenous local populations raised under traditional free-range village systems, although semi-intensive production systems have become increasingly widespread in recent years. Kars province consistently maintained the highest goose population in Türkiye during the last decade. However, goose production in Türkiye continues to face several important constraints, including low reproductive performance, hatchability problems, limited breeding stock availability, insufficient integrated slaughtering and processing infrastructure, and regionally concentrated consumption patterns. Despite its relatively limited contribution to total poultry production, goose breeding remains an important alternative poultry sector with substantial potential for sustainable expansion. Future development of the sector will largely depend on advances in genetic improvement, reproductive management, hatchability efficiency, modern production systems, processing infrastructure, and value-added product development.

***Keywords:*** *Goose production, Goose meat, Goose trade, Poultry sector*

## Introduction

Domestic geese (family: Anatidae, order: Anseriformes) are among the earliest domesticated poultry species and are generally considered to have originated mainly from two major ancestral lineages: the Greylag goose (*Anser anser*), associated predominantly with European domestic geese, and the Swan goose (*Anser cygnoides*), associated predominantly with Chinese domestic geese (Kozák, 2019; Wen et al., 2023). In addition, based on genetic, phenetic, phylogenetic, and historical analyses, Romanov (1995) proposed six major centers of domestication, breed formation, and dispersal for domestic geese, including the West-European center comprising breeds derived mainly from the western greylag goose (*Anser anser anser*); the Chinese center comprising Chinese and African geese originating largely from the Swan goose (*Anser cygnoides*) with possible influences from *A. anser, A. indicus, A. erythropus, and Cygnus olor*; the Euro-Asiatic center formed through complex hybridization between European and Chinese domestic geese and western and eastern Greylag geese; the Egyptian center represented by the ornamental domesticated Egyptian goose (*Alopochen aegypticus*); the North-American center involving the semi-domesticated Eastern Canada goose (Branta canadensis canadensis); and the Australian center associated with the local domestication of the Cape Barren goose (*Cereopsis novaehollandiae*) (Romanov, 1995). Differences in domestication history may also be associated with morphological and production differences between European and Chinese domestic geese. Chinese domestic geese are generally smaller and lighter, with relatively higher egg production, stronger legs and wings, and a characteristic knob at the base of the beak, whereas European domestic geese are typically larger and heavier with comparatively lower egg production (Wen et al., 2023). In general, compared with their wild ancestors, domesticated geese exhibit substantial increases in body weight and egg production, along with changes in reproductive behavior, including a shift from predominantly monogamous to more polygamous mating systems, and reduced migratory characteristics. Accordingly, considerable variation in body weight exists among domestic goose breeds, ranging from 2.3 kg in Philippine geese and 2.8 kg in ganders to 9.1-10.9 kg in Toulouse geese and 11.8-13.6 kg in ganders (Kozák, 2019). Considerable variation also exists in egg production traits among domestic goose breeds, ranging from 30 eggs per female annually in Pilgrim geese to more than 200 eggs per laying period in Huoyan geese (Buckland & Guy, 2002). In addition, fatty liver production represents one of the highest-value products derived from geese, with force-feeding practices applied for approximately 14-21 days increasing liver weight from around 80 g under normal physiological conditions to approximately 600-1000 g (Boz & Sarıca, 2018). Furthermore, feathers and down obtained from domestic goose populations constitute economically valuable by-products, with feather yield

generally ranging from 200-260 g in geese weighing 3.5-4.5 kg to 290-327 g in geese weighing 5-7 kg, while total plumage weight may reach 250-300 g per bird (Kozák, 2011).

Despite their long domestication history, their commercial importance has generally remained lower than that of chickens, turkeys, and ducks because goose production is still largely based on traditional and small-scale production systems in many countries (Kozák et al., 2010). Nevertheless, geese continue to represent an economically and culturally important poultry species in several regions of the world, particularly for meat, fatty liver, feather, and down production (Kozák, 2021). This study aims to provide a comprehensive overview of the current status, production characteristics, and global production and trade patterns of goose breeding in Türkiye and worldwide using the most recent available data.

***Goose Breeding in the World***

Geese are currently raised worldwide as multipurpose poultry species because of their adaptability and economic value, particularly in East and Southeast Asia and in some Eastern European countries, where goose meat has considerable economic and cultural importance (Saatci et al., 2021). In addition to meat production, geese are utilized for egg production, fatty liver (foie gras; typically >400 g in French standards; Fernandez et al., 2019), cooking fat, and feather products (feathers and down) (Kozák, 2021). Furthermore, they are also used as alarm animals for security purposes, in biological weed control systems, and for hobby or ornamental breeding (Cilavdaroğlu et al., 2020). Accordingly, domestic goose breeds can be broadly classified according to their predominant production purposes, including meat, egg, fatty liver, feather/down, and dual-purpose production types. Heavy breeds such as Emden, Toulouse, White Italian, Rhenish, Alsatian, Danish, Czech, and White Kołuda geese are mainly associated with meat production, whereas Landes and some Hungarian goose lines are widely used for fatty liver and feather production, respectively. Chinese geese are recognized for their reproductive and egg production performance, while breeds such as Rhenish, Slovak, Cuban, Roman, and Synthetic Ukrainian geese are valued for traits including rapid growth, lean meat production, adaptability, and suitability for commercial crossbreeding (Lewko et al., 2023; Nowicka and Przybylski, 2018). In China, commercially important native breeds include Huoyan, Sichuan White, Yili (China's only native *Anser anser* derived goose breed; Dong et al., 2023), and Wanxi White geese, and hybrid goose lines have also been developed to improve meat quality and production performance (Jun et al., 2023; Yan et al., 2023). Moreover, several improved goose breeds, hybrids, and commercial lines have been developed through breeding programs in different European countries, including White Kołuda geese and the W11, W31, and W33 lines in Poland, Babat Grey and Babat White geese in Hungary, Roman (Romanola/Italian) geese in Italy, Czech (Bohemian) and Novohradská geese in the Czech Republic, Pomeranian geese in Poland and Germany, Eskildsen geese in Germany, and Stow geese in the United Kingdom, and French White (INRA) goose lines in France, as well as the G35 white goose line developed for meat production and the G36 grey goose line developed for foie gras production in France (Cilavdaroğlu et al., 2020; Kırmızıbayrak, 2018). Nevertheless, goose production is still strongly influenced by environmental conditions and seasonal reproductive patterns and is constrained by several biological and reproductive limitations, including pronounced seasonality, broodiness behavior, relatively low egg production, low fertility, poor semen quality, and mate-selection behavior (Yan et al., 2025). Depending on the breed, geese generally produce 15-60 eggs per laying season, with egg weights ranging from 130 to 210 g and fertility rates varying between 53% and 98% (Önk & Kırmızıbayrak, 2019). Accordingly, various nutritional, managerial, and reproductive biotechnological approaches have been proposed to improve reproductive performance in geese (Akhtar et al., 2021; Shi et al., 2008).

Goose production systems vary considerably among countries depending on climatic conditions, production purposes, and management traditions. In China, traditional integrated fish-goose production systems (semi-intensive) remain the predominant production model, although the use of modern intensive closed-house systems has increased in recent years. In France, Eastern European countries (particularly Poland, Hungary, Romania, and Czechia), and South America, intensive and semi-intensive systems are widely practiced, whereas extensive pasture-based production remains common in certain regions of Europe and Africa. In Türkiye, traditional free-range village production systems are still maintained; however, intensive and semi-intensive goose production systems have become increasingly widespread in recent years (Sarı et al., 2021).

Figure 1 shows that chickens overwhelmingly dominated the global poultry population in 2024, accounting for 93.50% of the total population. In contrast, ducks, geese, and turkeys represented substantially smaller proportions at 4.31%, 1.29%, and 0.82%, respectively, while other poultry species accounted for only 0.08%.

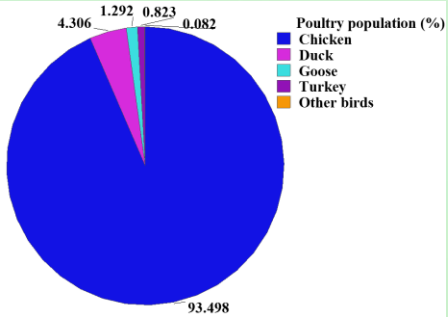



Figure 1. Proportional distribution of the world poultry population by species in 2024 (FAO, 2026)

Figure 2a shows that the global goose population increased from 375.03 million head in 2020 to 382.51 million head in 2024, despite a slight decline in 2022. This trend indicates the continued expansion of global goose production in recent years. Figure 2b demonstrates that, despite this numerical increase, the relative contribution of geese to the global poultry population declined slightly from 1.35% in 2020 to 1.29% in 2024. However, ducks maintained a relatively stable share at 4.3%, whereas the proportion of turkeys decreased from 0.96% to 0.82% during the same period.

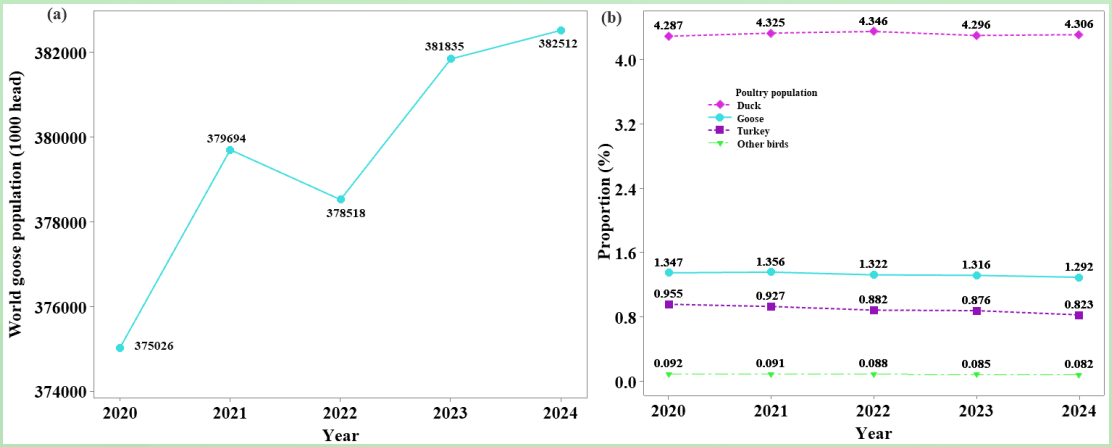



Figure 2. World goose population and its proportion within the poultry population between 2020 and 2024 (FAO, 2026)

The global goose population was estimated at approximately 359.12 million head worldwide (FAO, 2026). Asia dominated the world goose population in 2024, accounting for approximately 89.66% of the global total (342.95 million head). Africa and Europe contributed 6.07% (23.23 million head) and 3.79% (14.51 million head), respectively, whereas the Americas and Oceania represented only 0.45% and 0.02% of the world goose population (Figure 3).

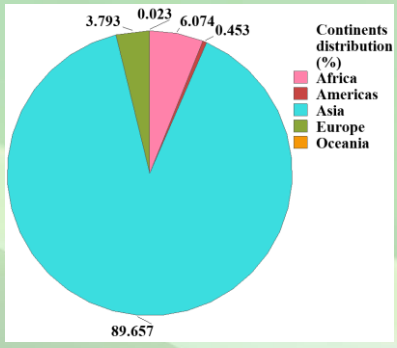



Figure 3. Proportional distribution of the world goose population by continent in 2024 (FAO, 2026)

As presented in Table 1, the global goose population in 2024 was overwhelmingly dominated by China, which accounted for 88.68% of the world total. Mozambique and Myanmar ranked second and third, respectively, whereas Türkiye represented 0.35% of the global goose population.

Table 1. Leading countries in world goose population and their proportional shares in 2024 (FAO, 2026)

| *Country* | *Goose population (1000 head)* | *Proportion (%)* |
|---|---|---|
| China | 333082 | 88.680 |
| Mozambique | 16473 | 4.386 |
| Myanmar | 5857 | 1.559 |
| Russian Fed. | 3577 | 0.953 |
| Egypt | 3524 | 0.938 |
| Madagascar | 3009 | 0.801 |
| Ukraine | 2964 | 0.789 |
| Türkiye | 1303 | 0.347 |
| Venezuela | 1000 | 0.266 |
| Iran | 984 | 0.262 |
| Other countries | 3828 | 1.019 |
| World total | 359128 | 100.000 |

Under organic dehesa production conditions in Spain, female live weight in goose populations ranged from 4.81 to 5.20 kg, whereas male live weight ranged from 5.13 to 6.11 kg at 30-32 weeks of age in Embden, Toulouse, and Embden × Toulouse F1 cross populations (Solé et al., 2015). In Lindovskaya goose populations reared under semi-intensive grazing conditions, female and male live weights reached 3.02 and 3.61 kg, respectively, at 16 weeks of age (Akbaş et al., 2020). In another study conducted under semi-intensive production conditions, with geese subjected to oat fattening during the final three weeks before slaughter, female and male live weights in White Kołuda (W31) goose populations reached 6.17 and 6.74 kg, respectively, at 16 weeks of age (Kucharska-Gaca et al., 2022). Similarly, in a Canadian study conducted exclusively on male goose strains and Embden-sired crosses reared under semi-intensive pasture-based production conditions, 16-week body weight ranged from 4.90 kg in the selected synthetic strain to 6.43 kg in the large Embden × selected Chinese cross, while dressing percentage (eviscerated carcass basis) varied between 60.1% and 62.5% (Shrestha & Grunder, 2005). In another study conducted on indigenous goose populations reared in the Afyon region of Türkiye, hot carcass yield values were 64.1% and 62.0% in males and females, respectively, whereas corresponding cold carcass yields decreased to 62.6% and 61.5% (Peşmen and Yönetken, 2020). However, lower carcass yield values were reported in other goose populations, including 59.0% in white Chinese and 56.8% in grey Chinese geese (Chaiwang et al., 2025), as well as hot carcass yield values of 59.2%, 58.1%, and 57.6% in Linda, Toulouse, and Mast goose populations, respectively (Aslan et al., 2024); both studies included equal numbers of male and female geese. Likewise, relatively high cold carcass yield values were also reported under semi-intensive production systems, with carcass yields of 82.1% and 77.4% in male and female Anatolian goose populations, respectively, whereas the corresponding values in Mast goose populations were 81.0% and 78.1% (calculated from Ürer et al., 2026).

Goose meat is considered a nutritionally valuable poultry product because of its high protein content (approximately 22%) and favorable fatty acid composition, particularly its richness in monounsaturated and polyunsaturated fatty acids. In addition, goose meat provides important minerals such as iron, zinc, phosphorus, iodine, and selenium, while goose fat contains relatively lower levels of saturated fatty acids compared with many other animal fats, thereby contributing to its nutritional and dietary value (Krunt & Zita, 2026). Although goose meat possesses high nutritional and economic value, its consumption remains relatively limited globally and is largely concentrated in particular regions and periods as part of traditional local consumption patterns (Gündüz et al., 2019). Nevertheless, compared with other poultry species, both goose production and goose meat consumption have remained relatively limited worldwide (Figure 1 and 4c). Average global goose meat consumption is estimated at 0.343 kg per capita per year. China represents the leading country in goose meat consumption, with an annual per capita consumption of approximately 1.73 kg, while France ranks among the major consumers, largely due to the traditional production and consumption of foie gras (Vorona & Makarynska, 2022).

Marked species-specific differences were evident in the global concentration patterns of poultry meat production in 2024 (Figure 4). Chicken meat production exhibited the most geographically dispersed production structure among the evaluated poultry species, with the United States (20.18 million t; 15.73%), China (16.07 million t; 12.52%), and Brazil (13.71 million t; 10.69%) accounting for the largest shares of total world production. Overall, the leading chicken meat-producing countries contributed 59.39% of global production, indicating a relatively diversified international production system (Figure 4a) (FAO, 2026).

In contrast, duck meat production demonstrated a markedly concentrated pattern dominated by China, which produced approximately 6.04 million t and accounted for 82.33% of world production, whereas the remaining leading producer countries individually contributed comparatively minor shares (Figure 4b). Goose meat production exhibited the highest degree of geographic concentration, with China alone producing 5.30 million t and accounting for 98.05% of total global production, reflecting an exceptionally centralized production structure (Figure 4c). Although the Russian Federation was represented among the countries with notable goose stock

populations in the FAO database (FAO, 2026), corresponding data on slaughtered animals, carcass yield, and goose meat production for 2024 were not available.

Global turkey meat production displayed an intermediate concentration pattern, led by the United States with approximately 2.32 million t corresponding to 45.55% of world production, followed by Germany (404 thousand t; 7.95%) and Poland (349 thousand t; 6.87%) (Figure 4d) (FAO, 2026). Collectively, these findings highlight substantial interspecific variation in the geographic distribution and concentration dynamics of global poultry meat production.

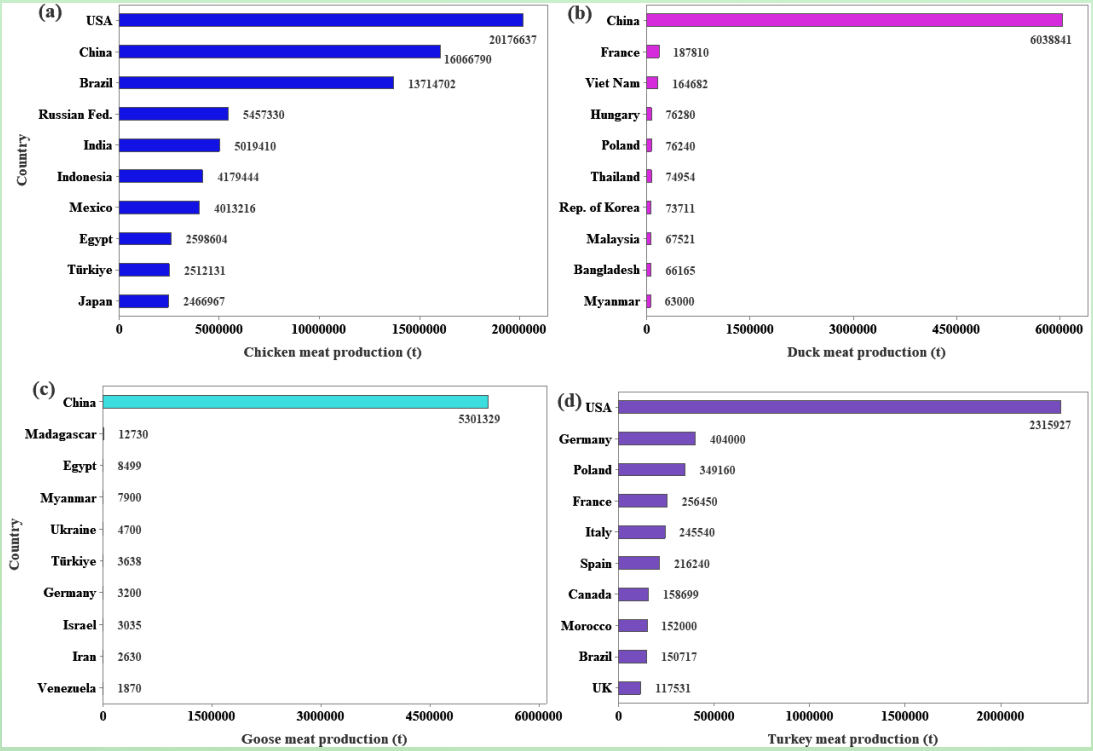



Figure 4. Leading poultry meat producing countries by species in 2024 (different X-axis scales) (FAO, 2026)

Germany, based on FAO official records, consistently exhibited the highest goose carcass weights between 2020 and 2024, ranging from 7.08 to 7.55 kg. Although based on FAO estimated values, China ranked second and showed a marked increase in carcass weight from 5.85 kg in 2020 to 6.89 kg in 2024, representing an increase of approximately 1.00 kg within five years and approaching the levels reported for Germany. However, Canada and the United Kingdom maintained relatively stable carcass weights at approximately 5.00 and 4.70 kg, respectively, whereas New Zealand, Norway, and France displayed comparatively lower values around 4.00-4.30 kg (Figure 5). According to FAO estimated values, Türkiye maintained a constant goose carcass weight of approximately 2.50 kg during the same period (FAO, 2026). Overall, substantial variation in goose carcass weight was observed among countries, with nearly a twofold difference even among the leading countries.

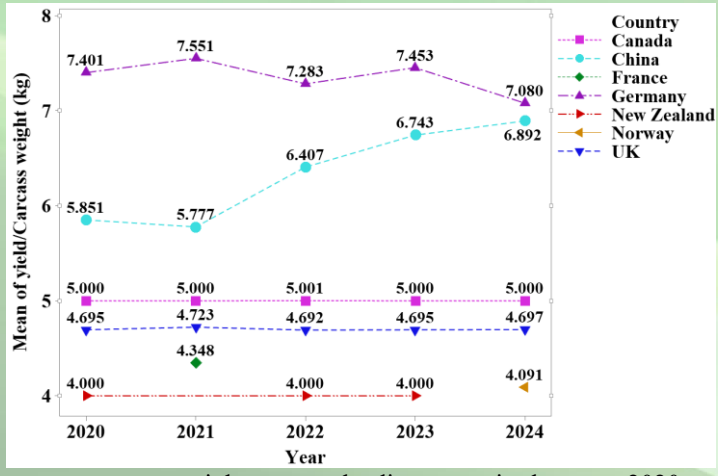



Figure 5. Changes in goose carcass weights among leading countries between 2020 and 2024 (FAO, 2026)

According to FAO official statistics for 2024, Albania and Hungary were the leading importing countries for live geese, importing 992 thousand and 907 thousand head, respectively, whereas Poland and Hungary were the leading exporting countries, exporting 488 thousand and 191 thousand head, respectively (FAO, 2026).

As presented in Table 2, the international trade of goose meat in 2024 was largely dominated by a limited number of countries. China and Germany accounted for 39.90% and 38.22% of global imports, respectively, whereas China (39.34%), Poland (37.35%), and Hungary (20.29%) emerged as the principal exporting countries in the global goose meat market.

Table 2. Leading countries importing and exporting goose meat in 2024 (FAO, 2026)

| *Importing countries* | *Import quantity (t)* | *Proportion (%)* | *Exporting countries* | *Export quantity (t)* | *Proportion (%)* |
|---|---|---|---|---|---|
| China | 15679.47 | 39.90 | China | 15433.45 | 39.34 |
| Germany | 15019.93 | 38.22 | Poland | 14650.10 | 37.35 |
| France | 2227.10 | 5.67 | Hungary | 7960.71 | 20.29 |
| Czechia | 1971.62 | 5.02 | Germany | 405.37 | 1.03 |
| Austria | 1110.10 | 2.82 | Austria | 200.13 | 0.51 |
| Other countries | 3290.79 | 8.37 | Other countries | 579.17 | 1.48 |
| World total | 39299.01 | 100.00 | World total | 39228.93 | 100.00 |

The global trade of edible goose offal in 2024 exhibited a marked regional concentration. Israel accounted for the largest share of global imports with 38.31%, followed by Germany (14.40%) and China (9.68%), whereas Hungary and Poland dominated global exports, representing 43.63% and 41.89% of total exports, respectively (Table 3).

Table 3. Leading countries importing and exporting edible goose offal (including liver) in 2024 (FAO, 2026)

| *Importing countries* | *Import quantity (t)* | *Proportion (%)* | *Exporting countries* | *Export quantity (t)* | *Proportion (%)* |
|---|---|---|---|---|---|
| Israel | 980.11 | 38.31 | Hungary | 367.90 | 43.63 |
| Germany | 368.42 | 14.40 | Poland | 353.19 | 41.89 |
| China | 247.63 | 9.68 | Belgium | 44.65 | 5.30 |
| Czechia | 192.56 | 7.53 | Germany | 35.35 | 4.19 |
| Belgium | 168.05 | 6.56 | France | 12.53 | 1.49 |
| Other countries | 601.91 | 23.52 | Other countries | 29.51 | 3.50 |
| World total | 2558.68 | 100.00 | World total | 843.13 | 100.00 |

The global market value of goose down is reported to range between 100-130 USD per kilogram. Furthermore, in European Union countries, the purchase price of goose fat is higher than that of butter (Vorona & Makarynska, 2022). Foie gras, regarded as one of the most valuable products derived from goose production, has an estimated market value of approximately 30-40 USD per kilogram. France accounts for nearly 80% of global foie gras production and consumption, while China, France, and Bulgaria are among the leading exporting countries. Moreover, notable production activities in Canada, Germany, and Poland, together with high consumer demand in countries such as Spain, Switzerland, and Belgium, indicate the widespread commercial importance of foie gras in international markets (Czibolya, 2015; Guemene & Guy, 2004; Karadağ, 2025). The international goose product market is further shaped by strong consumer demand for high-value specialty products, particularly in affluent European countries where foie gras and goose fat constitute important niche commodities (Dumlu, 2024).

***Goose Breeding in Türkiye***

Goose production is practiced across all regions of Türkiye, and indigenous Turkish geese constitute the majority of the national goose population, with goose breeding being primarily oriented toward meat production (Önk & Kırmızıbayrak, 2019; Kırmızıbayrak, 2020). Indigenous Turkish geese are generally classified according to plumage coloration, including white, black, pied (black–white), brown-yellow, and grey (ash-colored) varieties, which may be raised either separately or in mixed flocks (Akın and Çelen, 2020; Arslan, 2018; Boz and Sarıca, 2018; Cilavdaroğlu et al., 2020). These indigenous genotypes are generally characterized by relatively low growth and reproductive performance, with egg production typically remaining around 15-20 eggs per season. Nevertheless, considerable phenotypic variation exists among regional populations, and geese raised in Kars and Ardahan provinces, particularly in Ardahan-Sugöze, Kars Central, and Akyaka, are recognized for their comparatively larger body size and more developed body conformation (Kırmızıbayrak, 2018). In indigenous Turkish goose populations, female live weight ranged from 3.87 to 4.69 kg, whereas male live weight ranged from 4.54 to 5.73 kg between 16 and 18 weeks of age across intensive and free-range production systems (Boz et al., 2017).

In Türkiye, several exotic goose genotypes have been introduced over time, including 12,000 Romanola goose eggs imported from Italy in 1988 and 2,040 INRA goslings introduced through an FAO project in 1993. In recent years, producers have also introduced breeds such as Embden, Toulouse, African, and Chinese geese to meet

increasing demand, and crosses involving these genotypes and indigenous Turkish geese are still present in local populations (Boz & Sarıca, 2018).

Table 4. Goose population and estimated production data in Türkiye between 2016 and 2025 (FAO, 2026; TÜİK, 2026)

| *Year* | *Live goose population (head)*[a] | *Slaughtered geese (head)*[b] | *Goose meat production (kg)*[b] | *Goose carcass weight (kg)*[b] |
|---|---|---|---|---|
| 2016 | 933353 | 1020000 | 2548870 | 2.5 |
| 2017 | 978384 | 1072000 | 2679480 | 2.5 |
| 2018 | 1080190 | 1184000 | 2960470 | 2.5 |
| 2019 | 1157049 | 1273000 | 3181450 | 2.5 |
| 2020 | 1373960 | 1517000 | 3791670 | 2.5 |
| 2021 | 1477569 | 1636000 | 4089430 | 2.5 |
| 2022 | 1385507 | 1538000 | 3845730 | 2.5 |
| 2023 | 1328175 | 1479000 | 3697230 | 2.5 |
| 2024 | 1303026 | 1455000 | 3637660 | 2.5 |
| 2025 | 1263907 | - | - | - |

a: TÜİK official statistics, b: FAO estimated or imputed values obtained from FAOSTAT database.

Table 4 summarizes goose population data together with FAO estimated production data and derived ratios for Türkiye between 2016 and 2025. Goose carcass weight remained relatively stable at 2.50 kg. The live goose population in Türkiye increased markedly between 2016 and 2021, rising from 933,353 to 1,477,569 head (Table 1 and Figure 6a). After peaking in 2021, the population gradually declined to 1,263,907 head by 2025. Despite these fluctuations, geese consistently represented a relatively small proportion of the total poultry population in Türkiye, ranging from 0.28% to 0.38% (Figure 6b). Chickens overwhelmingly dominated the poultry population between 2016 and 2025 (>98%), whereas geese accounted for less than 0.40% of the total poultry population.

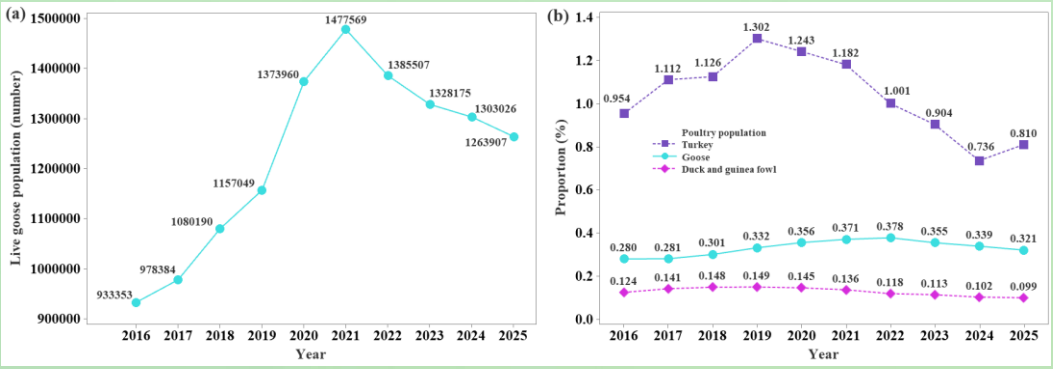



Figure 6. Live goose population and its proportion within poultry in Türkiye (TÜİK, 2026)

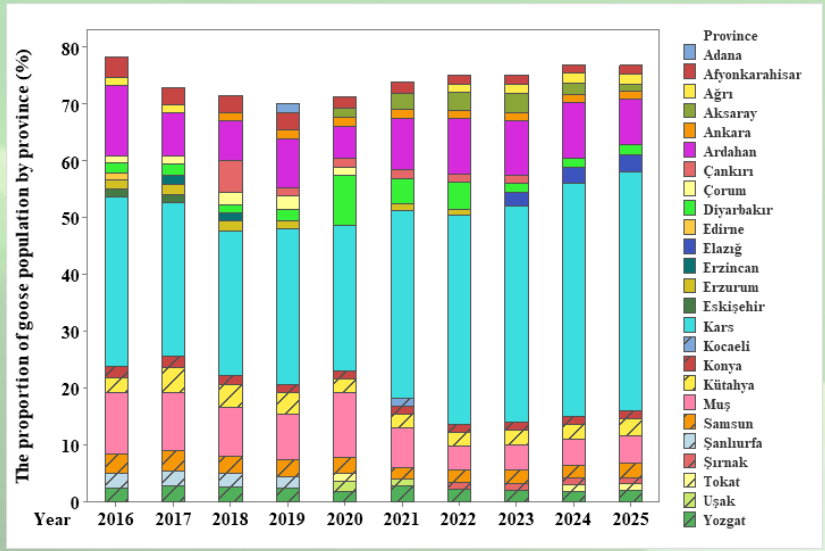



Figure 7. Leading 15 provinces by live goose population ratio over the past decade (alphabetically listed) (TÜİK, 2026).

During the last decade, Kars consistently maintained the highest live goose population in Türkiye, accounting for 29.91% (279,200 head) of the national goose population in 2016 and increasing to 42.14% (532,560 head) in 2025. Ardahan and Muş generally ranked second and third, respectively. Ardahan accounted for 12.25% (114,311 head) of the live goose population in 2016 and 8.13% (102,798 head) in 2025, whereas Muş accounted for 10.82% (100,965 head) and 4.85% (61,256 head) in the respective years. However, Diyarbakır ranked third in 2020 with 121,354 head (8.83%) and in 2022 with 64,919 head (4.69%), surpassing Ardahan and Muş in the respective years (TÜİK, 2026).

Afyonkarahisar, Samsun, Kütahya, Yozgat, Konya and Diyarbakır were consistently included among the leading 15 provinces with the highest live goose population throughout the evaluated years (Figure 7). Elazığ, which was not represented among the leading provinces in the earlier years, ranked sixth in 2023 (32,887 head; 2.48%) and fourth in both 2024 (37,874 head; 2.91%) and 2025 (37,825 head; 2.99%) (TÜİK, 2026).

Aksaray was represented among the leading provinces after 2020, ranking sixth in 2021 (41,870 head; 2.83%) and fifth in 2022 (44,150 head; 3.19%); however, its relative ranking declined to twelfth place in 2025. In contrast, Çankırı ranked fourth in 2018 (58,982 head; 5.46%) but did not maintain this position in the subsequent years and was not included among the top 15 provinces in 2024 and 2025. Edirne and Adana were represented among the leading 15 provinces only in 2016 and 2019, respectively (Figure 7).

Diyarbakır exhibited a marked increase in live goose population between 2020 and 2022, ranking third in both 2020 (121,354 head; 8.83%) and 2022 (64,919 head; 4.69%), and fourth in 2021 (65,342 head; 4.42%). However, its relative contribution declined in the subsequent years, falling to eleventh place in 2023 and ninth place in 2024 and 2025 (TÜİK, 2026).

These provinces are considered highly suitable for goose production due to their favorable climatic and rural production conditions, where traditional extensive production systems based on pasture grazing and limited concentrate feed use are still predominantly practiced. Nevertheless, increasing feed costs and the gradual decline of available agricultural and grazing lands continue to threaten the sustainability of goose production systems in Türkiye (Akın, 2023).

Despite the growing interest in goose products, demand remains limited and regionally concentrated in Türkiye. In addition, goose production is constrained by several factors, including low egg production, difficulties in obtaining fertile eggs, hatchability-related problems, the limited availability of breeding eggs and breeding stock, and the limited number of scientific studies conducted on geese (Akın, 2023; Saatci et al., 2021). Akın & Çelen (2020) further reported that governmental credit and support programs for goose production were largely concentrated in Kars and surrounding regions, while the absence of integrated production and slaughtering facilities similar to those in the broiler sector, together with the seasonally concentrated consumption pattern of goose meat, limited the sustainable development and commercialization of goose production in Türkiye.

## Conclusion

Global goose production remains a highly specialized branch of the poultry sector characterized by substantial diversity in breed structure, production purposes, and management systems. Although geese represent only a limited proportion of the total world poultry population, goose breeding continues to maintain economic and cultural importance in several regions, particularly in East and Southeast Asia and certain Eastern European countries. Current FAO statistics clearly demonstrate the exceptionally concentrated structure of the global goose sector, with China overwhelmingly dominating both goose population and goose meat production worldwide.

The literature reviewed in the present study indicates considerable variation among goose breeds and production systems with respect to live weight, carcass yield, and production characteristics. Furthermore, production performance differs substantially according to genotype, sex, and management conditions. Goose production systems worldwide range from traditional extensive pasture-based systems to modern semi-intensive and intensive commercial production models.

In Türkiye, goose breeding remains predominantly based on indigenous local populations raised under traditional village-type free-range systems, although semi-intensive systems have become increasingly widespread in recent years. Kars province continues to represent the principal goose production center of the country. However, the Turkish goose sector still faces several important limitations, including low reproductive performance, hatchability problems, insufficient breeding stock availability, limited integrated slaughtering and processing infrastructure, and seasonally concentrated consumption patterns.

The available literature and current production statistics indicate that goose breeding still possesses substantial development potential both globally and in Türkiye. Improvements in genetic resources, hatchability performance, modern production systems, and processing infrastructure may contribute considerably to the sustainable expansion and commercialization of goose production in the future.

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# Effects of Leaf Removal and Shoot Tipping Applied at Different Stages an Some Yield and Quality Characteristics of Kalecik Karası (*Vitis vinifera* L.) Grape Cultivar Grown under Ankara Conditions

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## Abstract

This study investigated the effects of summer pruning practices (leaf removal, shoot topping, and a combination of both) applied at two different phenological stages (bunch closure and veraison) on the yield and quality of the Kalecik Karası (*Vitis vinifera* L.) grape variety grown under Ankara conditions. The research was conducted at the Research and Application Vineyard of the Department of Horticulture, Faculty of Agriculture, Ankara University. The evaluated parameters included yield per vine, number of clusters per vine, berry weight, cluster length and width, total soluble solids (TSS), pH, titratable Acidity (TA), and total phenolic content. It was determined that the shoot topping treatment improved the values of the parameters examined regarding yield and important morphological characteristics. The highest number of clusters per vine (43.50 clusters/vine) was obtained from the shoot topping treatment applied during the bunch closure stage. In both phenological stages, TSS values were significantly higher in the leaf removal treatment compared to other treatments. For the leaf removal application, the highest total phenolic content was observed both the bunch closure and veraison stages. Correlation and principal component analysis (PCA) revealed strong relationships between cluster characteristics, yield, and fruit quality parameters. In conclusion, identifying optimal summer pruning timing for the Kalecik Karası variety provides a scientific basis for enhancing fruit quality and yield, offering valuable insights for viticultural practices in the Ankara region.

***Keywords:*** *Kalecik Karası, summer pruning, phenological stages, yield, quality, TSS*

## Ankara Koşullarında Yetiştirilen Kalecik Karası (*Vitis vinifera* L.) Üzüm Çeşidinde Farklı Dönemlerde Yapılan Yaprak Alma ve Uç Alma Uygulamalarının Bazı Verim ve Kalite Özelliklerine Etkileri

## Özet

Bu araştırmada, Ankara koşullarında yetiştirilen Kalecik Karası (*Vitis vinifera* L.) üzüm çeşidinde iki farklı fenolojik dönemde (iri koruk ve ben düşme) uygulanan yaz budamalarının (yaprak alma, uç alma, yaprak alma-uç alma) verim ve kalite üzerine etkisi araştırılmıştır. Çalışma, Ankara Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Araştırma ve Uygulama Bağında yetiştirilen üzümlerde gerçekleştirilmiştir. Denemede omca başına verim, omca başına salkım sayısı, tane ağırlığı, salkım boyu ve eni, suda çözünebilir kuru madde (SÇKM), pH, titre edilebilir asitlik (TEA), toplam fenolik madde parametreleri incelenmiştir. Uç alma uygulamasının verim ve önemli morfolojik özelliklerinde incelenen parametre değerlerini iyileştirdiği tespit edilmiştir. En yüksek omca başına salkım sayısı, iri koruk döneminde (43,50 adet/asma) uç alma uygulamasında elde edilmiştir. Uygulama yapılan her iki dönem içerisinde de yaprak alma uygulamalarından daha yüksek SÇKM değerini sağladığı belirlenmiştir. Yaprak alma uygulamasında hem iri koruk hem de ben düşme dönemlerinde en yüksek toplam fenolik bileşik sonuçları saptanmıştır. Korelasyon ve temel bileşenler analizi (PCA) sonuçları, salkım özellikleri, verim parametreleri ve meyve kalite parametreleri arasında güçlü ilişkiler bulunduğunu ortaya koymuştur. Sonuç olarak, Ankara ili bağcılığı için önemli bir tarımsal ürün olan Kalecik Karası üzüm çeşidinde en uygun yaz budaması zamanlarının belirlenmesi, bölge bağcılığına verim ve kalite artışı sağlayarak uygulamaların üreticilere kazandırılması açısından büyük önem taşımaktadır.

***Anahtar kelimeler:*** *Kalecik karası, yaz budaması, fenolojik, verim, kalite, SÇKM*

## Giriş

Kökeni Avrupa kıtasına dayanan ve en eski kültür bitkileri arasında yer alan *Vitis vinifera* L., insanlık tarihi boyunca kıtalar arası bir yayılım göstermiş ve ulaşılan yeni coğrafyalarda *Vitis* cinsine (*Vitis* spp.) ait farklı türlerin de tanımlanmasıyla bağcılık kültürü büyük ölçüde zenginleşmiştir (Sucu ve Yağcı, 2020). Bağcılık; yeryüzünde kuzey yarım kürede 30-50º, güney yarım kürede ise 30-40º enlem dereceleri arasında yapılmaktadır (Ünal ve ark., 2022).

Bitkisel üretimde önemli bir yere sahip olan üzüm, iklim ve toprak yönünden fazla seçici olmaması ve alternatif değerlendirme olanaklarına sahip olması nedeniyle dünyada ve Türkiye' de yaygın kültür bitkilerinden biridir (Semerci ve ark. 2015). Ülkemiz, küresel bağ alanlarının varlığı ve genişliği bazındaki dünya sıralamasında 6. basamakta konumlanırken, elde edilen toplam üzüm miktarı yönüyle dünya genelinde 7. sırada yer almaktadır (FAOSTAT, 2026). Türkiye' deki toplam meyve ürünleri, içecek ve baharat bitkileri üretim projeksiyonu içerisinde üzüm bitkisinin payı %23' tür ve bu oran 2.515.152 tonluk bir üretime denk gelmektedir. Üretilen toplam üzüm miktarının kullanım amaçlarına göre oransal dağılımı; sofralık tüketimde %58,11, kurutmalık kullanımda %32,74 ve şaraplık olarak değerlendirilen kısımda ise %9,15 olarak tespit edilmiştir (TÜİK, 2026). Üzüm; ülkemiz sınırları dahilinde taze (sofralık), kurutmalık ve şaraplık olarak doğrudan tüketime ve endüstriyel kullanıma sunulmasının yanı sıra, geleneksel mutfak kültürümüze bağlı olarak üzüm suyu, pekmez, sirke, pestil, köme ve köfter gibi katma değeri yüksek ürünlere dönüştürülerek de değerlendirilmektedir. (Aras Aşçı ve ark., 2021; Sezgin, 2021).

Ankara'nın Kalecik ilçesinde en iyi şekilde yetişebilen "Kalecik Karası" üzüm çeşidi İç Anadolu Bölgesindeki kaliteli kırmızı şaraplık çeşitlerimizin başında gelmektedir. Taneleri yuvarlak, siyah-mor renkte, kabukları kalın, etli kısmı yumuşak ve şıralıdır. Tanelerinde 1-2 çekirdek bulunur, genelde eylül ayında olgunlaşır (Başaran, 2006). İklim açısından, özellikle şaraplık üzüm yetiştiriciliği için ülkemizin en elverişli bölgeleri içerisinde kabul edilen İç Anadolu Bölgesi bağcılığında verim ve kalite arzu edilen derecede yüksek değildir. Birim alandan daha fazla miktarda ve daha kaliteli ürün elde edilmesi için farklı uygulamaların denenmesi ve bulguların değerlendirilerek yeni veya farklı yöntemlerin geliştirilmesi için başlayan çalışmalar halen devam etmektedir. Bu çalışmaların önemli bir bölümünü ise farklı ekolojilerde çeşitlere özgü yaz budaması çalışmaları oluşturmaktadır.

Asma yetiştiriciliğinde yapılan yaz budaması uygulamaları, uç alma, tepe alma, koltuk alma, yaprak alma ve salkımlarda uygulanan işlemlerdir. Uç alma uygulaması, genç omçaların taç oluşumunu ve şekillenmesini desteklemesinin yanı sıra, rüzgâr kaynaklı hasar riskini minimize etmek ve tane tutumunu artırmak amacıyla uygulanmaktadır. Yaprak alma uygulaması salkımların renklenmesinin, kaliteli ve homojen bir şekilde gerçekleşmesini sağlamak amacıyla uygulanmaktadır (Zinni, 2020; Bekar, 2016).

Bir bağda birkaç yıl seyrek tane tutumu gerçekleşiyor ise çiçeklenme öncesi uç alma uygulaması tavsiye edilmektedir. Çiçeklenme sonrası yapılan uç alma ise tane iriliğini artırmak, rüzgârdan kaynaklı hasar riskini önlemek, salkım ve yan yüzeydeki yaprakların gölgelenmesini engellemek gibi amaçlar için yapılmaktadır (Akural, 2016). Yeşil budama uygulamalarından uç alma ve yaprak seyreltme asma gözleri içerisindeki generatif oluşum üzerine teşvik edici veya engelleyici olabilmektedir.

Olgunlaşma sırasında asma salkım bölgesinde yapılan yaprak alma uygulaması ile tanelerin daha fazla ışık görmesine, tanelerde asiditenin azalmasına, tatlanma ve renklenmenin artmasına, ayrıca üzümlerin daha iyi havalanması ve nispi nemin azalmasından dolayı kurşuni küf *(Botrytis spp.)* vb. hastalıkların azaldığı gözlemlenmiştir (Sezgin, 2021). Altıntaş ve ark. (2023), yaptıkları çalışmada bağlarda yaz budamasının hastalıkları bir ölçüde engellemesinin olumlu etkilerinde söz etmişlerdir. Miele (2020) tarafından Merlot üzüm çeşidinde yaz budamasının; çiçeklenme döneminde yapılan tepe alma işleminin ve salkımların alt kısmında yer alan yaprakların tamamının alınmasının şarap bileşimindeki antosiyanin ve tanen miktarını, alkol oranını olumlu yönde etkilediği tespit edilmiştir.

Kalecik Karası üzüm çeşidinde verimliliğin artırılmasına yönelik çalışmalar günümüze kadar yapılmakla birlikte; bu çalışmalar daha çok tek yönlü olarak kalmış, verim ve kalite unsurlarının artırılmasına yönelik araştırmalar sınırlı sayıda kalmıştır. Şaraplık üzüm çeşitlerinde ekolojik koşullara uygun farklı uygulamalar ile özellikle kalitenin artırılmasına yönelik çalışmalar oldukça önem arz etmektedir. Bu araştırmalar içerisinde farklı şaraplık üzüm çeşitlerinde, farklı dönemlerde uygulanan yaz budamaları büyük bir payı oluşturmaktadır. Bu araştırma ile de Ankara ili koşullarında, Kalecik Karası üzüm çeşidi için farklı dönemlerde uygulanan bazı yaz budamalarının verim ve bazı kalite parametreleri üzerine etkisi belirlenmeye çalışılmıştır.

## Materyal ve Yöntem

### *Materyal*

Araştırmada, Ankara Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Araştırma ve Uygulama Bağındaki Kalecik Karası üzüm çeşidi bitkisel materyal olarak kullanılmıştır.

### *Yöntem*

Bu çalışma, 849 m rakıma sahip, Ankara ilinde yer alan Ankara Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Araştırma ve Uygulama Bağında 2021/2022 yılı üretim döneminde yürütülmüştür. Bağ alanında yıllık bakım ve teknikler; zamanında ve tekniğine uygun olarak yürütülmüştür. Kalecik Karası üzüm çeşidi parselinde 2 farklı dönemde gerçekleştirilen uygulamalar için toplam 48 adet omca kullanılmıştır. Her uygulama iki tekerrür halinde gerçekleştirilmiş ve her tekerrürde 24 omca kullanılmıştır.

Yaz budaması iki farklı fenolojik dönemde gerçekleştirilmiştir.

- İri Koruk (İK): Kalecik karası üzüm çeşidi iri koruk döneminin %50 oranında gerçekleştiği tarihlerde yapılmıştır.
- Ben Düşme (BD): Kalecik Karası üzüm çeşidi ben düşme döneminin %50 oranında gerçekleştiği tarihlerde yapılmıştır.

Gelişme dönemlerinde üç farklı yaz budaması uygulaması yapılmıştır:

- Kontrol (K): Her iki fenolojik dönemde yaprak alma ve uç alma uygulaması yapılmamıştır.
- Uç Alma (UA): İki fenolojik döneminde Kalecik Karası üzüm çeşidi uç alma işlemi son salkımdan 12. yapraktan sonra yapılmıştır.
- Yaprak Alma (YA): İki fenolojik döneminde Kalecik Karası üzüm çeşidi yaz sürgünündeki ilk 5 yaprak alınmıştır.
- Yaprak Alma- Uç Alma (YA-UA): İki fenolojik döneminde yaz sürgünündeki ilk 5 yaprak alınmış ve uç alma işlemi son salkımdan sonraki 12. yapraktan sonra yapılmıştır.



Şekil 1. Kalecik Karası parselinde budama uygulamasına ait görüntüler

*Çalışmada incelenen parametreler*

1. Verim unsurları

a) Omca Başına Verim (kg/omca): Hasat sırasında toplanan salkımların tartılarak omca sayısına bölünmesi ile elde edilmiştir.

b) Omca Başına Salkım Sayısı (adet/asma): Her bir deneme omcasındaki salkımlar sayılarak belirlenmiştir.

2. Morfolojik özellikler

a) Tane ağırlığı (g): Her omcadan tesadüfen alınan 100 adet tanenin ağırlıklarının ölçülüp 100'e bölünmesi ile belirlenmiştir.

b) Tane boyu (mm): Her omcadan tesadüfen alınan taneler kumpas yardımıyla ölçülerek belirlenmiştir.

c) Salkım boyu ve eni (cm): Her omcadan tesadüfen alınan 10'ar adet salkımın boyu ve eni cetvel yardımıyla ölçülerek belirlenmiştir.




Şekil 2. Üzümlerde ağırlık, en ve boy ölçümü

3. Kimyasal özellikler

Bu kapsamda yer alan analizler, asmaların değişik yöneylerinden alınan salkımların üst, orta ve alt kısmından seçilen 100 adet tane üzerinde gerçekleştirilmiştir.

a) SÇKM (%): El refraktometresi ile saptanmıştır.

b) pH: Mettler Toledo marka pH metre cihazı ile ölçüm yapılmıştır.
c) Titre edilebilir asit (TEA) miktarı (%): Otomatik titratör aleti ile belirlenmiştir.
d) Toplam fenolik madde: Üzüm sularında toplam fenolik bileşik analizleri Singletton ve Rossi (1965)'ye göre modifiye edilerek yapılmıştır. Okumalar "SHIMADZU" marka "UV-1208" model spektrofotometre cihazı ile 765 nm dalga boyunda yapılmıştır.

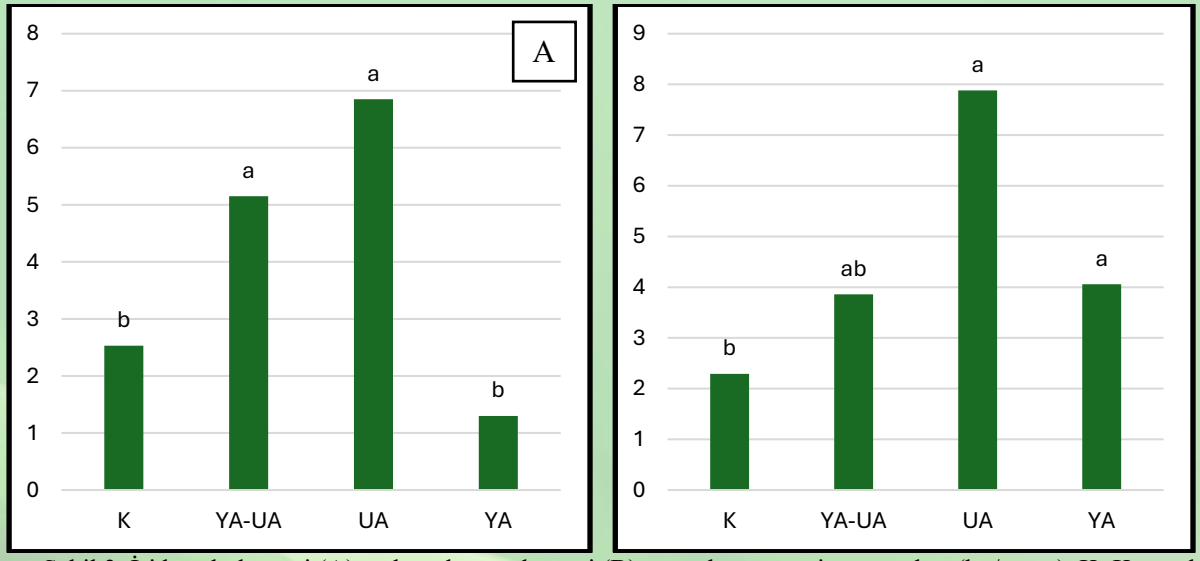
*İstatistiki Analiz*

Araştırma Tesadüf Blokları Deneme Deseninde kurulmuştur. İki farklı uygulama zamanı ve yaprak alma, uç alma ve yaprak alma-uç alma uygulaması yapılarak her blokta 12 omca ve iki tekerrürlü olacak şekilde çalışılmıştır. Elde edilen sonuçlar MSTAT-C ve JUMP istatistik paket programları ile değerlendirildikten sonra uygulamalar arasında görülen istatistiki farklılıklar p=0,05 önem derecesine göre varyans analizi ile ortaya konmuştur. Korelasyon analizi ve temel bileşen analizi SPSS (IBM SPSS Statistics 27.0) istatistik programı ile yapılmıştır.

**Bulgular**

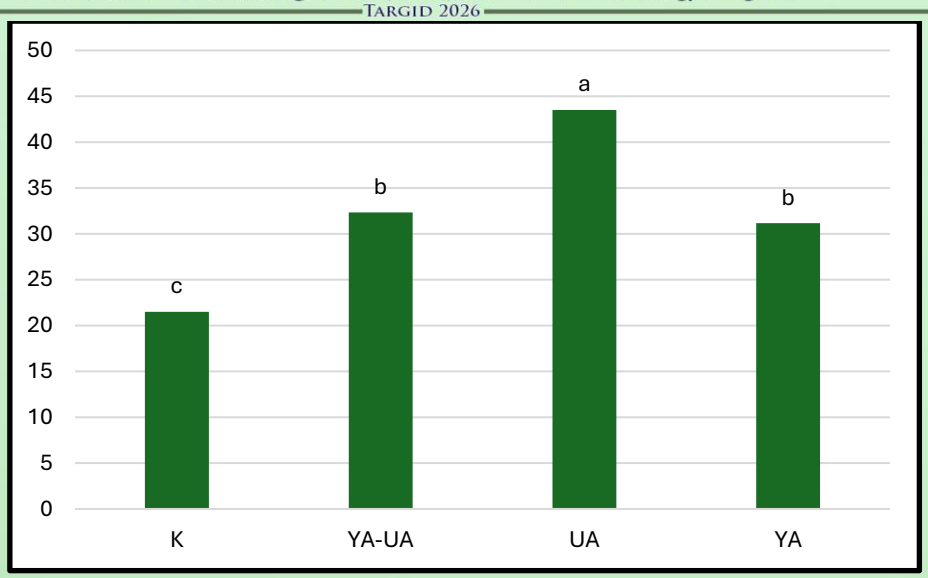
Ankara koşullarında yetiştirilen Kalecik karası üzüm çeşidinde farklı fenolojik dönemlerde kontrol, yaprak alma, uç alma ve yaprak alma- uç alma uygulamaları üzerine omca başına verim, omcadaki salkım sayısı, salkım eni-boyu, tane ağırlığı, tane boyu, pH, SÇKM, TEA ve toplam fenolik bileşik analizi incelenmiş ve veriler aşağıda değerlendirilmiştir.

Bu araştırmada Kalecik karası üzüm çeşidine ait iri koruk ve ben düşme döneminde yapılan yaz budaması uygulamalarının, omca başına verime ait ortalamalar arasında istatistiksel olarak önemli farklılıkların bulunduğu belirlenmiştir ($p \leq 0.05$) (Şekil 3). İri koruk döneminde omca başına verim sonuçları incelendiğinde, en yüksek verimin UA uygulamasında olduğu görülmüş; uygulama ile omca başına verim değeri 7,88 kg/omca olarak tespit edilmiştir. Ben düşme dönemindeki omca başına verim sonuçları incelendiğinde, en çok verim artışının iri koruk dönemi ile benzer şekilde UA uygulamasında olduğu görülmüştür. OBV değerleri arasındaki farklılıklar istatistik olarak önemli bulunmuştur. En yüksek omca başına verim değeri 6,85 kg/omca ile UA uygulaması ile elde edilmiştir. Verim değerleri arasındaki farklılıklar istatistik olarak da önemli bulunmuştur.



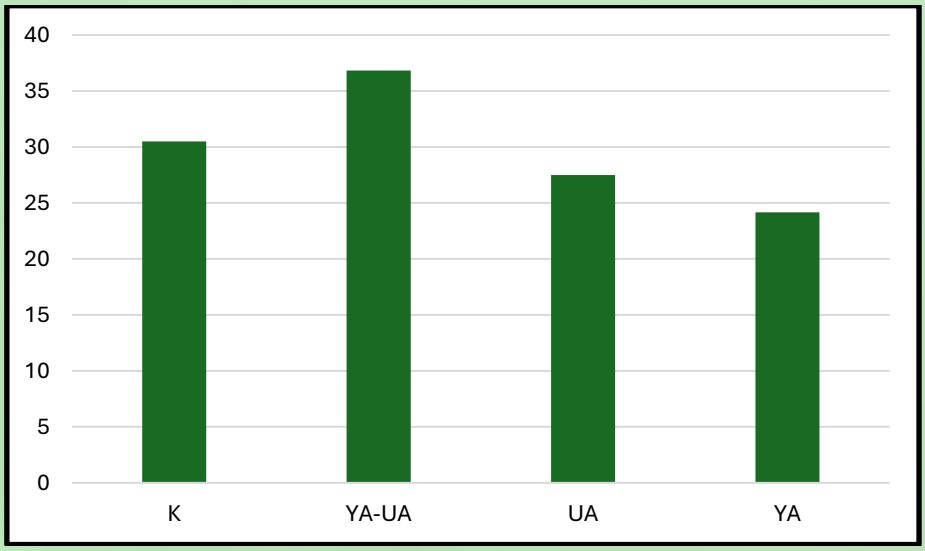

Şekil 3. İri koruk dönemi (A) ve ben düşme dönemi (B) omca başına verim sonuçları (kg/omca), K: Kontrol, YA-UA: Yaprak alma- Uç alma, UA: Uç alma, YA: Yaprak alma, SA: Salkım ağırlığı uygulamalar arasındaki fark 0,05 düzeyinde anlamlıdır.

Çalışmada Kalecik karası üzüm çeşidine ait iri koruk döneminde yapılan kontrol, yaprak alma, uç alma ve yaprak alma- uç alma uygulamalarının omca başına salkım sayısına (adet/asma) ait ortalamalar arasında istatistiksel olarak önemli farklılıklar bulunduğu saptanmıştır ($p \leq 0.05$) (Şekil 4). İri koruk döneminde omcadaki salkım sayısı sonuçları incelendiğinde, en fazla salkım sayısının UA uygulamasında olduğu görülmüş; ortalama 43,50 adet olarak hesaplanmıştır.




Şekil 4. İri koruk dönemi omca başına salkım sayısı sonuçları (adet/asma), K: Kontrol, YA-UA: Yaprak alma-Uç alma, UA: Uç alma, YA: Yaprak alma uygulamalar arasındaki fark 0,05 düzeyinde anlamlıdır.

Şekil 5'de ben düşme dönemi; kontrol, yaprak alma uç alma, uç alma, yaprak alma uygulamalarına ait omca başına salkım sayısına ilişkin bilgiler verilmiştir. Omcadaki salkım sayısı sonuçları incelendiğinde, ortalama en yüksek salkım sayısının YA-UA uygulamasında olduğu görülmüş; ortalama 36,83 adet olarak hesaplanmıştır. Uygulamalar arasındaki fark 0,05 düzeyinde anlamlı bulunmamıştır.




Şekil 5. Ben düşme dönemi omca başına salkım sayısı sonuçları (adet/asma), K: Kontrol, YA-UA: Yaprak alma-uç alma, UA: Uç alma, YA: Yaprak alma uygulamalar arasındaki fark 0,05 düzeyinde anlamlı bulunmamıştır.

Araştırmada, Kalecik Karası üzüm çeşidine ait iri koruk dönemine ait kontrol, yaprak alma- uç alma, uç alma, yaprak alma uygularının tane ağırlığı, salkım eni- boyu ve tane boyuna ait ortalamalar arasında istatistiksel olarak önemli farklılıklar bulunmamıştır. İri koruk döneminde farklı yaz budaması uygulaması yapılan omcalardaki tane ağırlığı en yüksek, UA uygulamasında 2,35 g olarak hesaplanmıştır. Tane boyu ortalamaları incelendiğinde en yüksek değer YA uygulamasında 15,20 mm olarak ölçülmüştür. Salkım eni ortalamaları incelendiğinde en yüksek değer 13,50 cm ile UA uygulamasında, salkım boyu ortalamaları incelendiğinde ise en yüksek değer 15,50 cm ile YA-UA uygulamasında ölçülmüştür. Çalışmada, Kalecik Karası üzüm çeşidinin ben düşme dönemine ait kontrol, yaprak alma- uç alma, uç alma, yaprak alma uygulamaları incelenmiş; salkım eni ortalamalarında, uygulamalar arasındaki fark istatistiki olarak önemli bulunmuş olup; tane ağırlığı, tane boyu ve salkım boyu arasındaki fark 0,05 düzeyinde anlamlı bulunmamıştır. Ben düşme döneminde farklı budama uygulaması yapılan omcalardaki tane ağırlığı UA uygulamasında 2,36 g olarak hesaplanmıştır. TB ortalamaları incelendiğinde de en yüksek değer UA uygulamasında 14,67 mm olarak ölçülmüştür. SE ortalamaları incelendiğinde en yüksek değer 14 cm ile UA uygulamasında, SB ortalamaları incelendiğinde ise en yüksek değer 16 cm ile YA-UA uygulamasında ölçülmüştür.

Çizelge 1. İri koruk ve ben düşme döneminde uygulama yapılan üzüm şıralarında hasat sonrası gerçekleştirilen tane ağırlığı (g), TB (mm), SE (cm), SB (cm) analiz sonuçları

| *Fenolojik Dönemler* | *Parametreler* | *K* | *YA-UA* | *UA* | *YA* |
|---|---|---|---|---|---|
| İri Koruk | Tane Ağırlığı (g) | 1,89 | 1,92 | 2,35 | 1,89 |
| | TB (mm) | 14,32 | 14,32 | 13,86 | 15,2 |
| | SE (cm) | 10,6 | 12,25 | 13,5 | 9 |
| | SB (cm) | 12,2 | 15,5 | 14,5 | 14,4 |
| Ben Düşme | Tane Ağırlığı (g) | 1,81 | 2,24 | 2,36 | 1,99 |
| | TB (mm) | 14,04 | 14,13 | 14,67 | 14,23 |
| | SE (cm) | 11,25 a | 12,90 a | 14 a | 8,75b |
| | SB (cm) | 14 | 16 | 15,5 | 13 |

K: Kontrol, YA-UA: Yaprak alma- uç alma var, UA: Uç alma, YA: Yaprak alma, TB: Tane boyutu, SE: Salkım Eni, SB: Salkım Boy; ben düşme dönemi salkım eni ortalamalarında, uygulamalar arasındaki fark istatistik olarak önemli bulunmuştur. Diğer uygulamalar arasındaki fark 0,05 düzeyinde anlamlı bulunmamıştır.

İri koruk ve ben düşme döneminde Kalecik Karası üzüm çeşidinin kontrol, yaprak alma- uç alma, yaprak alma, uç alma uygulamalarının SÇKM, TEA ve pH ait ortalamalar arasında istatistiki olarak farklılık bulunmamıştır. Ancak iri koruk döneminde YA uygulamasında 3,64 ile en yüksek pH değeri ölçülmüştür. SÇKM sonuçları incelendiğinde %25,20 ile en yüksek değerin yaprak alma omcalarında olduğu gözlemlenmiştir. TEA ortalama analiz sonuçlarında da en yüksek değer UA uygulamasında 0,060 mg/g olarak hesaplanmıştır. Ben düşme döneminde kontrol uygulamasında 3,74 ile en yüksek pH değeri ölçülmüştür. SÇKM sonuçları incelendiğinde %25,35 ile en yüksek değerin YA uygulamasında olduğu gözlemlenmiştir. TEA ortalama analiz sonuçlarında da en yüksek değer UA uygulamasında 0,054 mg/g olarak hesaplanmıştır.

Çizelge 2. İri koruk ve ben düşme döneminde uygulama yapılan üzüm şıralarında hasat sonrası gerçekleştirilen pH, SÇKM (%) ve Titre Edilebilir Asitlik (mg/g) analiz sonuçları

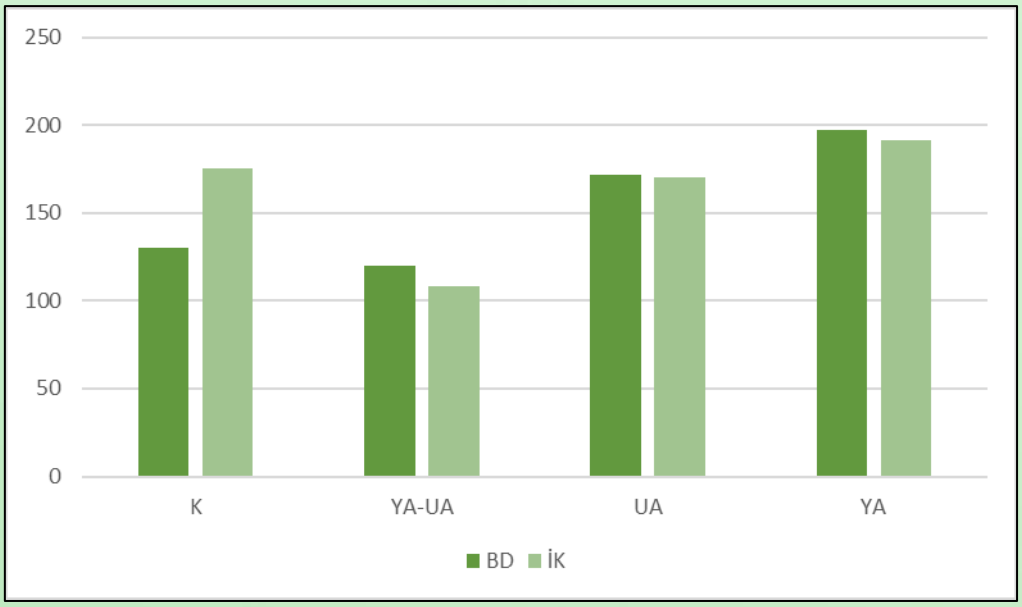
| *Fenolojik Dönemler* | *Parametreler* | *K* | *YA-UA* | *UA* | *YA* |
|---|---|---|---|---|---|
| İri Koruk | pH | 3,58 | 3,5 | 3,5 | 3,64 |
| | SÇKM (%) | 24,2 | 22,8 | 22,8 | 25,2 |
| | TEA (mg/g) | 0,055 | 0,044 | 0,060 | 0,039 |
| Ben Düşme | pH | 3,74 | 3,63 | 3,62 | 3,67 |
| | SÇKM (%) | 23,75 | 22,2 | 22,1 | 25,35 |
| | TEA (mg/g) | 0,039 | 0,047 | 0,054 | 0,049 |

K: Kontrol, YA-UA: Yaprak alma- uç alma, UA: Uç alma, YA: Yaprak alma, SÇKM: Suda Çözünür Kuru Madde Miktarı, TEA: Titre Edilebilir Asitlik; Uygulamalar arasındaki fark 0,05 düzeyinde anlamlı bulunmamıştır.

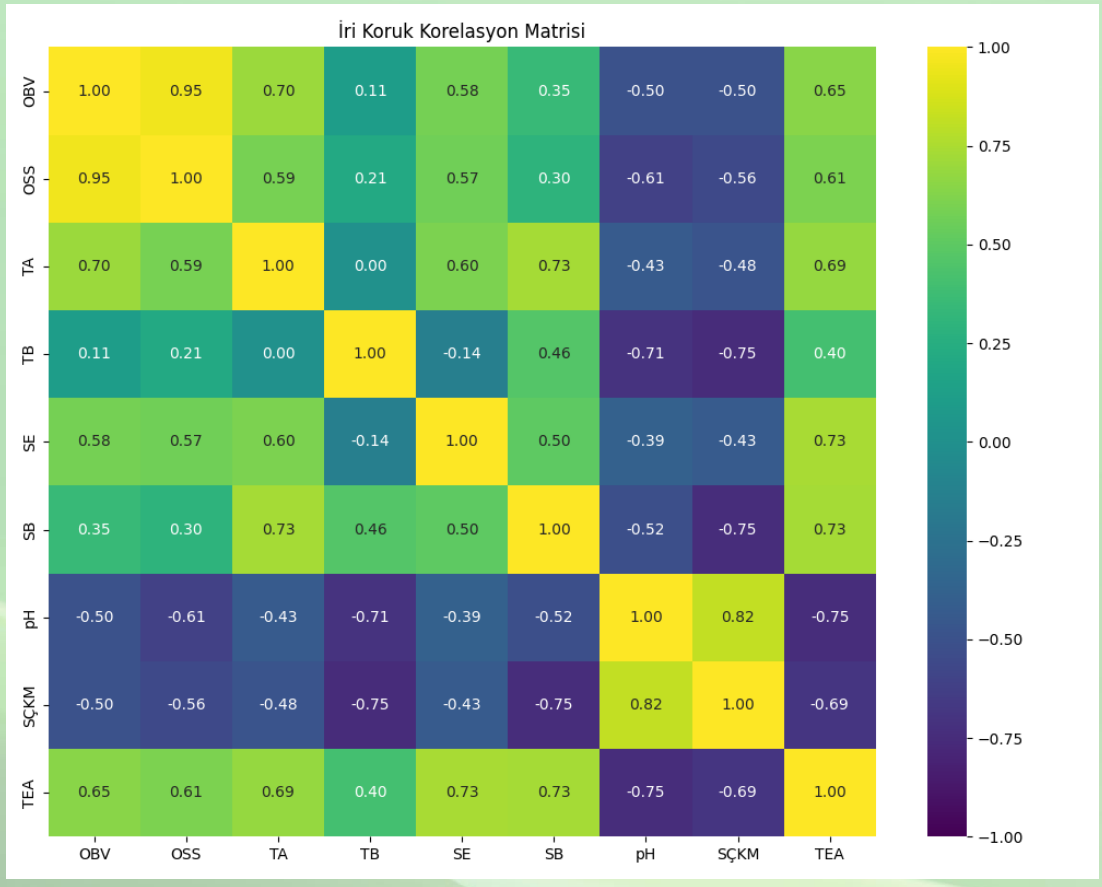
Kalecik Karası üzüm çeşidine ait iri koruk ve ben düşme dönemlerinde yapılan kontrol, yaprak alma- uç alma, yaprak alma, uç alma uygulamaları ortalamalarında, uygulamalar arasında istatistiksel olarak önemli farklılıklar bulunmamıştır. Toplam fenolik bileşik sonuçları incelendiğinde ben düşme döneminde yaprak alma uygulaması yapılan 197,3 mg GAE/l ile en yüksek toplam fenolik bileşik değeri elde edilmiştir. 191,5 mg GAE/l ile ikinci en yüksek değer; iri koruk döneminde yaprak alma uygulamasında tespit edilmiştir. Toplam fenolik bileşik sonuçları kontrol omcalarında; en yüksek toplam fenolik bileşik değeri İK dönemindeki 175,5 mg GAE/l olduğu hesaplanmıştır. UA-YA uygulaması bakımından tüm dönemler incelendiğinde; en yüksek değerin 119,75 mg GAE/l ile ben düşme döneminde olduğu görülmüştür. UA uygulaması bakımından toplam fenolik bileşik değerleri incelendiğinde de ben düşme dönemi 171,5 mg GAE/l ile en yüksek değer olarak tespit edilmiştir. YA uygulaması sonucunda hem İK hem de BD dönemlerinde yüksek miktarda toplam fenolik bileşik sonuçları elde edilmiştir.

Kalecik Karası üzüm çeşidinde incelenen parametreler arasındaki ilişkiler pearson korelasyon analizi ile değerlendirilmiştir (Şekil 7 ve Şekil 8). İri koruk döneminde uygulanan yaz budaması üzerine incelenen özellikler arasındaki ilişkiler değerlendirildiğinde, omca başına verim ile omca başına salkım sayısı arasında oldukça güçlü bir pozitif ilişki saptanmıştır (r=0.950). Benzer şekilde pH ile SÇKM arasında oldukça güçlü pozitif ilişki saptanmıştır (r=0.820). TA ile SB, TEA ile SE ve TEA ile SB arasında da yüksek düzeyde pozitif ilişki belirlenmiştir (r=0.730).

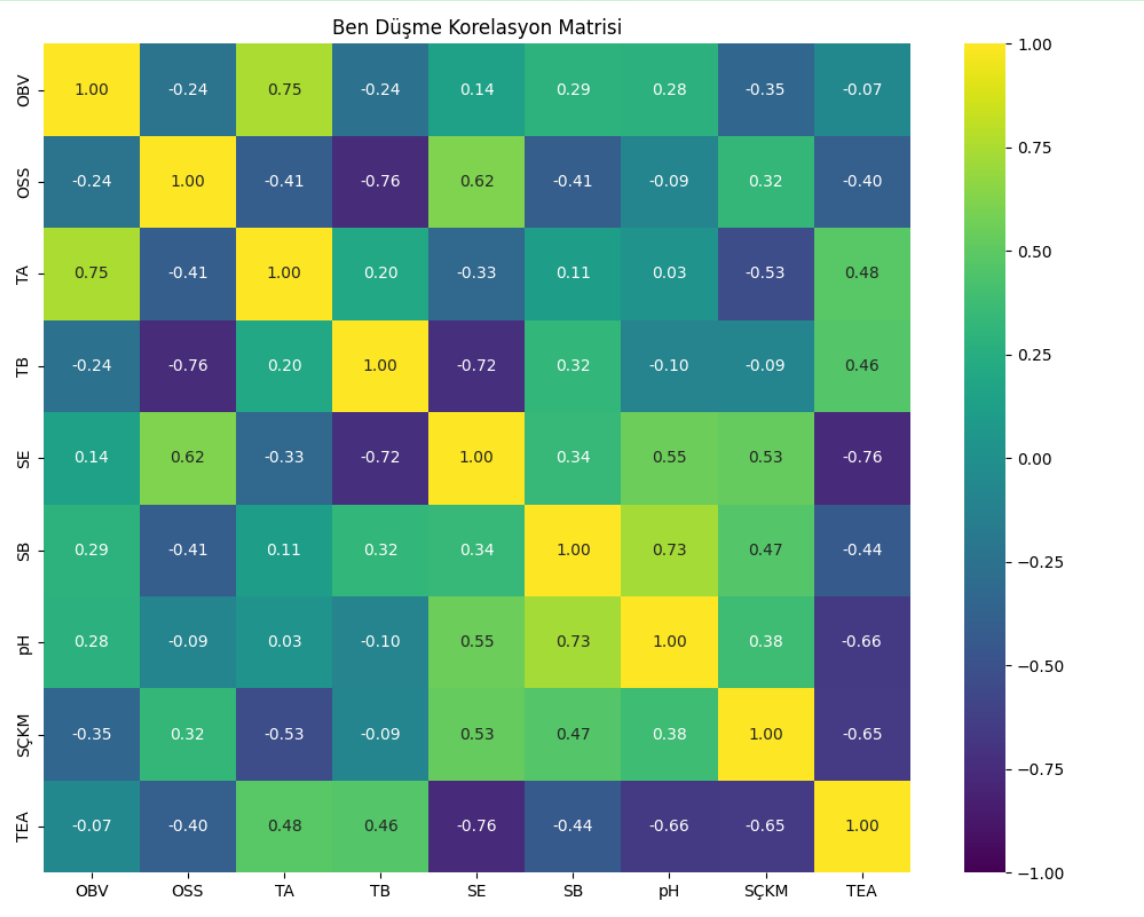
Ben düşme döneminde uygulanan yaz budaması üzerine incelenen özellikler arasındaki ilişkiler değerlendirildiğinde, omca başına verim ile tane ağırlığı arasında güçlü pozitif ilişki bulunmuştur (r=0.750). Benzer şekilde pH ile salkım boyu arasında güçlü pozitif ilişki tespit edilmiştir (r=0.730). Omca salkım sayısı ile tane boyu arasında güçlü negatif ilişki saptanmıştır (r=-0.760). Korelasyon haritalarından da (Şekil 7 ve Şekil 8) anlaşıldığı üzere iri koruk ve ben düşme döneminde verim bileşenleri ve meyve kalite parametreleri arasında yakın bir ilişki olduğu görülmektedir.




Şekil 6. Toplam fenolik bileşik değerleri sonuçları (mg GAE/l), K: Kontrol, YA-UA: Yaprak alma -uç alma, UA: Uç alma, YA: Yaprak alma (Şahit:0,082 alınmıştır)

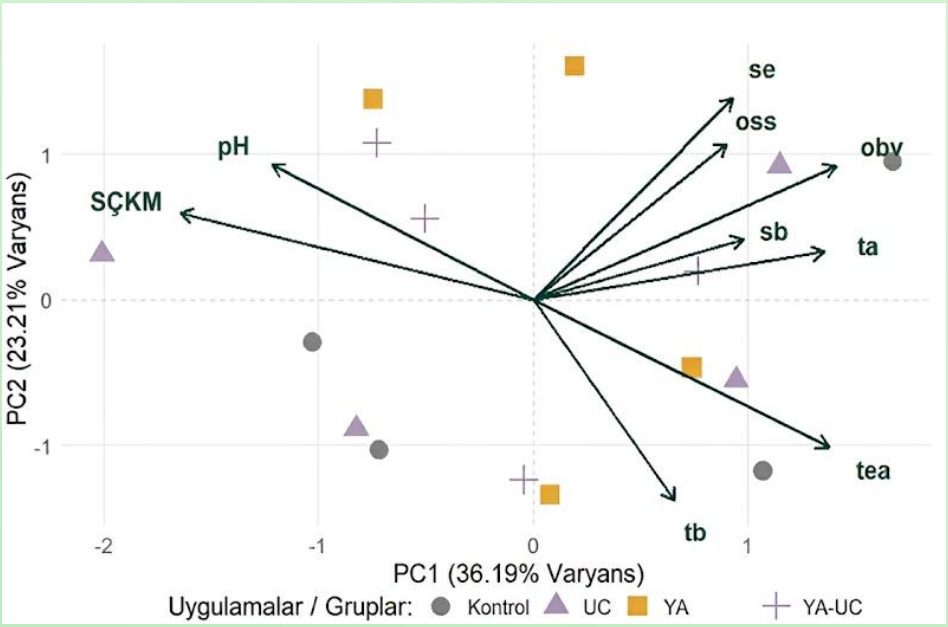



Şekil 7. Kalecik Karası üzüm çeşidinde iri koruk dönemi uygulanan yaz budaması üzerine incelenen parametrelere ait korelasyon haritası, OBV: Omca başına verim, OSS: omca salkım sayısı, TA: 100 tane ağırlığı, TB: Tane boyu, SE: Salkım eni, SB: Salkım boyu, SÇKM: Suda çözünebilir kuru madde, TEA: Titre edilebilir asitlik




Şekil 8. Kalecik Karası üzüm çeşidinde ben düşme dönemi uygulanan yaz budaması üzerine incelenen parametrelere ait korelasyon haritası, OBV: Omca başına verim, OSS: omca salkım sayısı, TA: tane ağırlığı, TB: Tane boyu, SE: Salkım eni, SB: Salkım boyu, SÇKM: Suda çözünebilir kuru madde, TEA: Titre edilebilir asitlik

Temel Bileşen Analizi (PCA) biplot grafiği, farklı yaz budaması uygulamalarının incelenen meyve kalite kriterleri ve morfolojik parametreler üzerindeki çok değişkenli etkilerini ve değişkenler arası ilişkileri açıklamak amacıyla oluşturulmaktadır. Analiz sonucunda elde edilen ilk iki temel bileşen, veri setindeki toplam varyansın %59.40'ını açıklamaktadır (PC1: %36.19, PC2: %23.21). Elde edilen bu kümülatif oran, uygulamalar ve parametreler arasındaki ana eğilimleri ortaya koymak için yeterli bir istatistiksel zemin sunmaktadır. Grafiğin sol ekseninde (-PC1) yer alan SÇKM (Suda Çözünür Kuru Madde) ve pH parametreleri birbiriyle dar bir açı yapmaktadır. Bu durum, şıradaki SÇKM birikimi ile pH seviyesi arasında güçlü bir pozitif korelasyon olduğunu doğrulamaktadır. Grafiğin sağ ekseninde (+PC1) kümelenen vektörler (OBV, TA, SB, OSS, SE, TEA, TB), kendi içlerinde pozitif yönlü bir ilişki sergilemektedir. SÇKM ve pH vektörlerinin, başta tane ağırlığı ve TEA olmak üzere sağ taraftaki gelişim parametreleriyle yaklaşık 180°C zıt açılar oluşturduğu görülmektedir. Bu bulgu, olgunlaşma dinamiklerinin tipik bir yansıması olarak üzümde SÇKM ve pH seviyeleri yükselirken, tane ağırlığında ve TEA oranında göreceli bir azalma meydana geldiğini istatistiksel olarak teyit etmektedir. Konsantrasyon etkisiyle tanelerin su kaybetmesi veya küçülmesi, SÇKM'nin oransal olarak artmasına neden olmuş olabilmektedir. Yaprak alma- Uç alma (YA-UA) uygulamaları ile özellikle Yaprak Alma (YA) sonuçlarının büyük bir kısmının grafiğin sol tarafında, SÇKM ve pH vektörlerinin aynı yönde kümelendiği görülmektedir. Bu durum yaprak alma uygulamalarının şıradaki kuru madde birikimini ve pH değerini arttırıcı yönde güçlü bir etkisi olduğunu göstermektedir. Kontrol grubu ve Uç Alma (UA) yapılan uygulamalar, grafiğin sağ kadranlarına doğru daha fazla yayılım göstermiştir. Bu grupların bir kısmının tane ağırlığı, TEA vd. fiziksel büyüme parametreleri yönünde konumlanması, bu asmalarda tane iriliğinin ve asitliğinin YA grubuna kıyasla daha yüksek seviyelerde olduğunu ifade etmektedir.




Şekil 9. Temel Bileşen Analizi

**Tartışma**

Uç alma uygulaması ile vejetatif sürgün ucu büyümesinin baskılanabileceği düşünülmektedir. Aktif büyüme konisinin uzaklaştırılmasının fotosentetik asimilatların doğrudan salkımları yönlendirilmesinde artışlar oluşturulabileceği düşünülmektedir. Bu durum özellikle iri koruk döneminde generatif gelişimi destekleyerek nihai verim parametrelerinde iyileşmeye yol açabilmektedir. Kalecik Karası üzüm çeşidinde iri koruk ve ben düşme dönemlerinde uygulanan yaz budamalarından uç alma uygulamasının verim parametrelerini olumlu olarak etkilediği saptanmıştır. Michele Palieri üzüm çeşidinde yapılan araştırmada, iri koruk döneminde uygulanan yaprak alma-uç alma yaz budamasının omca başına verimi arttırdığı belirlenmiştir (Korkutal ve ark., 2021). Diğer bir çalışmada, Chardonnary üzüm çeşidinde uygulanan uç alma işleminin verimi önemli ölçüde düşürdüğü saptanmıştır (Pulko ve ark., 2022). Bu çalışmada elde edilen bulgular söz konusu araştırmalar ile paralellik göstermektedir.

Asma yetiştiriciliğinde tane iriliğindeki azalmanın ben düşme öncesinde hücre genişlemesinin baskılanması sonucu olduğu düşünülmektedir. Çalışmada her iki fenolojik dönemde de yapılan uygulamalarda; salkım eni-boyu, tane ağırlığı ve tane boyu sonuçlarında sayısal farklılık gözlemlenmiştir. Şahin ve Dardeniz (2023), farklı yaz budaması uygulamalarının 'Atasarısı' üzüm çeşidinde en yüksek tane tutum sayısı ve tane tutum oranının uç alma- dip yaprak alma- koltuk alma uygulamasında elde edildiği; yaz budamalarının tane tutumuna, salkım sıklığına, tane eni, tane boyu ve tane ağırlığına olumlu etkisi olduğu belirlenmiştir. Çelik ve ark. (2026), çalışmasında tane ağırlığının tane tutumu döneminde yapılan yaprak alma uygulaması ile artırdığını saptamıştır. Akın (2018) tarafından yapılan bir çalışmada Alphonse Lavallée (*Vitis vinifera* L.) üzüm çeşidinde bazı yaz budama uygulamalarının ve hümik asit kullanımının en yüksek üzüm verimi, en yüksek salkım ağırlığı, en yüksek tane ağırlığına olumlu etkisi olmuştur. Başka bir çalışmada, Samancı Çekirdeksizinde 1/3 oranında yapılan uç alma ile tane ağırlığında ve hacminde artış, olgunluk ve renklenmede hızlanma meydana gelmiş; bilezik alma ile de tane hacmi, tane iriliği, tane sertliğinde artış olup renklenme ve olgunlaşmada hızlanma görülmüştür. Alphonse Lavallée üzüm çeşidinde ise ben düşme döneminde yapılan bilezik alma uygulamasının tane ağırlığını ve genişliğini arttırdığı gözlemlenmiştir (Çiftçi ve Çelik, 2023). Doğan (2025), salkım ve üzüm tane özelliklerinde iyileşmeler olduğunu, tane ağırlığında artış meydana geldiğini ve üzümlerin nakliyede daha dirençli olduğunu belirlenmiştir. Bu çalışmada elde edilen bulgular söz konusu araştırmalar ile paralellik göstermektedir.

Yapılan yaprak alma işleminin salkım içi güneşlenme ve havalanma üzerine artırıcı etkisinden dolayı şeker birikimi ve sekonder metabolit sentezinde teşvik edici değişiklikler olabileceği öngörülmüştür. Çalışmada iri koruk ve ben düşme dönemlerinde yapılan yaprak alma uygulaması üzümlerde SÇKM değerini olumlu yönde etkilediği belirlenmiştir. İncelenen diğer çalışmalarda, farklı üzüm çeşidi ve farklı yaz budamalarına göre sonuçlar farklılık göstermektedir. Öner (2014), araştırmasında Tekirdağ koşullarında Cabernet Sauvignon üzüm çeşidinde koltuk yaprakları alma uygulamasının toplam asitlik ve verimi artırdığını, SÇKM değerlerini azalttığını belirlemiştir. Pulko ve ark., (2022), SÇKM miktarının uç alma şiddeti arttıkça düştüğünü ve titre edilebilir asitlikte ise yükseldiğini saptamıştır. Alphonse Lavallee çeşidinde ise hafif uç alma uygulamasının SÇKM miktarını artırdığı bildirilmiştir (Çelik ve ark., 2026). Diğer bir çalışmada ise Karasakız üzüm çeşidinde salkım üzerinde daha fazla

yaprak bırakılmasının SÇKM'yi artırdığı ve TEA'i önemli ölçüde düşürdüğü tespit edilmiştir (Dardeniz ve ark., 2008). Dobrei ve ark. (2021) şaraplık üzümlerle ilgili yaptığı çalışmada yaz budamasının tomurcuk canlılığını ve tane brix değerini arttırdığını belirlemişlerdir.

Üzümlerde fenolojik bileşiklerin sentezi ışığa bağlı değişmektedir. Yapılan yaz budamasının toplam fenolik maddeyi etkilediği düşünülmektedir. Çalışmada sayısal olarak farklılıklar gözlemlenmiştir. Gün (2025), Horoz Karası üzüm çeşidi üzerine yaptığı çalışmada tane ağırlığı, tane boyu, pH, SÇKM, TEA seviyelerinde belirgin bir farklılık görülmemesine rağmen, S3 seviyesindeki salkım seyreltme uygulamasının toplam fenol kapasitesinde artış meydana geldiğini belirlemiştir. Alphonse Lavallee üzüm çeşidinde tane tutumu aşamasında yaprak alma uygulamasının çiçeklenme öncesi yaprak alma uygulamasına göre toplam fenolik madde miktarını artırdığı saptanmıştır (Çelik ve ark., 2026). Bu çalışmada elde edilen bulgular literatür ile paralellik göstermektedir.

## Sonuç

Yaz budamasının, üzümde ürün kalitesini ve tane iriliğini arttırma gibi birçok faydası olduğu bilinmektedir. Yapılan çalışmalar yaz budamasının birim alandan daha fazla verim ve kaliteli ürün elde edilmesi için yapılmasını tavsiye etmektedir. Araştırmada farklı fenolojik dönemlerde Kalecik Karası *(Vitis vinifera L.*) üzüm çeşidinde uygulanan farklı yaz budamasının verim ve kalite parametreleri üzerine etkileri araştırılmıştır. Araştırma projesi sonucunda Ankara ili için önemli bir ticari değere sahip olan Kalecik Karası üzüm çeşidinde yaz budaması uygulamalarının kalite parametrelerini artırdığı ve gerek iri koruk döneminde gerekse ben düşme döneminde gerçekleştirilecek yaprak alma uygulamalarının tavsiye edilebileceği görülmüştür. Verim parametreleri sonuçlarına göre ise en önemli verim parametrelerinden birisi olan omca başına verim değerini artıran uygulama olarak uç alma uygulaması önerilmektedir.

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# Trophic Positioning and Habitat Ecology: A Comparative Review of PFAS Bioaccumulation Dynamics in Pelagic versus Demersal Fish Species

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## Abstract

T Global concern and pose a significant threat to aquatic ecosystems and to the health of human consumers. Due to their extraordinary chemical stability and amphiphilic nature, these persistent chemicals are effectively biomagnified in marine and freshwater food webs. However, the spatial distribution of PFAS in the water column leads to different bioaccumulation pathways between pelagic and bottom species. Understanding these habitat-specific variations is essential for an accurate assessment of the environmental risks and for the development of targeted frameworks for food safety monitoring. Pelagic species that are present in the upper water column are primarily exposed to waterborne fractions of PFAS and highly mobile, water-soluble short-chain alternatives through the uptake of pelagic food webs and by the fishery. As these species often exhibit high metabolic rates and extensive migration patterns, their PFAS profiles are strongly influenced by horizontal spatial changes in water contamination and by rapid trophic translocation. Conversely, demersal and benthic species (e.g. flatfish, cod and turbot) are found close to the seabed and are therefore very vulnerable to long-chain variants of PFOS. The demersal food web is intrinsically linked to the detrital routes and to the organisms living in the sediment, which results in a long-term, chronic exposure matrix for pelagic and demersal fish species. Recent toxicokinetic studies show that while pelagic fish often show a rapid response to changing surface water concentrations, demersal species tend to have significantly higher steady state bioconcentration factors and a higher body weight of hydrochlorofluorocarboxylic and sulphonic PCBs. This comparative analysis highlights that the primary drivers of tissue distribution of PFAS are habitat ecology, interaction with sediments and trophic distribution. Therefore, in order to accurately mitigate the risks of chemical exposure in human food, reliable environmental monitoring and safety regulations need to implement stratified sampling strategies, distinguishing between pelagic and demersal habitats.

***Keywords:*** *PFAS, PFOS, Pelagic Fish, Demersal Fish, Bioaccumulation, Sediment-Water Interface, Seafood Safety.*

## Introduction

The bioaccumulation of per- and polyfluoroalkyl compounds in aquatic organisms is a multifaceted process driven by a complex systems including biological, chemical, and environmental differences (Flinders et al., 2025). Especially, structural properties such as perfluoroalkyl chain length and the nature of the polar functional group differ the bioaccumulation potential of these contaminants. In addition to these structural determinants, the intrinsic pharmacokinetic mechanisms--in particular the high affinity for proteins such as serum albumin and liver fatty acid binding proteins, combined with the active translocation by membrane transporters--control the specific distribution, bioaccumulation and elimination kinetics of these compounds in the body (Jia et al., 2022; Ng & Hungerbühler, 2013, 2014). In addition, functional group type is often a more important predictor of internal distribution patterns than length of the chain itself, resulting in different concentrations in tissues such as liver, blood and bile. (Armitage et al., 2016; Shi et Furthermore, the co-exposure of congener molecules with different chain lengths modulates the accumulation kinetics. The uptake of long-chain compounds may be competitively inhibited and the steady state bioconcentration factors of the short-chain analogs can be significantly reduced. The inhibitory effect is due to the fact that long chain congeners have higher binding affinities for important transport proteins such as serum albumin and liver fatty acid-binding proteins, and thus effectively compete with their shorter chain counterparts for these limited sites and membrane transporters. (Wen et al., 2017). Thus, the prevalence of more hydrophobic, long-chain PFAS in environmental mixtures can significantly influence the uptake and tissue-specific accumulation of shorter-chain analogs, a factor that requires meticulous consideration when assessing the cumulative risks associated with complex PFAS contamination profiles (Wen et al., 2017).

In addition to these internal transport dynamics, the trophic position of the species and the distinctive configuration of the food web further influence biomagnification potential, with greater accumulation often seen in organisms at higher trophic levels (Kelly et al., 2024). Moreover, molecular affinity for subcellular components—such as the distribution between protein-rich albumin and polar lipid fractions—significantly impacts the thermodynamic accumulation potential of certain PFAS congeners (Fremlin et al., 2023).

The current knowledge about PFAS accumulation on different fish species is mostly focusing on explain the accumulation based on the relevant compounds characteristic and cover very limited scope, but for the better understanding of how these pollutants cause the risk in fish by food chain to public; investigation of bioaccumulation mechanisms in depth is necessary. Thus, this review is aimed explain the mechanism of PFAS accumulation in the model food matrices, fish tissues by considering from both sides; from fish side such as living area, proximate composition and physical properties, from chemical pollutant side such as mechanic and physicochemical characteristics.

**Physicochemical Properties of PFAs Driving Chemical Bioaccumulation**

Buildup of PFAS in living things depends less on greasiness than once thought. Instead, it hinges on how long the molecule's tail is, what kind of charged tip it carries, along with its grip strength on proteins and cell layers. Molecules with longer tails plus sulfur-based tips tend to stick harder, leave the body slowly, and grow more concentrated up food chains. On the flip side, those with shorter arms or redesigned parts move faster through plants, slip into water readily. Predicting their behavior means building tools that track exactly how they cross membranes, latch onto bodily structures, flush out over time. Specifically, the molecular docking of these substances to transport proteins is often driven by a combination of exothermic van der Waals forces, electrostatic interactions, and hydrogen bonding (Fan et al., 2025). These binding affinities generally scale with increasing perfluoroalkyl chain length, significantly influencing the subsequent rate of elimination and the overall tissue-specific residue patterns (Brunn et al., 2023; Ma et al., 2023). Moreover, compound-specific variations in partitioning are further underscored by evidence that branched versus linear isomers exhibit distinct transfer efficiencies and pharmacokinetic profiles, which may complicate standardized assessments of bioaccumulation across diverse taxa (Jouanneau et al., 2021).

**The Toxicokinetics of PFAS: Protein Binding vs. Phospholipid Partitioning**

The unique physical and chemical characteristics of per- and polyfluoroalkyl substances (PFAS) dictate their unique toxicokinetic profiles in biological systems. PFAS have a hydrophobic, oleophobic fluorinated carbon tail, and a hydrophilic head group. In contrast to traditional lipophilic persistent organic pollutants (POPs) that tend to bioaccumulate in adipose tissues, PFAS are characterized by a high affinity for protein-rich and membrane dense compartments . The standardized distribution, tissue bioaccumulation, and biological life cycle of PFAS are determined by this delicate balance between two main mechanisms, i.e. specialized protein attachment and non-specific phospholipid membrane fragmentation.

Short- to long-chain PFAS have a tissue tropism for the plasma and liver over adipose tissue, due to their extremely high binding affinity to serum albumin, fatty acid-binding proteins (FABPs), and many other cellular transporters (Leung, 2023; Lewis, 2022; Hu, 2024). The structure of the perfluoroalkyl carboxylic acids (PFCAs, generally C6-C10) and related functional groups show the greatest binding affinity to serum protein, which is highly structure-dependent (Yao, 2025).

**Phospholipid Membrane Interaction and Partitioning**

The way phospholipid membranes interact with things is really interesting. When you have a chain of fluorinated carbon the way it stays in the membrane changes. It goes from interacting with proteins to just becoming part of the membrane because of phospholipids. This is especially true for long chains of PFAS. When this happens the PFAS builds up in the membrane, which's a big reason why it accumulates in living things like fish. This has been seen in organisms and fish like in the work of Shi in 2018 Sun in 2022 and Alfa in 2024.When PFAS has a long chain it is hard for the body to get rid of it through the kidneys. This is because the PFAS becomes part of the membrane and the kidneys have a time removing it. As a result the kidneys are not able to clear out the PFAS as shown by Shi in 2018.

The way medium and long chain PFAS act in cell membranes is also worth looking at. These PFAS are able to dissolve in the part of the membrane that does not like water. This happens because of the way the molecules are structured not just because they are attracted to each other. The phospholipid membrane and PFAS interact in a way that is governed by the structure of the molecules, than just a simple attraction.

**PFAS Accumulation in Fish: Tissue Tropism & Habitat Disparities**

Fresh water fish tend to accumulate much higher levels of PFAS (per- and poly-fluoroalkyl substances) than marine species, owing to their closer proximity to industrial pollution sources and the smaller volume of dilution in rivers and lakes. Based on U.S. EPA data, average totals range from 9,500 to 11,800 ng/kg in freshwater fillets versus generally negligible parts-per-billion levels in ocean-going marine fish. But recent ecotoxicological studies

show that the severity of this chemical burden is governed by a fish's specific internal anatomy. PFAS are highly proteinophilic (protein binding) compounds and therefore do not distribute homogenously among fish tissues. Evidence from peer-reviewed research indicates that bioaccumulation is maximized in the highly vascular, protein-rich organs: liver (l), viscera (v), and gonads/gills Dynamic tissue-distribution characterization reveals that liver amounts of primary legacy substances such as PFOS routinely reach 60 to 350 times higher than levels in the surrounding edible muscle fillet.

**Influence of Fish Proximate Composition on the Bioaccumulation and Proteinophilic Partitioning of Per- and Polyfluoroalkyl Substances (PFAS)**

The mechanism for the bioaccumulation of per-and polyfluoroalkyl substances (PFAS) into fish diverges from the typical lipophilic partitioning model and incorporates the interaction between the chemicals' properties, the habitat where the organism lives, and the organism's internal physiological traits. There is a global pattern between species and the type of habitat they inhabit: freshwater fish contain significantly higher levels of PFAS compared to marine organisms because freshwater is often closer to industrial and consumer use and sources of contamination; furthermore, lacustrine and riverine environments have lower dilution capabilities. Examining individual tissues in a global scale assessment of fillet samples shows an opposite relationship between crude lipid content and PFAS concentrations: leaner muscle has higher chemical load. This occurs because PFAS, unique to other pollutants of concern to fish, is proteinophilic, which means they preferentially partition onto functional proteins such as serum albumin or liver fatty acid-binding proteins. Thus, the chemical has a high affinity for protein-binding instead of accumulating within storage lipids and partitions strongly into the blood plasma and to blood organs like the liver and kidneys.Consequently, traditional predictive models using total lipid or total protein as simple normalization metrics fail to capture real-world variability (Giari et al., 2023; Taiwo et al., 2025). Because PFAS bind selectively to distinct functional proteins rather than bulk protein mass, total protein content does not serve as a reliable standalone predictor (Babut et al., 2017). Instead, mechanistic and food-web models indicate that for long-chain variants, bioaccumulation is heavily driven by phospholipid partitioning, with renal elimination remaining negligible (Taiwo et al., 2025). For these long-chain compounds, the primary route of uptake shifts systematically from branchial gills to dietary ingestion as chain length increases, significantly slowing elimination kinetics—a process successfully simulated by advanced mechanistic models substituting specific protein/phospholipid fractions for lipid values (Valsecchi et al., 2020; Taiwo et al., 2025).

Aside from pure proximate composition, other organisms differences can be best explained by properties of the organism itself, choice of diet, transporter protein, and the habitat of the transporter protein (Liang, 2022). Many studies of river and lake systems have confirmed through stable isotope analysis that trophic position and foraging ecology strongly predict PFAS signatures, much more so than fish size or bulk composition (Macorps, 2022; Xing et al., 2023; Levanduski et al., 2026). Carnivores, tertiary consumers more readily accumulate PFAS body burdens than omnivores and lower trophic level counterparts (Liang, 2022) as is evidenced by higher accumulation in catfish, an apex predator, compared to their conspecific carp counterparts across all body tissues (Lewis, 2022). Structural and commercial considerations significantly further differentiate PFAS accumulation independent of mere fat levels as wild fish have substantially higher accumulation in their bodies compared to farmed varieties and the inclusion or omission of skin in a fish fillet also structurally influences the final PFAS pattern(Figueroa-Muoz, 2024).

**Conclusion**

This review deeply investigated the key factors for bioaccumulation of PFAs in fish tissue. The results and compare assessment could be beneficiary for different disciplines from food science to environmentalist and gastronomy.

***Author Contribution Statement***

Please indicate how each author contributed to this work and at what stage. For example:

ETAT: Data collection, investigation, formal analysis, and writing the original draft, supervision, conceptualization, methodology, review and editing

***Conflict of Interest***

The author declares no conflict of interest

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ID: 832

# Impact of Organic and Inorganic Fertilization on the Growth and Yield Dynamics of Tomato (*Lycopersicon esculentum*) in the Young Meghna Estuarine Floodplain, Bangladesh

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## Abstract

Tomato (*Lycopersicon esculentum*) productivity is heavily reliant on strategic nutrient management to optimize both vegetative growth and reproductive performance. Efficient nutrient application is also essential for reducing yield gaps while maintaining soil biodiversity in subtropical floodplain agroecosystems. This study evaluated the comparative effects of organic and inorganic fertilizers on the growth and yield of tomato at Noakhali Science and Technology University, Bangladesh (AEZ-18: Young Meghna Estuarine Floodplain), during the *Rabi* season from December 2022 to March 2023. The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications and three treatments: $T_1$ = control (no fertilizer), $T_2$ = recommended organic fertilizer (cow dung at 10 t $ha^{-1}$), and $T_3$ = recommended inorganic fertilizers (urea, TSP, MP, gypsum, zinc, and boric acid at BARI-recommended doses). Results revealed that fertilizer application significantly improved all measured growth and yield parameters. The inorganic fertilizer treatment ($T_3$) showed the highest performance, with maximum plant height (81.08 cm), leaf number (52.56), and enhanced flowering efficiency. Yield-contributing traits such as fruit length, diameter, and individual fruit weight were also highest under $T_3$, resulting in a yield of 2.61 kg per plant compared to 1.36 kg per plant in the control. Although inorganic fertilizers provided immediate yield benefits, the findings highlight the importance of integrated nutrient management for sustaining soil health and ensuring long-term productivity in the AEZ-18 region of Bangladesh. These findings provide practical insights for improving tomato productivity in coastal floodplain systems and have broader implications for sustainable intensification in similar agroecological regions worldwide.

***Keywords:*** *Tomato productivity, Inorganic fertilizer, Organic fertilizer, Yield attributes, Sustainable agriculture*

*ID: 833*

# Assessment of Biodiversity and Climate Change among Food Marketers in Akure Metropolis, Nigeria

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## Abstract

The study analyzed the assessment of biodiversity and climate change among food marketers in Akure metropolis, Nigeria. A two-stage sampling procedure was used to select 120 food marketers from the study area. Primary data were used for this study through a well-structured questionnaire, focus group discussions (FGDs), and environmental monitoring devices**.** The collected data were analyzed using descriptive statistics, analysis of variance (ANOVA), and a multiple regression model. The results showed that 78.3% of respondents were female, with an average age of 46.97 years. Oja Oshodi shows the highest overall biodiversity, while Oja Oba has a larger share of marketers in the lowest biodiversity range (≤ 1.0). Oja Araromi shows moderate to high biodiversity, and Oja Arakale presents a mixed distribution with a significant portion of its markers in both the none and moderate biodiversity categories. The ANOVA results indicate no significant differences in the biodiversity of food crops among the four markets. The ANOVA of key climate change indicators (CO, $CO_2$, temperature, relative humidity) across three locations in each of the four markets yielded significant results for all indicators. The results further revealed that Gender, Level of Education, membership in an association, and Carbon dioxide ($CO_2$) had a significant influence on the marketers' sales. The study concludes that sales/day are affected by biodiversity and climate change, suggesting that, at a minimum, $CO_2$ is associated with sales/day and that the introduction of diverse food crop products in the study area and Nigeria at large is occurring.

***Keywords:*** *Biodiversity, Climate change, Food marketers, Analysis of variance, Margalef index, Akure metropolis*

*ID: 834*

# The Role Digital Economy in Enhancing Egg Quality

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## Abstract

The main of this review was to examine the roles of digital economy in enhancing the egg quality. The leading global egg producing countries are China, India and United States, the bulk of the egg consumption varies over the past few decades due to changes in preferences and technology development. A Systemic approach was employed to identify the global sustainability and consumption patterns of eggs in developed countries, digital methods adopted, and branding. Egg nose detection, spectral analysis technology, computer fission technology, multi-fusion sensor technology, and artificial intelligence technology were the modern digital techniques employed to measure egg quality in developed countries. All these approaches were found to be effective in egg quality controls especially in terms of freshness and shell strength and other related attributes. The study concludes that holistic approaches ranging from nose electronic detection to current use of algorithms using artificial intelligence promotes the freshness, egg shell strength and other physical attributes of eggs in terms of quality in developed countries. The study recommends the needs for developing countries to adopt the modern digital methods using in developed countries for egg quality improvement.

***Keywords:*** *digital economy, egg quality*

## Introduction

Poultry eggs not only play the most fundamental nutritional role in national life but are also widely used in bread, pastry, baking, and other food industries, because of their exceptional processing qualities, such as foaming, emulsification, gel properties, and their active ingredients can be used to create new egg products and functional foods (Liu *et al.,* 2024). China dominated the global egg production in 2024, due to large-scale agricultural and technical improvements, accounting for 49% of the total (29.4 million tons), with low-cost feed and population increase, India produced 142.7 billion eggs, demonstrating notable growth. Flock reductions and avian influenza outbreaks caused a 3.9% decrease in U.S. production (76.8 billion eggs). The EU and Brazil contributed 6.66 million tons (−1.83%) and 45.8 billion eggs (+4.8%), respectively, reflecting regional issues such feed prices and animal welfare regulations. The average annual intake of eggs per person worldwide was 162, with differences across high-consumption areas (Patnaik *et al.* 2024). The sector faces substantial obstacles in maintaining quality across the supply chain. As perishable goods, eggs gradually lose their freshness from the farm to the table, which is made worse by improper temperature and humidity controls during storage and transit (Chi *et al.* 2024).

This deterioration raises the dangers to food safety and reduces their economic value. China currently lacks uniform laws for determining the shelf life of eggs, despite growing public awareness of food safety and nutritional health. Market practices rely on post-laying storage length as a freshness indicator, which is an inadequate metric that ignores handling-related mechanical damage or environmental factors that promote bacterial infection. The main quality factor, freshness, has a direct impact on the edibility, nutritional value, and processing appropriateness of eggs (Kapse et al. 2022). In particular, albumen proteins have functional qualities that are crucial for the production of food, such as the ability to foam and gel, which deteriorate with decreasing freshness. Since 1985, China, a sizable nation that both produces and consumes poultry eggs, has led the world in annual poultry egg production (Liu *et al.* 2024).

Processing poultry eggs has become a major problem since they are hard to transport, store, and preserve. By experimenting with various processing techniques and including nutrients that the human body need, new egg products can be produced. Additionally, egg products can be fortified and nutritionally modified to satisfy consumer expectations; examples of this include eggs that have been enhanced with iodine and selenium. Additionally, poultry eggs are a prominent topic of study for both domestic and international scholars due to their exceptional foaming, gelation, and emulsification capabilities in bread, cake, cream, and other consumables (Yang *et al.* 2023).

**Egg Quality and physical factors**

Several factors were connected to the poultry bird origin, food, flock age, or training technique which affects the quality of table eggs (Drabik *et al.* 2021). Regardless of them, eggs undergo biophysical and chemical changes shortly after laying, which have a detrimental impact on egg quality. According to Drabik *et al.* (2018), the primary cause of the change in egg mass is the evaporation of water from the contents of the egg through gas exchange between the contents and the external environment. Distributors and manufacturers of table eggs may face a variety of issues since weight loss during storage happens regardless of weight grade or storage circumstances.

Eggs with weights near the lower limits of the weight classes listed in the Regulation may lose weight during the trade; this change, even if it results from natural reasons, is regarded as adulteration. The quality variations also impact certain aspects of the egg's morphology. The albumen dense fraction is thinning, and its mass and pH have changed (Vlěcková, *et al.* 2019). Relative humidity is equally important in this regard because high levels of it prevent other changes in egg quality by reducing water evaporation from the egg content (Jones *et al.* 2005). Alternative techniques for protecting eggs from adverse quality changes during storage have also grown in favor. They can be divided into two major categories: those that use shell pore sealers (Drabik *et al.* 2021) or those that alter the atmosphere in the egg environment (Jia *et al.* 2019).

The main objective was to examine the roles of digital economy in enhancing the egg quality.

Thus, the purpose of this review is to: -

a. Examine existing literature: The goal of this review is to provide thorough research into sustainability and consumption eggs, modern digital methods adopted in developed economies, and branding of egg consumption pattern.

b. Composing relevant data from reliable sources and evaluating reputable articles are part of planned results synthesis.

The reputable research platform Research Gate is used in this review. An analysis of the research gate database was done. Target focus areas include global egg consumption, variation in egg consumption patterns, digital methods adopted for egg quality management, and branding of egg qualities in digital economies. We obtained pertinent material from several academic disciplines by using these keywords. Articles that were reviewed in a specific quantity included reports, conference proceedings, and journal articles.

**Global Eggs consumption and sustainability**

Chicken eggs are produced and consumed in large quantities in China. The country was ranked first in the globe in 2023 with 35.63 million tons of chicken eggs produced, coupled with a well-balanced combination of proteins, vitamins, minerals, trace elements, and other nutrients, poultry eggs are a natural source of life energy and complete nourishment. Customers from Brazil, China, India, Mexico, Spain, and the United States of America (USA) were interviewed for a recent study that assessed customers' willingness to pay for a more sustainable egg supply chain (Sánchez-Bravo *et al.*, 2021). The overall findings indicated that 34.8% of customers were willing to pay a premium of at least 20% and 47.8% were willing to pay at least 5% more for a more sustainable egg supply. Only a few numbers of customer said they would not be interested in paying extra for improvements in the sustainability of the egg supply; however, there were some disparities between the countries, with at least 25% of customers in the USA and Spain refusing to pay any premium. The consumers are now more concerned with the sustainability of the food chain and the effects of their decisions on the environment. The quality of eggs in developed economies like China, USA, and others were influenced by the sustainability behaviors of the consumers which are dependent trending development.

**Global variation in Egg Consumption Quality patterns**

The worldwide egg industry was able to produce an extra 46 eggs per person (+29.9%) between 2000 and 2020, despite the fact that the world's population increased by 26.9% and reached 7.795 billion in 2020. By running marketing and promotional programs that draw more customers to the egg category and raise the frequency of consumption, national associations of egg producers play a crucial role in boosting egg consumption (Guyonnet, 2019a). Large-scale marketing research studies offer incredibly useful customer insights, such as who they are, where they get eggs, when and where they eat them, and why they do or do not eat more eggs (Guyonnet, 2012). These businesses are able to divide the market into several consumer categories, such as egg lovers and non-consumers, based on the answers to these numerous questions, and then develop the most effective messages for each consumer category. These businesses can create highly successful campaigns to target every customer segment by combining print, social media, radio, and television advertising (Guyonnet, 2020a). For example, between 2010 and 2020, consumption of eggs increased by 119 in Brazil, 111 in Colombia, and 59 in Canada as a result of this all-encompassing approach to egg marketing and promotion (International Eggs Commission, 2021).

**Digital methods adopted for Egg Quality**

***Electronic Nose Detection***

Sensor arrays made up of several gas-sensitive components that are individually selectively responsive to particular volatile substances are used in electronic nose (e-nose) technology (Zhai, *et al.*, 2024). It evaluates odor patterns produced with the deterioration of egg freshness by simulating human olfaction (Yang *et al.*, 2023). Hydrogen sulfide, carbon dioxide, and organic acids are among the volatile substances that escape through the shell pores as eggs age, changing the composition of the gas (Konkol *et al.*, 2022). In order to assess freshness, the sensor array first detects these changes, converts them into electrical signals, and then uses pattern recognition algorithms to examine the data.

***Spectral Analysis Technology***

Spectral analysis technology is a non-destructive detection technique that provides information on chemical composition, structural features, and physical qualities by using the absorption, scattering, or emission characteristics of materials at particular wave lengths (Lu and Lu, 2017). Based on the principles of light interaction (transmission, refraction, and reflection), it evaluates the freshness of eggs by examining optical processes during light penetration and developing mathematical models for quality assessment (Huang *et al.*, 2024).

***Computer Vision Technology***

According to Wiley and Lucas (2018), computer vision technology uses image processing techniques to extract, analyze, and interpret visual information from picture data. Critical areas including the air cell and yolk undergo morphological changes when egg freshness declines (Rho and Cho, 2024). This method allows for accurate and effective freshness evaluation by analyzing egg photos using algorithms and models to extract important visual aspects and correlate them with freshness labels. Deep learning techniques and conventional methods make up the current computer vision-based image processing techniques.

***Multi-Fusion Sensor Technology***

Drayer and Howard, (2012) reported that in order eliminate systemic uncertainties and produce accurate observational results and integrated intelligence, multisensory fusion technology uses computational techniques to automatically analyze and synthesize information and data from multiple sensors or sources under specific criteria (Drayer and Howard, 2012). It has been extensively used in robotics, intelligent inspection, military systems, industrial monitoring, object identification and tracking, image analysis, and automatic target recognition. Based on the degree of information processing, the architecture of multi-sensor fusion detection systems can be divided into three hierarchical frameworks: data-level fusion (direct integration of raw sensor data), feature-level fusion (extraction and combination of salient features from preprocessed data), and decision-level fusion (aggregation of outputs from individual sensor-based decision models) (Karvir, 2010).

***Artificial Intelligence (AI) Technology***

Artificial intelligence is one of the modern techniques employed in developed countries to improve overall egg quality analysis and automate the egg mirage process, Helene *et al.* (2025) investigated conventional methods of poor egg quality by presenting a novel solution that combines Artificial Intelligence (AI) and Internet of Things (IoT) technologies. The web-based application uses a Convolutional Neural Network (CNN) algorithm to deliver real-time feedback on egg quality. In addition to revolutionizing egg quality monitoring in the poultry sector, this research's accomplishment establishes a standard for integrating AI and IoT to solve challenging issues in agricultural practices, such as power management. Artificial intelligence (AI) developments are driving an extraordinary technological transformation in the traditional egg deep-processing sector. Ali *et al.* (2024) observed that by examining spectrum characteristics associated with Haugh units, AI-powered hyperspectral imaging (HSI) improves freshness grading and defect detection related to eggs fractures and stains. Production line scheduling and operational efficiency are being optimized by AI technologies such as data analytics and machine learning algorithms. For example, real-time data analysis makes it possible to make predicted changes to production schedules that minimize waste and guarantee alignment with market expectations (Ameh, 2024). Artificial intelligence is using its special advantages to enable revolutionary advances in all facets of egg processing, including production, management, and sales. Thus, egg qualities are improved in developed countries with the introduction of basic algorithms.

**Branding of Egg quality among consumers in Digital Economies**

Guyonnet (2019b) asserts that the global sector will further improve its economic sustainability while offering consumers safe and nutritious foods by concentrating on marketing campaigns that highlight the value and advantages of branded shell eggs, either by national organizations of producers or by egg companies. The great

majority of shell eggs are sold in bulk, either by weight or by unit, in major egg-producing nations like China, India, and Indonesia (Guyonnet, 2020b). The need to convert consumers from these "generic" eggs to branded eggs will be one of marketing's main responsibilities because the sale of branded goods usually results in higher revenues for egg farmers and packers. There will be numerous potentials to increase the worldwide market share of beautifully packaged, branded shell eggs because younger generations of consumers no longer prioritize pricing when making purchases.

## Conclusion and Recommendations

This study therefore concludes that egg consumption level had increase over the years globally and consumers are now concerned about the egg quality and well as the physical attributes which when properly managed during production could improve the freshness of the eggs. Holistic approaches ranging from nose electronic method to current use of algorithms using artificial intelligence promotes the freshness, egg shell strength, and other physical attributes of eggs in terms of quality in developed countries. The study recommends the needs for developing countries to adopt the modern digital methods using in developed countries for egg quality improvement. There is also need to train egg producers and consumers on the significance of egg quality measure to ensure sustainability from both ends.

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# Optimizing Layer Feed Composition for Improved Egg Quality

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## Abstract

Nutrition plays a significant role in poultry management especially for laying hen who produce eggs which are of high economic value to producers, there is a need to improve the quality of eggs produced by these laying eggs through adequate nutritional measures. The main aim of this review was to examine optimization of layer feed composition for improved egg quality. A Systemic approach was employed to identify organic and inorganic feed composition used by laying hens, feed optimization of laying hens, and factors influencing laying hen's eggs optimization. The findings reveal that both organic and inorganic feed components had significant impacts on laying in egg quality especially in the terms of egg weight, shell thickness, and Haugh unit. Mineral feed additions like Zinc helps to improve laying eggs during high stress conditions, while methionine acts as antioxidants. Crude protein promotes enzymatic activities which promotes egg formation. Feed optimization improves feeding efficiency and egg quality of laying hens. Several factors influence laying hens egg quality ranging from feeding, genetic history, and environment. The study concludes that a combination of mineral and organic trace feed composition coupled with proper feed optimization patterns daily could improve the egg qualities of laying hens. The study recommends the need for poultry producers to adopts nutrition feeding plan which accommodates important feeding composition and optimization plans.

***Keywords:*** *Optimization, Eggs, Quality, Layers, and hen*

## Introduction

The best strategy to maintain high hen-day output, eggshell integrity, and internal quality throughout long laying cycles is to optimize each hen's daily consumption of accessible nutrients rather than depending only on dietary percentages in feed (Poudel, 2025). In order to maximize laying hen productivity and egg quality, microminerals are crucial to poultry nutrition (Atmaja et al. 2024). boosting egg production is the top aim for farmers, breeding firms, and feed companies. This is currently achieved by boosting older hens' lay persistence and egg quality stability (Yves, 2015). Due to their low cost, eggs have become a major consumer product globally, have significant economic worth, and are a great source of animal protein [Mottet and Tempio, 2017]. Interest in the internal nutritional makeup of eggs, such as the amount and percentage of omega-3 and omega-6 unsaturated fatty acids in the egg yolk, is growing as living standards in both industrialized and developing nations continue to rise (Gao et al. 2023). Breeders and producers are paying more attention to the crucial function that dietary oils play in livestock and poultry feed as a result of ongoing advances in the performance of these animals.

The need to satisfy the various demands of consumers, the poultry sector is now concentrating on ways to increase egg quality and boost egg production performance. In order to prevent laying birds from taking energy from sources such as their body protein, which would lower their production level and cause them to lose weight, the birds should be fed feeds with the proper energy level. This happens as a result of a lack of energy compromising its muscle mass. Even though these associations are widely recognized in commercial feed formulation today, it would be ideal to confirm the somewhat outdated findings using contemporary hybrid layers in order to measure the impacts and improve suggestions (Jeroch, 2011). Laying hens must consume more protein and energy in the morning (AM) and more calcium (Ca) in the afternoon/evening (PM) to produce eggs than during the rest of the day. To better address the needs of hens than with traditional approaches, poultry experts are attempting to implement a novel feeding strategy known as AM/PM or split feeding (Akter, *et al.* 2025). Due to the high initial costs, agricultural technology in the past was mostly centered on animal production, which required larger investments and higher feeding costs.

However, nutritional strategies to more precisely meet poultry nutrient requirements are becoming crucial for economic sustainability due to growing economic challenges like fluctuating egg prices (Moss *et al.* 2021) and the fact that feed accounts for more than 65% of the costs associated with live poultry production (Wilkinson, 2018). When accounting for calcium retention, hens need 4 g of calcium per day and export 2.2 g per egg (Nys., 2015). Giving layer hens distinct morning and evening feeds may help them obtain the particular nutrients required at different times of the day because the retention time of feed in the digestive tract of poultry is notably short,

averaging about 5 to 6 hours (Svihus and Itani, 2019). Improved egg quality and layer skeletal health are two productivity and financial advantages of this morning (AM)/evening (PM) feeding method.

The main objective was to assess optimizing layer feed composition for improved egg quality.

Thus, the purpose of this review was to: -

a. Examine existing literature: The goal of this review is to provide thorough research into layer feed composition, use of organic and inorganic feed composition for egg quality, feed optimization in layer hens, and factors influencing layer hen egg quality.

b. Composing relevant data from reliable sources and evaluating reputable articles are part of planned results synthesis.

The reputable research platform Research Gate was used in this review. An analysis of the research gate database was done. The main aim was on optimization of poultry feeds especially laying hens, organic and inorganic feed composition, feed optimization in relation to egg quality, and factors influencing laying hens eggs quality. Relevant information from several academic disciplines by using these keywords. Articles that were reviewed in a specific quantity included reports, conference proceedings, and journal articles.

**Inorganic and organic feed composition on Laying hen eggs quality**

According to Alagawany *et al.* (2016), crude protein (CP) is crucial for maintaining laying performance because it provides the essential amino acids needed for tissue repair, enzyme activity, and egg production. Organic trace minerals can increase eggshell thickness and decrease fecal mineral retention without adversely affecting layer hen productivity, according to Qiu *et al.* (2020). But nutrient needs are dynamic; they change during different stages of production and are impacted by environmental stresses. In order to decrease metabolic heat load during heat stress (> 30°C), lower FI requires greater nutrient density diets with higher concentrations of energy and amino acids but lower CP (Teyssier *et al.* 2022).

Zinc (Zn) is a vital trace mineral that supports laying hens under stress and is involved in over 300 enzymatic pathways. Its physiological roles include immunological control, metabolic homeostasis maintenance, and antioxidant defense (Mohebbifar *et al.* 2025). By increasing shell thickness, egg weight, Haugh unit, and feed conversion ratio (FCR), studies have demonstrated that zinc supplementation improves laying performance and egg quality (Torki *et al.* 2015). Excessive CP decreases impair both performance and egg quality, although moderate reductions can reduce nitrogen excretion and metabolic heat production (Alagawany *et al.* 2016).

Reduced-CP diets can be balanced by supplementing with feed-grade amino acids, especially methionine (Met), the first limiting amino acid in corn-soybean meal diets (Zhang et al. 2024). According to Kachungwa Lugata *et al.* (2022), Met plays a crucial role in antioxidant defense and methylation activities, and its demand increases significantly under stressful conditions. Because they complement one another, adding both organic and inorganic trace minerals to chicken feed can be a beneficial solution. While Kong *et al.* (2022) also highlights chelation technology that can increase the bioavailability of minerals, Razanova *et al.* (2023) demonstrates that organic trace minerals in the form of chelates can improve growth performance and egg quality.

**Feeds optimization in layer hen and egg quality**

The optimization of laying hen production and egg quality using a combination of inorganic and organic trace mineral supplementation was investigated by Atmaja *et al.* (2025). The results showed that while adding a mix of inorganic and organic trace minerals (ITMs and OTMs) to the feed can greatly increase the weight and thickness of the eggshells, it has no discernible impact on the laying hens' overall performance. Using a nutritional combination of crude protein, methionine, and zinc, Mohebbifar *et al.* (2023) evaluated the optimization of laying hen productive performance and egg quality under cold stress. The study demonstrates that reducing crude protein enhanced laying performance under cold stress with negligible impacts on the majority of egg quality characteristics; nevertheless, interactions between crude protein and methonine affected albumen quality and egg shape index. Diets containing a specific percentage of the three combinations in the proper proportion had the best results. Akter et al. (2025) claim that morning (AM): afternoon (PM) intake shows that selective feeding takes place between treatments ($p < 0.001$) and that the degree of selection is dependent on the variation in nutrient levels between AM and PM diets. This study found that laying hens' feed efficiency and egg quality are improved when AM/PM feeding is optimized. According to Utami and Akbar (2025), adding fermented leucaena leucocephala (FLM) to laying quail diets efficiently increases nutrient utilization and improves egg production and quality without increasing feed intake; the ideal inclusion level for optimizing benefits seems to be less than 10%.

**Factors influencing feed composition among layer hens egg quality**

The environment, genetic composition, and feed consumption all have an impact on egg laying. Methionine content caused egg weight to rise, plateauing at 0.36–0.38%. It is easier to adapt the supply of protein and amino

acids to changes in feed intake and hen requirements at a particular age when the daily requirement is estimated in relation to egg weight. The internal quality of the egg (Haugh unit, vitelline membrane) was impacted by hen age, however the primary cause for ending the production period will be a decline in eggshell quality. The most effective and straightforward method to improve eggshell quality and reduce excessive bone loss is to provide an ideal supply of both fine and coarse particle sizes (Nys., 2015). Due to increased excretion levels, the usage of inorganic trace elements has a negative influence on the environment and low bioavailability (G. Lv et al., 2023). According to Adeyemo (2015), egg quality generally refers to all of an egg's attributes that influence a consumer's acceptance of it, or to different standards placed on eggs. A high-quality egg is thought to have a density somewhat higher than that of water, hence it shouldn't float in water. Egg weight increases with each extra gram of protein consumed daily (Bouvarel *et al.*, 2011). In order to prevent competition between pullet growth and egg feed requirements, early sexual maturity and selection for low consumption hens involve controlling feed intake capacity, pullet growth curve, and weight at the beginning of laying. The laying hens provision of essential amino acids and protein also affects egg weight. The health and welfare of hens should also be considered by producers. Hen diet is essential for maximizing the exceptional genetic potential of contemporary chickens in terms of high egg quality and productivity while preventing fatty liver syndrome and bone demineralization (Nys, 2015). Laying hens dietary habits have an impact, which may support sustained egg production and increase producer profitability.

## Conclusion and Recommendations

Feed optimization improves feeding efficiency and egg quality of laying hens. Several factors influence laying hens egg quality ranging from feeding, genetic history, and environment. The study concludes that a combination of mineral and organic trace feed composition coupled with proper feed optimization patterns daily could improve the egg qualities of laying hens. The study recommends the need for poultry producers to adopts nutrition feeding plan which accommodates important feeding composition and optimization plans.

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# Determination of the Properties of Water Kefirs Produced from Various Plant-Based Extracts

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## Abstract

Interest in milk kefir and water kefir important sources of probiotics has grown even further since the COVID-19 pandemic. In addition to the probiotics they contain, the metabolites formed during fermentation also make significant contributions to a healthy lifestyle. The aim of this study was to ferment orange and lemon peel extracts with water kefir and to determine the chemical and microbiological properties of the resulting product over a 15-day shelf life. The lemon and orange peels were extracted after undergoing specific pretreatment steps; the resulting liquid was sterilized by adding 1% turmeric and black pepper for the lemon peel extract and 1% cinnamon for the orange peel extract. A 5% water kefir grain culture was added at 25°C, and the mixture was left to ferment at this temperature for 36 hours. The pH, dry matter, ash, lactic acid, and acetic acid content of the organic acids in the pre- and post-fermentation samples were determined by liquid chromatography; additionally, the contents of *Lactobacilli* spp., *Lactococci* spp., and yeast were detected. The product's dry matter content was 1%. The initial pH values were 4.53 for lemon and 5.80 for orange extracts, and these decreased significantly to 3.50 and 3.06, respectively, by the end of fermentation. Consistent with this, an increase in lactic acid bacteria was observed. The Lactococcus spp. content in our lemon and orange extracts was determined to be 7.3 log CFU/ml and 7.3 log CFU/ml on day 1, and 7 log CFU/ml for both extracts on day 15. The *Lactobacillus* spp. content was determined to be 6.69 and 8.3 log CFU/ml on day 1, and 6.68 and 7.3 log CFU/ml on day 15. The lactic acid content of kefir obtained from lemon and orange-peel extracts was determined to be 804.370 g/L, and the acetic acid content was determined to be 548.401 g/L and 691.892 g/L, respectively. The results obtained indicate that agricultural food industry waste, such as orange and lemon peels, can be considered a potential raw material for the production of functional and fermented beverages. It was determined that fermentation using water kefir contributes positively to the production of microbiologically safe and sensorially acceptable products.

***Keywords:*** *Lemon peel, orange peel, water kefir, fermentation, lactic acid bacteria*

## Farkli Bitki Temelli Ekstraktlarından Üretilen Su Kefirlerinin Özelliklerinin Belirlenmesi

## Özet

Probiyotiklerin önemli kaynakları olan süt kefiri ve su kefirine olan ilgi, COVID-19 pandemisinden sonra daha da artmıştır. İçerdikleri probiyotiklerin yanı sıra fermantasyonla oluşan metabolitler de sağlıklı yaşama önemli katkılar sağlamaktadır. Bu araştırmada, portakal ve limon kabuğu ekstraktının su kefiri ile fermantasyonu gerçekleştirilmesi ve elde edilen ürünün 15 gün raf ömrü süresince kimyasal ve mikrobiyolojik özelliklerinin belirlenmesi amaçlanmıştır. Limon ve portakal kabukları, belirli ön işlemlerden geçirilerek ekstrakte edilmiş, elde edilen ekstrakt %1 oranında limon kabuğu için zerdeçal ve karabiber, portakal kabuğu için %1 oranında tarçın eklenerek sterilize edilmiştir. 25 °C'de %5 oranında su kefiri dane kültürü ilave edilerek bu sıcaklıkta 36 saat süresince fermantasyona bırakılmıştır. Fermantasyon öncesi ve sonrası örneklerde pH, kuru madde, kül, laktik asit ve asetik asit olmak üzere organik asit içerik likit kromatografik olarak belirlenmiş; ayrıca *Lactobacilli* spp., *Lactococci* spp. ve maya içerikleri tespit edilmiştir. Ürünün kuru madde içeriği %1 olarak bulunmuştur. Başlangıç pH değerleri limon için 4,53, portakal için 5,80 'den fermantasyon sonunda sırasıyla pH 3,50 ve 3,06'a önemli düzeyde azalmıştır. Uyumlu olarak, laktik asit bakterilerinde artış belirlenmiştir. Limon ve portakal ekstraklarının *Lactococcus* spp. içeriği 1. gün 7,3 logkob/ml ve 7,3 logkob/ml, 15. günde iki ekstrakt için 7.0 logkob/ml olarak belirlenmiştir. Fermente limon ve portakal ekstraklarının *Lactobacilli* spp. içeriği sırasıyla 1. gün 6,69 ve 8,3 logkob/ml, 15. gün 6,68 ve 7,3 logkob/ml olarak belirlenmiştir. Limon ve portakal kabuğu ekstraklarından elde edilen kefirin laktik asit içeriği 804,370 g/L, asetik asit içeriği 548,401 g/L olarak belirlenmiştir. Elde edilen sonuçlar, portakal ve limon kabuğu gibi tarımsal gıda endüstrisi atıklarının fonksiyonel ve fermente içecek üretiminde potansiyel bir ham madde olarak katma değerle değerlendirilebileceğini ortaya koymaktadır. Su kefiri ile yapılan fermantasyonun mikrobiyolojik açıdan güvenli ve duyusal olarak kabul edilebilir ürünler elde etme yönünde olumlu katkılar sunduğu belirlenmiştir.

***Anahtar Kelimeler:*** *Limon kabuğu, portakal kabuğu, Su kefiri, Fermantasyon, Laktik asit bakterileri*

## Giriş

Son yıllarda fonksiyonel gıdalara artan ilgi klasik olarak üretilen gıda ürünlerinin dahi insan sağlığına artı katkılar sağlaması gerekliliğini ön plana çıkarmıştır. Probiyotik bakterilerin, insan sindirim sistemini düzenlemesinin ve bağışıklık sistemini desteklemesinin yanı sıra sağlık üzerine birçok olumlu etkilerinin olduğu bilimsel araştırmalarda tespit edilmiştir. Probiyotiklerin önemli kaynakları olan süt kefiri ve su kefirine olan ilgi, COVID-19 pandemisinden sonra daha da artmıştır. İçerdikleri probiyotiklerin yanı sıra fermantasyonla oluşan metabolitler de sağlıklı yaşama önemli katkılar sağlamaktadır. Süt bazlı kefir ürünleri üzerinde yapılan pek çok çalışmadan elde edilen sonuçlara göre, şeker kaynağı olarak laktoz içermesi, kolesterol düzeyi; laktoz intolerans ve kolesterol sorunları olan kişilerde sağlık problemlerine neden olabildiğini göstermekte, içeriğinde çeşitli bitkisel besin kaynaklarına kolaylıkla adapte olabilen dost bakteri ve mayaların varlığı, düşük kalori içeriği, sıfır kolesterol ve laktoz içermeyen su kefirinin, süt bazlı kefir ürünlerine karşın bir alternatif olarak düşünülmesini sağlamaktadır, ayrıca vegan ve/veya vejetaryenler için de önemli bir probiyotik kaynağı olarak dikkat çekmektedir (Değirmencioğlu, 2020). Su kefiri karakteristik olarak *Lactobacillus* spp., ve *Lactococcus* spp. gibi çeşitli laktik asit bakterileri, asetik asit bakterilerini ve *Saccharomyces* spp. gibi mayaları içerir. Su kefiri önemli bir bitkisel probiyotik kaynağıdır. Özellikle hayvansal ürünlere alerjisi/intoleransı ve vegan beslenme anlayışında olan bireyler için önemli bir kaynaktır. Su kefiri, kimyasal yapısı, fiziksel görünümü ve mikrobiyal içeriği bakımından süt kefirinden farklı bir kefirdir (Gökırmaklı ve Güzel Seydim, 2022). Su kefiri, karbonhidrat içeren bir solüsyonun su kefiri tanecikleri ile 21–25°C'de 48–96 saat fermente edilmesiyle üretilmektedir. Su kefiri içecekleri elde etmek için elma, mandalina, hurma, ayva, üzüm, kivi, hindistan cevizi, bal, üzüm pekmezi, zencefil ve soğan gibi çeşitli besin kaynakları substrat olarak kullanılarak, içerik ve duyusal özellikleri birbirinden farklı ürünler elde edilebilmektedir. Yapılan araştırmalarda, su kefiri tüketiminin irritabl bağırsak sendromuna karşı olumlu etkilere sahip olabileceğini ve bağırsak sağlığını koruyucu, kolesterol düşürücü, antidiyabetik ve antihiperlipidemik, antimikrobiyal, anti inflamatuar, antioksidan ve hepato-protektif özelliklere sahip olduğunu bulunmuştur (Guzel-Seydim ve Gökırmaklı, 2021).

Limon kabuğu, içerdiği yüksek miktardaki biyoaktif bileşenler nedeniyle önemli bir fonksiyonel gıda hammaddesi olarak kabul edilmektedir. Yapısında başta C vitamini, fenolik bileşikler, flavonoidler, diyet lifleri, uçucu yağlar, karotenoidler ve potasyum, kalsiyum, magnezyum, fosfor, demir ve çinko gibi mineral maddeler bulunmaktadır. Ayrıca limon kabuğu, selüloz, hemiselüloz ve lignin gibi yapısal polisakkaritler bakımından da zengin bir kaynaktır. Sağlık açısından değerlendirildiğinde, limon kabuğunda bulunan fenolik bileşikler ve C vitamini güçlü antioksidan özellik göstererek serbest radikallerin neden olduğu oksidatif stresi azaltmayı desteklemektedir. Bu sayede hücre hasarının önlenmesine katkı sağlayarak kardiyovasküler hastalıklar, diyabet, obezite ve bazı kanser türlerine karşı koruyucu etkiler gösterebileceği bildirilmektedir. Bunun yanında, yüksek diyet lifi içeriği bağırsak sağlığını desteklemekte, sindirimi düzenlemekte, tokluk hissini artırmakta ve glikoz ile kolesterol emilimini yavaşlatarak metabolik hastalıkların kontrolüne katkıda bulunmaktadır (Mercan, 2025). Ayrıca çözünür liflerin bağırsak mikrobiyotası tarafından fermente edilmesiyle oluşan kısa zincirli yağ asitleri de bağırsak ve genel vücut sağlığı üzerinde olumlu etkiler oluşturmaktadır. Benzer şekilde, portakal kabuğu, meyve suyu endüstrisinde büyük oranda atık olarak ortaya çıkmasına rağmen, yüksek besin değeri ve zengin biyoaktif bileşen içeriği sayesinde fonksiyonel gıda üretiminde önemli bir hammadde olarak değerlendirilmektedir. Portakal kabuğu; diyet lifi, fenolik bileşikler, flavonoidler, pektin ve doğal antioksidanlar bakımından oldukça zengindir. Ayrıca çeşitli vitaminler, mineraller ve biyolojik olarak aktif fitokimyasallar içermektedir (Erdoğdu, 2023).

Sürdürülebilir gıda üretimi ve gıda atıklarının katma değerli ürünlere dönüştürülmesi, günümüzde hem bilimsel çalışmaların hem de gıda sanayisinin temel araştırma alanlarından biri hâline gelmiştir. Meyve ve sebze işleme süreçleri sonucunda ortaya çıkan kabuk, posa, çekirdek ve benzeri yan ürünler; fenolik bileşikler, diyet lifi, vitaminler ve antioksidanlar bakımından zengin içeriklere sahip olmalarına rağmen çoğunlukla atık olarak değerlendirilmektedir. Bu durum yalnızca ekonomik kayıplara yol açmakla kalmayıp çevresel sürdürülebilirlik açısından da çeşitli sorunları beraberinde getirmektedir. Buna karşın, söz konusu yan ürünlerin fonksiyonel gıda üretiminde değerlendirilmesi hem döngüsel ekonomi yaklaşımını desteklemekte hem de yüksek katma değerli yeni ürünlerin geliştirilmesine olanak sağlamaktadır. Bu nedenle, gıda endüstrisinden kaynaklanan bitkisel yan ürünlerin yeniden kullanımı, çevresel etkilerin azaltılması ve kaynak verimliliğinin artırılması açısından önemli bir strateji olarak görülmektedir (Mirabella ve ark., 2014).

Son yıllarda gerçekleştirilen çalışmalar, meyve ve sebze kökenli ekstraktların su kefiri üretiminde alternatif substrat olarak kullanılabileceğini ve fermentasyon sürecini olumlu yönde etkileyebileceğini göstermektedir. Farklı bitkisel kaynaklardan elde edilen ekstraktların fermentasyon ortamına ilave edilmesiyle besin bileşiminin zenginleştiği, probiyotik mikroorganizmaların canlılığının arttığı ve elde edilen ürünlerin fonksiyonel özellikleri ile antioksidan aktivitelerinin geliştiği bildirilmektedir. Ayrıca bu doğal bileşenlerin, su kefirinin aroma, tat ve genel duyusal özelliklerini iyileştirerek tüketici beğenisini artırabileceği ifade edilmektedir (Agirman ve ark., 2024; Vicenssuto ve Castro, 2020).

Bu çalışma kapsamında, gıda sanayisinde yan ürün olarak açığa çıkan limon ve portakal kabuklarının fonksiyonel gıda üretiminde kullanım olanaklarının değerlendirilmesi amaçlanmıştır. Ayrıca, söz konusu

narenciye kabuklarının sürdürülebilir bir yaklaşımla katma değerli ürünlere dönüştürülmesine katkı sağlayabilecek özelliklerinin ortaya konulması hedeflenmiştir.

## Materyal ve Yöntem

Limon ve portakal kabukları Isparta ilindeki yerel pazarlardan sağlanmıştır. Analizlerde kullanılan tüm besiyerleri ve kimyasallar analitik saflıkta olup Merck (Almanya) firmasından temin edilmiştir.

### Fermente Limon ve Portakal Kabuğu Ekstraktı Üretimi

Yaklaşık 200'er gram limon ve portakal kabuğu 1 L içme suyu içerisinde hazırlanmış, limon kabuklarına %1 oranında taze zerdeçal ile %1 oranında sarımsak ilave edilmiştir. Portakal kabuklarına %1 oranında tarçın ilave edilmiştir. Karışımlar 15 dakika kaynatıldıktan sonra süzme işlemi yapılıp, kabuklar ve su ayrılmıştır. Sonrasında ekstraktlar 121°C'de 15 dakika sterilize edilmiştir. Oda sıcaklığına getirilen ekstraktlara hacimce %5 oranında su kefiri danesi ilave edilmiş ve 25°C'de 36 saat fermantasyona bırakılmıştır. Fermantasyon öncesi ve sonrasında pH ölçümleri gerçekleştirilmiştir.

### Su Kefiri Üretimi

Su kefiri daneleri Danem Süt ve Süt Ürünleri Ltd. Şti. (Isparta, Türkiye)'nden temin edilmiştir. Su kefiri üretimi, %10 sakkaroz çözeltisine %15 oranında su kefiri danesi ilavesiyle gerçekleştirilmiş ve karışım 25°C'de pH 4,6'ya ulaşıncaya kadar (36–48 saat) fermente edilmiştir.

### Mikrobiyolojik Analizler

Lactobacillus spp., Lactococcus spp. Koliform ve maya-küf sayımları uygun seçici besiyerleri kullanılarak gerçekleştirilmiştir. Örnekler seri dilüsyonlara tabi tutulmuş, uygun inkübasyon koşullarında inkübe edilmiş ve sonuçlar koloni oluşturan birim (log KOB/mL) olarak ifade edilmiştir.

### Mikrobiyal Tanımlama

Bakteriyel tür tanımlaması için PacBio tabanlı tam uzunlukta 16S rRNA gen dizileme yöntemi uygulanmıştır. Maya ve fungal türlerin belirlenmesinde ITS bölgesi PCR ile çoğaltılmış ve Sanger dizileme yöntemi kullanılmıştır. Elde edilen diziler biyoinformatik analizlerle değerlendirilerek tür tanımlamaları yapılmıştır.

### Kimyasal Analizler

Besin değeri analizleri TÜBİTAK Marmara Araştırma Merkezi'nde gerçekleştirilmiştir. pH ölçümleri pH metre ile yapılmıştır. Toplam kuru madde ve kül içerikleri AOAC yöntemlerine göre belirlenmiştir.

Organik asit analizleri HPLC sistemi kullanılarak gerçekleştirilmiştir. Toplam fenolik madde miktarı Folin–Ciocalteu yöntemiyle belirlenmiş ve sonuçlar mg GAE/100 mL olarak ifade edilmiştir. Toplam antioksidan kapasite ABTS yöntemine göre ölçülmüş ve sonuçlar TEAC değeri olarak verilmiştir. Fenolik bileşenlerin profili HPLC-DAD sistemi ile belirlenmiştir.

### Duyusal analiz

Ürünlerin duyusal özellikleri (tat, koku ve görünüş) 10 kişilik panelist grup tarafından değerlendirilmiştir. Değerlendirmeler 10'lu hedonik skala kullanılarak yapılmış olup, 1 "çok düşük beğeni" ve 10 "çok yüksek beğeni"yi ifade etmektedir. Elde edilen skorların ortalamaları alınarak sonuçlar hazırlanmıştır.

## Bulgular

### pH Değişimi

Limon ve portakal kabuğu ekstraktları kullanılarak üretilen su kefirlerinde fermentasyon süreci boyunca pH değerlerinde azalma meydana gelmiştir. Limon kabuğu ekstraktı içeren örneklerde başlangıç pH değeri 4,53 iken fermentasyon sonunda 3,50'ye düşmüştür. Benzer şekilde, portakal kabuğu ekstraktı ilave edilen örneklerde başlangıçta 6,38 olarak belirlenen pH değeri fermentasyon sonunda 4,27 olarak tespit edilmiştir. Her iki örnekte de gözlenen pH düşüşü, fermentasyon sırasında mikroorganizmaların faaliyetleri sonucu organik asit oluşumunun arttığını ve ortamın daha asidik bir yapıya ulaştığını göstermektedir. Bununla birlikte, limon kabuğu ekstraktı içeren örneklerin fermentasyon sonunda portakal kabuğu ekstraktı içeren örneklere kıyasla daha düşük pH değerine sahip olduğu belirlenmiştir.

### Besin Değeri Bulguları

Analiz sonuçlarına göre her iki ekstraktın da önemli kısmını su oluşturmaktadır (≈99,25 g/100 g). İnorganik madde içeriği oldukça düşük olup kül oranı 0,16 g/100 g olarak belirlenmiştir. Temel besin bileşenleri açısından ekstraktların oldukça fakir bir yapıya sahip olduğu görülmüş; protein ve yağ tespit edilememiş, diyet lifi ise eser

düzeydedir (<0,65 g/100 g); çünkü posa ekstraksiyondan sonra ayrılmıştır. Karbonhidrat içeriği 0,64 g/100 g düzeyinde bulunmuştur. Bu kompozisyona bağlı olarak her iki ekstraktın enerji değeri düşük olup yaklaşık 3 kcal/100 g olarak tespit edilmiştir.

**Mikrobiyolojik Bulgular**

Mikrobiyolojik analiz sonuçları log kob/mL cinsinden değerlendirildiğinde, limon kabuğu ekstraktı (karabiber ve zerdeçal ilaveli) içeren su kefiri örneklerinde Lactobacillus spp. sayısının depolama süresince 7,00 log kob/mL'den 6,70 log kob/mL'ye gerilediği belirlenmiştir. Aynı örneklerde Lactococcus spp. içeriği 7,30 log kob/mL'den 7,00 log kob/mL'ye azalırken, maya sayısı ise 6,70 log kob/mL'den 2,48 log kob/mL'ye azalmıştır. Portakal kabuğu ekstraktı (tarçın ilaveli) içeren örneklerde ise Lactobacillus spp. sayısı 8,32 log kob/mL'den 7,30 log kob/mL'ye gerilemiş, Lactococcus spp. sayısı 7,30 log kob/mL'den 7,00 log kob/mL'ye azalmıştır. Maya sayısında daha belirgin bir azalma gözlenmiş ve değerler 6,30 log kob/mL'den 2,30 log kob/mL'ye gerilemiştir. Buna karşın, her iki örnekte de analiz süresince koliform bakteri ve küf tespit edilmemiştir. Elde edilen bulgular, depolama süresince laktik asit bakterilerinin canlılıklarını koruduğunu, maya içeriğindeyse önemli düzeyde azalma meydana geldiğini göstermektedir.

**Mikrobiyal Tanımlama Analiz Bulguları**

Limon ve portakal örneklerinin mikrobiyota profilleri benzer bir temel mikrobiyal flora göstermiştir. Her iki örnekte de Gluconobacter, Acetobacter ve Saccharomyces varlığı tespit edilmiştir. Ayrıca Lacticaseibacillus spp. belirlenmiştir. Bu sonuçlar, her iki örnek matrisinin asetik asit bakterileri ve mayalar için uygun bir mikrobiyal yapı sunduğunu göstermektedir.

**Organik Asit Analizi**

Organik asit sonuçlarına göre fermente limon kabuğu ekstraktında baskın organik asit laktik asit (804,370 ppm) olup bunu asetik (548,401 ppm), sitrik (489,961 ppm) ve askorbik asit (27,294 ppm) izlemiştir; malik ve fumarik asit tespit edilmemiştir. Fermente portakal kabuğu ekstraktında ise en yüksek düzeyde asetik asit (691,892 ppm) belirlenmiş, ayrıca sitrik (32,355 ppm), askorbik (20,097 ppm) ve fumarik asit (0,243 ppm) saptanmıştır; laktik ve malik asit tespit edilmemiştir. Bulgular, limon ve portakal kabuğu ekstraktlarının su kefiri fermentasyonu sırasında farklı organik asit profilleri oluşturduğunu ve bunun mikrobiyal metabolizmanın substrat tipine bağlı olarak değiştiğini göstermektedir. Bu sonuçlar fermantasyonun başarıyla gerçekleştiğini ve laktik asit bakterilerinin aktif metabolik faaliyet gösterdiğini ortaya koymaktadır.

**Duyusal Analiz**

Duyusal analiz sonuçlarına göre limon ve portakal örnekleri tat, koku ve görünüş parametreleri açısından değerlendirilmiştir. Tat (ortalama 5 puan) ve koku (ortalama 8 puan) puanları her iki örnek için de benzer bulunmuştur. Görünüş açısından ise özellikle renginden dolayı portakal örneği 10 ortalama puan ile limon örneğine (8 puan) kıyasla daha yüksek bir kabul edilebilirlik göstermiştir.

## Sonuç

Bu çalışmada limon ve portakal kabuğu ekstraktlarının su kefiri fermentasyonunda kullanımı gerçekleştirilmiş ve elde edilen ürünlerin mikrobiyolojik, kimyasal ve duyusal özellikleri değerlendirilmiştir. Fermantasyon süreci sonunda her iki örnekte de pH değerlerinde belirgin bir düşüş gözlenmiş olup, limon ekstraktında pH 4,53'ten 3,50'ye, portakal ekstraktında ise 6,38'den 4,27'ye gerilemiştir. Bu durum, su kefiri mikroorganizmalarının aktif metabolik faaliyet gösterdiğini ve organik asit üretiminin gerçekleştiğini ortaya koymuştur. Mikrobiyolojik analizlerde her iki örnekte de Lactobacillus spp. ve Lactococcus spp. varlığının korunduğu, maya popülasyonunun ise depolama süresince canlılığını sürdürdüğü belirlenmiştir. Ayrıca koliform bakteri ve küf gelişiminin tespit edilmemesi, ürünlerin mikrobiyolojik açıdan güvenli olduğunu göstermiştir. Organik asit profili açısından limon ekstraktında laktik asit baskın olurken, portakal ekstraktında asetik asit daha yüksek düzeyde belirlenmiştir. Bu sonuçlar, fermentasyon metabolizmasının kullanılan substrata bağlı olarak değiştiğini doğrulamaktadır. Besin değeri analizleri her iki ekstraktın düşük makro besin içeriğine ve düşük enerji değerine sahip olduğunu göstermiştir. Düşük enerji içerikli, biyoaktif bileşenleri içeren içecekler günümüzün tercih eğilimidir. Genel olarak elde edilen bulgular, limon ve portakal kabuğu ekstraktlarının su kefiri üretiminde alternatif substrat olarak değerlendirilebileceğini ve fonksiyonel özelliklere sahip fermente ürünlerin geliştirilmesine katkı sağlayabileceğini göstermektedir. Bu araştırmada ekstraktlara besin bileşenleriyle zenginleştirme yapılmadan probiyotiklerin gelişimi tespit edilmiştir. Ürünler duyusal özellikler bakımından geliştirilmeye açıktır.

**Yazar Katkı Beyanı**

Her yazarın bu çalışmaya hangi aşamada ve nasıl katkı sağladığı belirtilmelidir. Örneğin: Ramazan ERÇELİK: Veri toplama, araştırma, biçimsel analiz ve ilk taslak yazımı

Prof. Dr. Zeynep Banu SEYDİM: Proje yönetimi, danışmanlık, kavramsallaştırma, metodoloji, gözden geçirme ve düzenleme

**Çıkar Çatışması**

Yazarlar herhangi bir çıkar çatışması olmadığını beyan eder.

**Kaynaklar**

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# Fermentation of Beet Extract with Water Kefir

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## Abstract

The aim of this study was to carry out the fermentation of beet extract with water kefir and to determine the chemical and microbiological properties of the resulting product over a 15-day shelf life. Beet peels were extracted after undergoing specific pretreatment steps, and the resulting liquid was sterilized after addition of 0.5% ginger and garlic. A 5% water kefir grain culture was added at 25°C, and the mixture was left to ferment at this temperature for 36 hours. The pH, dry matter, ash, lactic acid, and acetic acid content of the organic acids in the pre- and post-fermentation samples were determined by liquid chromatography; additionally, the contents of Lactobacilli spp., Lactococci spp., and yeast were detected. The product's dry matter content was 2%. The initial pH value decreased significantly from 6.36 to 4.23 at the end of fermentation. Correspondingly, an increase in lactic acid bacteria was observed. The Lactococcus spp. content was determined to be 7.3 log CFU/mL on day 1 and 7.26 log CFU/mL on day 15. The lactic acid content of the kefir obtained from beet extract was determined to be 1.880 g/L, and the acetic acid content was 1.636 g/L. Additionally, within the scope of sensory analysis studies, panelists evaluated the product's appearance, odor, and taste characteristics, and it was determined that the overall acceptability level was appropriate. The results obtained demonstrate that agricultural food industry waste, such as beet peel, can be considered a potential raw material to produce functional and fermented beverages. It was determined that fermentation using water kefir contributes positively to the production of microbiologically safe and sensorially acceptable products.

***Keywords:*** *Beet, Water kefir, Fermentation, Lactic acid bacteria*

## Pancar Ekstraktının Su Kefiri ile Fermantasyonu

### Özet

Bu çalışmada, pancar ekstraktının su kefiri ile fermantasyonu gerçekleştirilmesi ve elde edilen ürünün 15 gün raf ömrü süresince kimyasal ve mikrobiyolojik özelliklerinin belirlenmesi amaçlanmıştır. Pancar kabukları, belirli ön işlemlerden geçirilerek ekstrakte edilmiş, elde edilen sıvı %0,5 oranında zencefil ve sarımsak eklendikten sonra sterilize edilmiştir. 25 °C'de %5 oranında su kefiri dane kültürü ilave edilerek bu sıcaklıkta 36 saat süresince fermantasyona bırakılmıştır. Fermantasyon öncesi ve sonrası örneklerde pH, kuru madde, kül, laktik asit ve asetik asit olmak üzere organik asit içerik likit kromatografik olarak belirlenmiş; ayrıca Lactobacilli spp., Lactococci spp. ve maya içerikleri tespit edilmiştir. Ürünün kuru madde içeriği %2 olarak bulunmuştur. Başlangıç pH değeri 6,36 değerinden fermantasyon sonunda 4,23'e önemli düzeyde azalmıştır. Uyumlu olarak, laktik asit bakterilerinde artış belirlenmiştir. Lactococcus spp. içeriği 1. gün 7,3 log kob/ml, 15. günde 7,26 log kob/ml olarak belirlenmiştir. Pancar ekstraktından elde edilen kefirin laktik asit içeriği 1,880g/L, asetik asit içeriği 1,636 g/L olarak belirlenmiştir. Ayrıca, duyusal analiz çalışmaları kapsamında panelistler tarafından ürünün görünüş, koku ve tat özellikleri değerlendirilmiş ve genel kabul düzeyinin uygun olduğu belirlenmiştir. Elde edilen sonuçlar, pancar kabuğu gibi tarımsal gıda endüstrisi atıklarının fonksiyonel ve fermente içecek üretiminde potansiyel bir ham madde olarak değerlendirilebileceğini ortaya koymaktadır. Su kefiri ile yapılan fermantasyonun mikrobiyolojik açıdan güvenli ve duyusal olarak kabul edilebilir ürünler elde etme yönünde olumlu katkılar sunduğu belirlenmiştir.

***Anahtar Kelimeler:*** *Pancar, Su kefiri, Fermantasyon, Laktik asit bakterileri*

## Giriş

Fonksiyonel gıdalar, temel besleyici özelliklerinin yanında insan sağlığı üzerinde olumlu etkiler sağlayan ve bazı hastalık risklerinin azaltılmasına katkıda bulunan ürünler olarak tanımlanmaktadır. Son yıllarda tüketicilerin sağlıklı yaşam ve dengeli beslenmeye yönelik ilgisinin artması, fonksiyonel gıda pazarının hızla büyümesine neden olmuş; özellikle probiyotik içeren fermente ürünler bu alanda önemli bir yer edinmiştir (Gezginç ve Gök, 2016; Azizi ve ark., 2021). Fermente içecekler, içeriklerindeki yararlı mikroorganizmalar ve fermantasyon sırasında oluşan biyoaktif bileşikler sayesinde fonksiyonel gıdalar arasında öne çıkmaktadır.

Su kefiri, şekerli ortamda kefir danelerinin gerçekleştirdiği fermantasyon sonucu elde edilen, hafif asidik karaktere sahip, düşük alkollü ve doğal olarak karbon dioksit içeren ferahlatıcı bir içecektir. Su kefiri daneleri; laktik asit bakterileri, mayalar ve asetik asit bakterilerinden oluşan karmaşık bir mikrobiyal topluluğu içeren polisakkarit yapılı granüllerdir (Laureys ve De Vuyst, 2014). Bu mikroorganizmalar fermantasyon süresince sakarozu kullanarak laktik asit, asetik asit, etanol, karbondioksit ve çeşitli aroma bileşiklerini üretmekte, böylece ürünün hem duyusal hem de fonksiyonel özelliklerini geliştirmektedir (Fiorda ve ark., 2017).

Su kefiri, süt bazlı kefirden farklı olarak laktoz içermemesi nedeniyle vegan bireyler ile süt alerjisi veya laktoz intoleransı bulunan tüketiciler için önemli bir alternatif probiyotik ürün olarak değerlendirilmektedir (Dahiya ve Nigam, 2023). Ayrıca su kefirinde bulunan laktik asit bakterileri ve mayalar arasındaki simbiyotik ilişki, fermantasyon süresince mikrobiyal canlılığın korunmasına ve ürünün raf ömrünün uzamasına katkı sağlamaktadır (Gulitz ve ark., 2011; Gulitz ve ark., 2013).

Su kefiri danelerinin yapısında bulunan dekstran, mikroorganizmalar için koruyucu bir ortam oluşturmaktadır. Bu yapı başta Leuconostoc ve Lactobacillus türleri olmak üzere çeşitli mikroorganizmalar tarafından sentezlenmekte ve danelerin karakteristik fiziksel özelliklerinin oluşmasında rol oynamaktadır (Davidović ve ark., 2015; Fels ve ark., 2018). Su kefirinin mikrobiyal yapısında ağırlıklı olarak Lactobacillus spp., Lactococcus spp., Leuconostoc spp., Saccharomyces spp. ve çeşitli asetik asit bakterileri yer almaktadır. Bu mikroorganizmaların oluşturduğu metabolitler ürünün probiyotik ve fonksiyonel özelliğinin temelini oluşturmaktadır.

Su kefirinin sağlık üzerine etkileri uzun yıllardır araştırılmaktadır. Yapılan çalışmalar, kefirin antimikrobiyal, antioksidan, immünomodülatör ve antikanserojen özellikler gösterebildiğini ortaya koymuştur. Fermantasyon sırasında oluşan organik asitler ve çeşitli biyoaktif bileşiklerin patojen mikroorganizmaların gelişimini baskıladığı; probiyotik mikroorganizmaların ise bağırsak mikrobiyotasını düzenleyerek bağışıklık sistemini desteklediği bildirilmektedir (Hosono ve ark., 1990; De LeBlanc ve ark., 2007, Güzel Seydim ve ark.,2021). Ayrıca bazı araştırmalar probiyotik ürün tüketiminin serum kolesterol seviyelerinin düşürülmesine katkıda bulunabileceğini göstermiştir (Guo ve ark., 2011).

Günümüzde sürdürülebilir gıda üretimi ve gıda atıklarının değerlendirilmesi konusu da araştırmaların önemli odak noktalarından biri hâline gelmiştir. Meyve ve sebze işleme sanayisinde ortaya çıkan yan ürünler, yüksek miktarda biyoaktif bileşen içermelerine rağmen çoğu zaman ekonomik değer oluşturulmadan uzaklaştırılmaktadır. Bu yan ürünlerin fonksiyonel gıda üretiminde değerlendirilmesi hem çevresel yükün azaltılması hem de ekonomik katma değer oluşturulması açısından önem taşımaktadır (Mirabella ve ark., 2014).

Kırmızı pancar, içerdiği betalain pigmentleri, fenolik bileşikler, flavonoidler ve diyet lifi nedeniyle besinsel ve fonksiyonel açıdan önemli bir sebzedir. Özellikle pancar kabuklarının toplam fenolik madde ve antioksidan kapasite bakımından oldukça zengin olduğu bildirilmektedir (Kujala ve ark., 2000; Bangar ve ark., 2022). Bununla birlikte pancar işleme süreçlerinde oluşan kabuklar çoğunlukla atık olarak değerlendirilmekte ve içerdiği değerli bileşenlerden yeterince yararlanılamamaktadır.

Son yıllarda gerçekleştirilen çalışmalar, meyve ve sebze bazlı ekstraktların su kefiri üretiminde başarıyla kullanılabildiğini ve bu hammaddelerin probiyotik mikroorganizmaların gelişimini desteklediğini göstermektedir. Özellikle farklı bitkisel kaynaklardan elde edilen ekstraktların su kefiri fermentasyonunda kullanılmasıyla mikrobiyal canlılığın arttığı ve ürünlerin fonksiyonel özelliklerinin iyileştiği bildirilmektedir (Agirman ve ark., 2024; Vicenssuto ve Castro, 2020). Bu nedenle pancar kabuğu ekstraktının su kefiri üretiminde kullanılması hem sürdürülebilirlik hem de fonksiyonel ürün geliştirme açısından önemli bir araştırma konusu olarak değerlendirilmektedir.

Bu çalışmada, pancar kabuğu ekstraktı kullanılarak su kefiri üretimi gerçekleştirilmiş ve elde edilen ürünün mikrobiyolojik, fizikokimyasal ve duyusal özellikleri incelenmiştir. Çalışmanın, tarımsal bir atık olan pancar kabuğunun katma değerli bir fonksiyonel içeceğe dönüştürülmesine yönelik bilimsel veriler sağlayarak sürdürülebilir gıda sistemlerine katkı sunması amaçlanmıştır.

## Materyal ve Yöntem

Çalışmada kullanılan su kefiri daneleri, Danem Ltd. Şti. (Isparta, Türkiye)'nden temin edilmiştir. Kırmızı pancar kabukları Isparta ilindeki yerel pazarlardan sağlanmıştır. Analizlerde kullanılan tüm besiyerleri ve kimyasallar analitik saflıkta olup Merck (Almanya) firmasından temin edilmiştir.

### Fermente Pancar Kabuğu Ekstraktı Üretimi

Yaklaşık 200 g pancar kabuğu 1 L içme suyu içerisinde hazırlanmış ve karışıma %0,5 oranında taze zencefil ile %0,5 oranında sarımsak ilave edilmiştir. Karışım 15 dakika kaynatıldıktan sonra süzme işlemi yapılıp, kabuklar ve su ayrılmıştır. Ayrılan ekstrakt 121°C'de 15 dakika sterilize edilmiştir. Oda sıcaklığına soğutulan ekstrakta hacimce %5 oranında su kefiri danesi ilave edilmiş ve 25°C'de 36 saat fermantasyona bırakılmıştır. Fermantasyon öncesi ve sonrasında pH ölçümleri gerçekleştirilmiştir.

**Mikrobiyolojik Analizler**

Mikrobiyolojik analizlerde *Lactobacillus* spp., *Lactococcus* spp., koliform bakteriler ve maya-küf sırasıyla; MRS Agar, M17 Agar, VRB Agar ve PDA besiyerlerinde uygun inkübasyon sıcaklık ve süreleri uygulanarak belirlenmiştir. Sonuçlar log KOB/mL olarak ifade edilmiştir.

**Kimyasal Analizler**

pH ölçümleri SCHOTT marka pH metre ile gerçekleştirilmiştir. Toplam kuru madde ve suda çözünür kuru madde analizleri sırasıyla nem tayin cihazı ve refraktometre kullanılarak belirlenmiştir. Kül tayini AOAC (2000) yöntemine göre 550°C'de gerçekleştirilmiştir. Organik asit analizleri HPLC (Shimadzu LC-2050) cihazında C18 kolon kullanılarak yapılmıştır. Laktik asit, asetik asit, malik asit, fumarik asit ve askorbik asit miktarları belirlenmiştir.

**Duyusal Analiz**

Ürünün duyusal kabul edilebilirliği, 10 kişilik panelist grup tarafından 10'lu hedonik skala kullanılarak değerlendirilmiştir. Panelistler ürünü tat, koku ve görünüş parametreleri açısından bireysel olarak puanlamış, elde edilen verilerin ortalamaları alınarak raporlanmıştır.

## Bulgular ve Tartışma

**pH Değişimi**

Fermantasyon sonunda pH değeri 6,36'dan 4,23'e azalmıştır. Bu azalma, laktik asit bakterilerinin ortamdaki besin bileşenlerini kullanarak metabolik olarak organik asitleri üretmesinden kaynaklanmaktadır. Düşük pH aynı zamanda ürünün mikrobiyolojik stabilitesini artırmaktadır.

**Mikrobiyolojik Bulgular**

Üründe ortalma *Lactococcus spp*. sayısı 7,3 log kob/mL, *Lactobacillus spp*. sayısı ise 8,3 log kob/mL olarak belirlenmiştir. Depolama sürecinde önemsiz azalma olmasna rağmen probiyotik mikroorganizmalar canlılıklarını korumuştur.

Maya sayısı başlangıçta 4,78 log kob/mL olarak belirlenmiş ve 15 gün sonunda 4,30 log kob/mL seviyesine gerilemiştir. Ürünlerde koliform ve küf tespit edilmemiştir.

**Kuru Madde ve Kül Analizi**

Toplam kuru madde miktarı %2,09, suda çözünür kuru madde miktarı ise %1,5 olarak belirlenmiştir. Kül miktarı ölçülebilir düzeyde bulunmamıştır.

**Organik Asit Analizi**

HPLC analizlerinde laktik asit miktarı 1,880 g/L, asetik asit miktarı ise 1,636 g/L olarak tespit edilmiştir. Bu sonuçlar fermantasyonun başarıyla gerçekleştiğini ve laktik asit bakterilerinin aktif metabolik faaliyet gösterdiğini ortaya koymaktadır.

**Duyusal Analiz sonuçları**

Duyusal analiz sonuçlarına göre ürünün en yüksek beğeni puanını görünüş parametresinden aldığı (≈9/10), koku puanının ise 7,5–8,0 aralığında olduğu belirlenmiştir. Tat parametresi ise diğer özelliklere kıyasla daha düşük bulunarak 6,0–6,5 seviyelerinde değerlendirilmiştir. Genel olarak ürünün görsel ve aromatik özelliklerinin ön planda olduğu, buna karşılık tat profilinin daha zayıf kaldığı görülmüştür.

## Sonuç

Bu çalışmada, pancar kabuğu ekstraktının su kefiri ile fermantasyonu gerçekleştirilmiş ve elde edilen ürünün mikrobiyolojik, fizikokimyasal ve duyusal özellikleri değerlendirilmiştir. Fermantasyon sonucunda pH değerinin 6,36'dan 4,23'e düşmesi, su kefiri mikroorganizmalarının aktif metabolik faaliyet gösterdiğini ve organik asit üretiminin gerçekleştiğini ortaya koymuştur. Mikrobiyolojik analizlerde, *Lactobacillus spp*. ve *Lactococcus spp*. popülasyonlarının yüksek seviyelerde korunduğu belirlenmiş, ayrıca mayaların depolama süresince canlılığını sürdürdüğü tespit edilmiştir. Koliform ve küf gelişiminin gözlenmemesi ise ürünün mikrobiyolojik kalitesi açısından olumlu bir sonuç olarak değerlendirilmiştir. Organik asit analizlerinde laktik asit (1,880 g/L) ve asetik asit (1,636 g/L) tespit edilmiş olup, bu sonuçlar fermantasyonun başarıyla gerçekleştiğini desteklemiştir. Duyusal değerlendirmelerde ürünün görünüş özelliği yüksek kabul görmüş, koku ve tat özellikleri ise kabul edilebilir düzeylerde bulunmuştur. Özellikle pancar kabuğundan kaynaklanan doğal renk oluşumu, ürünün tüketici açısından çekiciliğini artırmıştır. Ürünün nispeten düşük olan tat puanı, doğal lezzet bileşenlerinin ilaveleriyle iyileştirilebilecektir. Elde edilen sonuçlar, tarımsal bir yan ürün olan pancar kabuğunun su kefiri üretiminde

başarıyla kullanılabileceğini göstermektedir. Bu yaklaşım, hem gıda atıklarının değerlendirilmesine hem de fonksiyonel özelliklere sahip yeni fermente içeceklerin geliştirilmesine katkı sağlayabilecek sürdürülebilir bir yöntem sunmaktadır.

## Yazar Katkı Beyanı

Her yazarın bu çalışmaya hangi aşamada ve nasıl katkı sağladığı belirtilmelidir. Örneğin: Ramazan ERÇELİK: Veri toplama, araştırma, biçimsel analiz ve ilk taslak yazımı Prof. Dr. Zeynep Banu SEYDİM: Proje yönetimi, danışmanlık, kavramsallaştırma, metodoloji, gözden geçirme ve düzenleme

Eyüp ELVEREN: Veri toplama, araştırma

## Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması olmadığını beyan eder.

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# Floristic Composition, Community Structure and Biological Spectrum of Vegetation at Sarsawa Hills District Kotli Azad Jammu and Kashmir

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## Abstract

Sarsawa Hills in District Kotli, Azad Jammu and Kashmir, Pakistan, harbor significant subtropical vegetation, yet their floristic composition and community structure remain poorly documented, limiting conservation and ecological management efforts. This study aimed to characterize the plant diversity, phytosociology, biological spectrum, and leaf size spectra of vegetation across ten sites at Sarsawa Hills (altitude 700–1150 m) during 2023–2024. Field surveys were conducted using the quadrat method, and plant communities were identified through the Importance Value Index (IVI); life forms were classified following Raunkiaer's system while leaf size classes were determined according to standard leaf spectra categories. A total of 92 plant species belonging to 83 genera and 41 families were recorded, with Amaranthaceae, Asteraceae, and Lamiaceae as the leading families (9 species each), followed by Fabaceae (8 species). Ten distinct plant communities were identified, with *Cynodon dactylon* as the dominant species (IVI = 60.00), followed by *Olea ferruginea* (IVI = 14.70) as co-dominant and *Dalbergia sissoo* (IVI = 13.1) as an associated component. Analysis of the biological spectrum revealed Therophytes as the dominant life form (41.3%), followed by Nanophanerophytes (19.6%), and Hemicryptophytes and Megaphanerophytes (16.3% each); no Chamaephytes were recorded. Microphylls dominated the leaf size spectrum (35.9%), followed by mesophylls (23.9%) and nanophylls (14.1%). These results indicate that Sarsawa Hills support rich subtropical floral diversity shaped by a warm climate, moderate altitude, and ongoing anthropogenic pressures including overgrazing and deforestation. The dominance of Therophytes and microphylls reflects the seasonal, dry-adapted character of the vegetation, and the findings provide a baseline for biodiversity conservation and sustainable land management in the region.

***Keywords:*** *Floristic composition, community structure, biological spectrum, Importance Value Index, Raunkiaer life forms, Sarsawa Hills.*

*ID: 844*

# The Potency of Baker's Yeast (*Saccharomyces cerevisiae)* As Biosorption Agent of Cadmium and Chromiun in *Kindirmo* in Kano State, Nigeria.

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## Abstract

Heavy metals in food, presents a threat to food safety and human health due to their high toxicity which can cause severe illness and death. *Kindirmo* is a traditional fermented milk product similar to yoghurt, which is widely consumed in Nigeria. The application of biosorbents like bacteria, yeast, and algae is a method of heavy metal decontamination in foods and water. In this study, *Kindirmo* was produced with baker's yeast (*S. cerevisiae*). Furthermore, the *kindirimo* was intentionally spiked with Cadmium and Chromium separately and in combination in different concentrations of 50, 60, 70, 80, 90 and 100 µg/ml. The efficacy of the yeast in decontaminating the fermented milk product was assayed using Atomic Absorption Spectrometry to detect the residual metals after 4, 8, 12 and16 hours. The highest removal was noted at the first 4 hours in 50 µg/ml concentration with 76 % efficiency, showing a rapid onset removal for Cadmium. At 90 µg/ml in 16 hours 70 % removal was achieved which was the highest removal rate in Chromium. The removal rate in combination was at 35 µg/ml where Chromium was totally removed, while over 80 % of Cadmium was removed at 45 µg/ml. For both metals, there was higher removal affinity at 8-16 hours, showing a prolonged biosorption process. This study has shown that *S. cerevisiae* has great potential for chromium and cadmium removal from *Kindirmo*, and is a green environmental friendly tool for heavy metal decontamination.

***Keywords:*** *Kindirmo, Saccharomyces cerevisiae, chromium, cadmium, biosorption, decontamination*

*ID: 845*

# Comparative Evaluation of Phenolic Compound Profiles and Antioxidant Properties of Two Truffle Species (*Tuber uncinatum* and *Tuber brumale*)

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## Abstract

Truffles are valuable macrofungi known for their distinctive aroma and potential bioactive properties. In recent years, attention has increasingly focused on the phenolic composition and antioxidant potential of truffle species for applications in functional foods and nutraceuticals. This study compared the phenolic profiles and antioxidant capacities of methanolic extracts from *Tuber uncinatum* and *Tuber brumale*. The total phenolic content (TPC), antioxidant activity (FRAP and DPPH assays), and individual phenolic compounds were assessed. Results showed substantial differences between the two species in phenolic composition and antioxidant characteristics. Among the identified phenolic compounds, tyrosol was the dominant component in both species. *T. brumale* had significantly higher tyrosol content (383.62 ± 6.41 mg/kg DW) than *T. uncinatum* (115.88 ± 3.48 mg/kg DW). In contrast, (+)-catechin and chlorogenic acid were detected only in *T. uncinatum*, at 18.55 ± 0.29 and 2.07 ± 0.05 mg/kg DW, respectively. Oleuropein and 3-hydroxytyrosol were markedly higher in *T. uncinatum*, whereas syringic acid was comparatively higher in *T. brumale*. *T. brumale* exhibited higher FRAP (30.31 ± 1.19 mg TE/g DW), DPPH (38.30 ± 0.74 mg TE/g DW), and TPC (27.89 ± 0.87 mg GAE/g DW) than *T. uncinatum* (FRAP: 24.70 ± 1.14 mg TE/g DW, DPPH: 32.16 ± 0.99 mg TE/g DW, and TPC: 16.71 ± 0.66 mg GAE/g DW). The elevated antioxidant capacity of *T. brumale* may be associated with its substantially higher tyrosol concentration and overall phenolic content. Overall, the findings demonstrate that both truffle species are important sources of phenolic compounds with notable antioxidant potential. In particular, *T. brumale* showed promising antioxidant characteristics, whereas *T. uncinatum* exhibited a more diverse phenolic profile.

***Keywords:*** *Truffle, Tuber uncinatum, Tuber brumale, Phenolic compounds, Antioxidant activity*

*ID: 846*

# Water Source Types as Drivers of Asset Accumulation Trajectories Among Pastoralist Households in Semi-Arid areas of Monduli District Tanzania

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## Abstract

This study examined the association between livestock drinking water source types and household asset ownership among pastoralist households in semi-arid areas of Monduli District, Tanzania. Specifically, the study assessed how reliance on improved and unimproved livestock water sources influences household asset accumulation among pastoral communities. A cross-sectional research design was employed, involving 367 pastoralist households selected from Makuyuni and Moita wards. Quantitative data were collected using household surveys and field observations. A household asset index was constructed using tetrachoric Principal Component Analysis (PCA), and households were categorized into low, medium, and high asset ownership groups. A generalized ordered logit model was applied to examine the association between livestock water source types and household asset ownership. The findings revealed that households relying on improved livestock water sources were less likely to belong to higher asset ownership categories compared to households using unimproved water sources. The results suggest that wealthier pastoralists benefit from flexible access to dispersed natural water sources that support livestock mobility and extensive grazing strategies. Household income positively influenced asset ownership, while off-farm income was negatively associated with higher asset groups, indicating its role as a coping strategy among poorer households. The study concludes that livestock water access and pastoral mobility jointly shape household asset accumulation. Therefore, pastoral development interventions should integrate water planning with mobility support and rangeland management strategies**.**

***Keywords:*** *Pastoralism; Livestock water sources; Asset accumulation; Semi-arid rangelands; Household livelihoods*

*ID: 847*

# RSM-Optimized Spray-Dried Microencapsulated *Lactiplantibacillus plantarum* LP-RB5: Enhanced *In-Vitro* Survivability and Storage Stability

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## Abstract

This study aims to optimize microencapsulation of *Lactiplantibacillus plantarum* (LP-RB5) and with maltodextrin using spray drying. The microencapsulation efficiency of LP-RB5 was significantly affected by probiotic concentration, wall material content, and inlet air temperature. The response surface methodological-based optimal microencapsulation parameters were 31% probiotic concentration, 18% maltodextrin, and spray drying conditions with an inlet air drying temperature of 147 °C, feed flow rate (10 mL/min), and an outlet air temperature of 90 °C. The impact of microencapsulation on encapsulation efficiency, physicochemical properties of microcapsules, and cell survivability in simulated gastrointestinal digestion during six-month storage were evaluated. The findings revealed that LP-RB5 cells encapsulated with maltodextrin in a 1.5:1 (w/w) core-to-wall ratio offered the highest yield (28.53%) and encapsulation efficiency (88.38%). The optimized LP-RB5 powder demonstrated final viability (9 $Log_{10}$ CFU/g), microbial reduction (1.3 $Log_{10}$ CFU/g), 4.56% moisture content, 0.29 $a_w$ water activity, and 94.72% water solubility index. Scanning electron microscope observations of LP-RB5 and LP-8014 powders possessed smooth and dent spherical microcapsule morphologies with uniform particle sizes ($D_{[3,2]}$)=28.77 and 29.40 µm, respectively. *L. plantarum* cells in LP-RB5 had a survival rate of 80.11% during digestion and 93% after storage at freezer and chiller temperatures, indicating better survivability and stability. Understanding the survivability of microencapsulated LP-RB5 under these circumstances is crucial for assessing its potential as a functional ingredient in food and nutraceutical products.

***Keywords:*** *Microencapsulation, spray-dried powder, morphological characterization, SEM, microbial stability, cell viability.*

*ID: 848*

# The use of allelic variants of seed storage proteins in the production of sunflower parental lines

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## Abstract

The effectiveness of heterosis-based sunflower breeding is significantly enhanced when evaluating and developing lines and hybrids by selecting plants based on allelic variants of the electrophoretic spectra of seed storage proteins. The selection of plants based on allelic variants in the experiments was carried out using the method of electrophoresis of sunflower seed reserve proteins, as described by A. F. Popereya (Institute of Plant Breeding and Genetics, Ukraine). The studies conducted enabled the determination and identification of allelic variants in the electrophoretic patterns of the sunflower genotypes under investigation. During the study, the following loci were identified on electrophoregrams: HEL 1, HEL 2, HEL 3, HEL 4, HEL 5 and HEL 6. The creation of interspecific sunflower hybrids made it possible to expand genetic diversity and isolate genotypes with the new HEL 7, HEL 8, HEL 9 and nk loci. Based on the selection of plants according to protein spectra, new source material and new parental lines: inbred maternal lines and pollen fertility restorer lines with a high level of typicality (99.0–100.0%) were created. The use of new inbred lines in breeding combinations. The identification of sunflower genotypes based on allelic variants of the electrophoretic spectra of sunflower seed storage proteins addresses issues relating to improving the efficiency of plant evaluation and selection, expanding genetic diversity, improving the typicality of parental lines and hybrids, and obtaining parental lines that yield hybrids with high yield potential.

***Keywords:*** *Sunflower-1, Protein electrophoresis spectrum-2, Selection-3, Line-4, Typicality-5, Yield-6*

The effectiveness of breeding work in the development of sunflower hybrids is based on the evaluation and selection of plants according to both morphological traits and allelic variants of the electrophoretic spectra of seed storage proteins. The use of protein spectra in hybrid breeding allows for the expansion of genetic diversity and the creation of new, identified parental lines.

The experiments identified allelic variants in the electrophoretic patterns of sunflower seed storage proteins, which enabled the identification, isolation and creation of new breeding material and new self-pollinated parental lines. The studies conducted demonstrated and confirmed that the genes encoding the synthesis of storage proteins are clustered as a single component or as a single group (block). Alleles of genes controlling polypeptide synthesis are represented on electrophoregrams as bands (components) within a block. A block of electrophoretic components serves as a phenotypic marker for the corresponding locus at the protein molecule level. The alleles on the electrophoregrams exhibited different mobility and intensity, which made it possible to identify different series of allelic variants for each cluster, to identify homozygotes for each allele (a characteristic of self-pollinated lines), and heterozygotes (a characteristic of hybridity). Alleles at each locus were arranged in order of increasing mobility and decreasing molecular weight from HEL1 to HEL 6.

Analysis of the electrophoregrams during the course of the study showed that the components of sunflower seed storage proteins (helianthinins) are controlled by at least six loci: HEL1, HEL2, HEL3, HEL4, HEL5 and HEL6. The HEL1 and HEL6 genes are represented by one (homozygous) or two alleles (heterozygous). The HEL3 and HEL5 genes are always represented by a single allele. Differences in these genes can be observed in the intensity of the components of the electrophoretic spectra. The HEL2 gene is represented by two components. The HEL4 gene is represented on electrophoregrams by a series of components of varying mobility and intensity and controls the synthesis of the polypeptide block of the fourth electrophoretic spectrum. The group of sunflower seed storage proteins encoded by the HEL4 locus exhibits the greatest polymorphism of helianthinins among all identified and described sunflower samples when subjected to electrophoretic separation.

An analysis of changes in the allelic variants of protein electrophoretic spectra during self-pollination and the hybridisation of the resulting parental lines established that the electrophoretic component cluster serves as a phenotypic marker for the corresponding polygenic locus at the protein molecule level and is equivalent to the concept of an ‘allele’ for a clustered locus.

The production of interspecific hybrids involving annual wild sunflower forms has enabled the isolation and creation of new breeding material with established and identified allelic variants of the Hel 7, Hel 8, HEL 9 and nk loci, which has significantly contributed to the expansion of genetic diversity in the breeding process.

The evaluation and selection of plants based on allelic variants of the electrophoretic spectra of helianthinins, combined with an assessment of morphological traits, enabled the creation of the maternal sterile lines GE59A,

GE6A, GE195A, and nine sunflower pollen fertility restorer lines with a high level of genetic purity (99.0–100.0%), which are used in breeding to produce hybrid combinations. Using these sunflower lines, promising hybrids have been developed, which are currently undergoing competitive variety trials. The yield of the resulting sunflower hybrids ranges from 3.5 to 4.3 t/ha.

From a population of plants with identical and indistinguishable morphological traits of the hybrid combination of the interspecific hybrid Maxim × H. praecox, two pollen restorer lines, GE 106Rf and GE 3-38Rf, were isolated and established based on differences in the allelic variants of electrophoretic spectra. The sunflower line 106Rf, derived from line 3-38Rf, is characterised by a shorter growing season. In terms of growing season during, line 106Rf belongs to the third early maturity group. The growing season of plants from this line lasted 99 days, which is 15 days shorter than that of line 3-38Rf. The line 3-38Rf belongs to the 5th mid-season group, has a growing season of 114 days, and is characterised by a more higher level of yield potential in the hybrids combinations.

The lines obtained, when used in crossing combinations, yielded hybrids with varying levels of yield. Hybrid combinations produced using the pollen fertility restorer line GE 3-38Rf yield between 4.0 and 4.5 t/ha. Hybrid combinations produced using the pollen fertility restorer line GE 106Rf yielded between 3.10 and 3.54 t/ha.

When creating parental self-pollinated sunflower lines, analysis of allelic variants of the electrophoretic spectra of seed storage proteins demonstrates the necessity and effectiveness of evaluating and selecting plants based on protein spectra to improve genetic purity and bring the newly created breeding material to a homozygous state with a high level of typicality.

*ID: 849*

# Effect of Different Choline Chloride-Based Deep Eutectic Solvents on Chitosan Extraction from Blue Crab Shells (*Callinectes sapidus*)

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## Abstract

Deep eutectic solvents (DESs) have recently attracted considerable attention as environmentally friendly alternatives to conventional extraction systems for chitin and chitosan production. In this study, the effects of different choline chloride-based DES systems on chitosan extraction from blue crab (*Callinectes sapidus*) shells were investigated. Fructose, ethylene glycol, and urea were used as hydrogen bond donors (HBDs) in combination with choline chloride (ChCl) at a molar ratio of 1:2. Chitin extraction was performed using DES systems, followed by alkaline deacetylation to obtain chitosan. The structural characteristics of the resulting chitosan samples were evaluated by Fourier transform infrared spectroscopy (FTIR), and the degree of deacetylation (DD) was calculated from characteristic absorption bands. In addition, chitosan yield, deproteinization (DP), and demineralization (DM) efficiencies were determined. FTIR spectra confirmed successful conversion of chitin to chitosan across all DES systems. Characteristic absorption bands corresponding to O–H/N–H stretching, amide groups, and polysaccharide backbone vibrations were clearly observed. ChCl:Fructose exhibited the highest DD value (90.85%), followed by ChCl:E.Glycol (90.53%) and ChCl:Urea (89.82%). ChCl:Fructose also showed the highest chitosan yield (48.60%) and the highest deproteinization efficiency (40.78%). Regarding demineralization performance, ChCl:Urea showed the highest efficiency (28.62%), followed by ChCl:E.Glycol (26.18%) and ChCl:Fructose (10.30%). The results demonstrated that the chemical nature of the hydrogen bond donor significantly affected the extraction behavior, deacetylation efficiency, and physicochemical properties of the resulting chitosan. Among the evaluated systems, the fructose-based DES showed the most balanced extraction performance due to its high DD, chitosan yield, and deproteinization efficiency.

***Keywords:*** *Chitosan, Deep eutectic solvent, Crab shell, FTIR, Deacetylation degree, Green extraction*

*ID: 850*

# Assessment of Frost Damage in Walnut Orchards in Kaman District of Kırşehir Province Following the Extreme Cold Wave in Türkiye in 2025

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## Abstract

This study was conducted in Kaman, the largest district of Kırşehir province (Türkiye) by surface area, located between 39°21′ N latitude and 33°43′ E longitude. Walnut cultivation, which plays a pivotal role in the regional economy, is highly vulnerable to late spring frosts. Frost events occurring during the spring period cause severe yield losses by damaging the male and female flowers, as well as the young shoots of walnut trees. Within the scope of this study, the impacts of the severe frost event that occurred in the walnut orchards of Kaman district in the spring of 2025 were investigated. Extensive field observations and orchard examinations were carried out to determine the extent of frost damage on the male flowers, female flowers, and terminal buds of walnut trees across different age groups. Additionally, the elevation, slope, and aspect characteristics of the sampling sites were evaluated using Geographical Information Systems (GIS). The results revealed that frost damage was predominantly intensified during the flowering period and varied significantly among orchards depending on topographical features. Notably, in the orchard coded G40, located at an elevation of approximately 980 m, renewed shoot development and limited fruit formation were observed following the frost event. The outcomes of this study are expected to contribute to frost risk assessment and optimal orchard site selection for sustainable walnut cultivation in the region.

***Keywords:*** *Kaman, Walnut, Frost damage, Phenological stage, Yield loss, Agricultural risk management.*

## Türkiye'de 2025 Yılında Yaşanan Ekstrem Soğuk Hava Dalgasının Ardından Kırşehir İli Kaman İlçesinde Ceviz Bahçelerinde Meydana Gelen Don Hasarının Değerlendirilmesi

## Özet

Bu çalışma, Kırşehir ilinin yüzölçümü bakımından en büyük ilçesi olan ve 39°21′ kuzey enlemi ile 33°43′ doğu boylamı arasında yer alan Kaman'da gerçekleştirilmiştir. Bölge ekonomisinde stratejik bir öneme sahip olan ceviz yetiştiriciliği, geç ilkbahar donlarından olumsuz yönde etkilenmektedir. İlkbahar döneminde meydana gelen don olayları; ceviz ağaçlarının erkek ve dişi çiçeklerinin yanı sıra genç sürgünlerine de zarar vererek ciddi verim kayıplarına yol açmaktadır. Bu araştırmada, 2025 yılı ilkbaharında Kaman ilçesindeki ceviz bahçelerinde yaşanan şiddetli don olayının etkileri incelenmiştir. Farklı yaş gruplarındaki ceviz ağaçlarının erkek çiçek, dişi çiçek ve uç tomurcuklarında meydana gelen don hasarını belirlemek amacıyla detaylı saha gözlemleri ve bahçe incelemeleri yürütülmüştür. Ayrıca, örnekleme yapılan alanların rakım, eğim ve bakı (yön) özellikleri Coğrafi Bilgi Sistemleri (CBS) kullanılarak analiz edilmiştir. Araştırma sonuçları, don hasarının özellikle çiçeklenme döneminde yoğunlaştığını ve topografik özelliklere bağlı olarak bahçeler arasında belirgin farklılıklar gösterdiğini ortaya koymuştur. Özellikle yaklaşık 980 m rakımda yer alan G40 kodlu bahçede, don olayı sonrasında yeniden sürgün gelişimi ve sınırlı düzeyde meyve oluşumu tespit edilmiştir. Bu çalışmadan elde edilen bulguların, bölgedeki ceviz yetiştiriciliğinde don riski yönetiminin geliştirilmesine ve doğru bahçe yeri seçimi kriterlerine katkı sağlaması öngörülmektedir.

***Anahtar Kelimeler:*** *Kaman, Ceviz, Don hasarı, Fenolojik aşama, Verim kaybı, Tarımsal risk yönetimi.*

*ID: 851*

# Growth, Blood and Histopathological Changes in Broiler Fed Aflatoxin

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## Abstract

Aflatoxins are lethal secondary products that have negative influences on growth, health, and organ functioning of broiler chickens, especially in diets based on maize. This study was focused on the protective effect of activated charcoal against the toxicity of aflatoxins on broilers. A total of one hundred one-day-old Arbor Acres chicks were randomly allocated to five diet treatments based on T1: basal control diet, and T2-T5: basal diet with supplementation of aflatoxin, 200 ppb, activated charcoal, 0, 0.5, 1.0, 1.5, and 2.0%. At day 42, blood samples would be taken to assess serum biochemical and hematological parameters, and 6 birds would be euthanised to evaluate the liver and kidney histopathology. Biochemistry of serum did not have any significant differences ($P > 0.05$) between ALT (2.93-6.48 IU /L), total protein (2.97-3.65 g/dL), albumin (0.87-1.27 g/dL), cholesterol (108.86-138.79 mg/dL), glucose (132.61-148.11 mg/dL), or calcium (8.94-9.47 mg/dL). AST levels showed tremendous disparities in comparison with treatments, and HDL, LDL, uric acid, alkaline phosphatase, creatinine, and phosphorus showed significant (P 0.05) alterations, which is suggestive of a dose-dependent physiological reaction to activated charcoal. This did not significantly affect haematological indices such as packed cell volume (28.83-32.83 %), hemoglobin (9.27-10.62%), and red blood cell count ($2.9–3.34 \times 10^6$/µL). The count of white blood cells ranged between 15.36 - 17.83 x $10^9$/µL, platelet between 13.52 - 16.80 x $10^3$, and lymphocyte percentages were highest in T5 (65.17%) and lowest in T4(59.33%). Dose-dependent hepatic and renal lesions were observed by histopathology with moderate degeneration in 1.0 percent charcoal (T3) and almost all tissue being preserved in 2.0 percent (T5), as in the control group. The results show that the protective effect on the negative effects of aflatoxin-contaminated diets is induced by the addition of activated charcoal to the food and reduces the negative effects on broiler chicken growth, physiological indices, and protein integrity. Maximum charcoal supplementation improves the resistance to aflatoxicosis and promotes the health of poultry in polluted feeding environments.

***Keywords:*** *Aflatoxin, Broiler chicken, Activated charcoal, Growth performance, histopathology, Serum biochemistry.*

## Introduction

The aflatoxins are one of the most dangerous mycotoxins that put food and feed systems at risk worldwide, and aflatoxin B1 (AFB1) was recognised as the most powerful and most common one (Gobikanila *et al*., 2025). The observed types of secondary metabolites are mainly the products of the fungal taxa of Aspergillus flavus and A. parasiticus in the case of exposure to the warm and humid environmental conditions that favour fungal growth (Klich, 2007; Guo *et al*., 2023). It is particularly prone to tropical and subtropical areas, since the climate patterns of these areas favor the development of fungi in crop growing, harvesting, storage, and processing stages. Therefore, aflatoxin pollution continues to limit maize-based poultry production systems, especially in developing economies.

The nutritional dietary intake with aflatoxins has been associated with impaired performance, reduced feed efficiency, immunosuppression, hepatotoxicity, and increased incidences of mortality in broiler chickens (Moloi *et al*., 2024). Chronic aflatoxicosis, which is not only prevalent in field conditions but also more common than acute toxicity, normally develops insidiously and compromises physiological resistance over time (Peles *et al*., 2019; Nazhand *et al*., 2020). Feed contamination impairs protein synthesis, causes oxidative stress, and upsets metabolic homeostasis, which eventually affects the declining productivity and profitability of poultry enterprises (Jobe *et al*.,2023; Moloi *et al*., 2024). These negative effects highlight the importance of using effective methods of mitigating diets.

Mycotoxins, such as aflatoxins, ochratoxins, fumonisins, deoxynivalenol, or zearalenone, are structurally heterogeneous, low-molecular-weight, fungal metabolites with severe toxicological effects (Solis-Cruz *et al*., 2018). These are ubiquitous, which makes it difficult to prevent them entirely and requires other mitigation measures (Salvo *et al*., 2018). Out of these toxins, aflatoxins are especially worrisome as they are hepatotoxic and carcinogenic in nature, having been proven to have a well-associated correlation with hepatocellular carcinoma in humans (Pyshyev *et al*., 2021).

Several strategies have been put forward to reduce the toxicity imposed by aflatoxins in poultry feeds, such as better agronomic systems, superior storage systems, and the addition of toxin binders. Adsorbents like bentonite, hydrated sodium -calcium aluminosilicates, and products of yeast work by adsorbing aflatoxin to absorb aflatoxin in the gastrointestinal tracts, thus lowering the systemic absorption (Kolawole *et al*., 2022; Mgbeahuruike et al., 2023). Among them, one has been drawn to activated charcoal because of its high adsorption capacity, porosity, and the ability to bind to the spectrum (Ahmed *et al*., 2022).

Activated charcoal works by binding aflatoxins in the digestive system, reducing bioavailability and toxic effects (Genli *et al*., 2022)

This study aims to advance resilience in the poultry production system by helping to develop resilient systems to dose-dependent protective potential of activated charcoal in addition to its application in sustainable feed safety actions.

## Materials and Methods

### *Study Location*

The current experiment was conducted in the Teaching and Research Farm of the University of Ibadan, Ibadan, Oyo State, Nigeria. The location is a tropical climate with a distinct rainy and dry season. The experiment was run in the period between 22 $^0$C and 30 $^0$C ambient temperature, the relative humidity was 40 per cent to 55 per cent or less, and it was carried out during the dry season (December 2024 to January 2025). These were deemed to be favorable to the production of broilers and continued to be monitored within the period of the experiment.

### *Management and Experimental Birds*

One hundred 1-day chicks were purchased in a well-known commercial hatchery located in Ibadan. The birds were randomly allocated to five dietary treatment groups on arrival, with each consisting of 20 chicks and five birds per replicate in a randomised complete block design. The experiment lasted 42 days. The poultry pen was well-ventilated; the size of the pens was 2 m x 2m. Wood shavings was used as bedding material. Feed and water were given *ad libitum*. The normal (routine) management procedures, such as check-ups, inoculation, deworming, and strict biosecurity procedures, were established to provide maximum welfare and to limit the chances of exogenous contamination.

### *Production of Aflatoxin and Maize Preparation with Inoculums*

*Aspergillus flavus* (isolate 3228), a toxigenic strain of this fungus, was obtained in the Mycotoxin Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. The fungus culture was cultivated on V8 agar medium, which had 50 mL of V8 vegetable juice diluted by 950 ml of sterile distilled water and corresponding pH adjusted to 6.0. Plates were inoculated following autoclaving at 121 $^0$C and incubated at 37 $^0$C over a period of seven days. The spores were picked in 0.1% Tween 20 solution and diluted to 2 x 10 6spores/mL with a hemocytometer. Maize grains were washed with clean water for 10 minutes and autoclaved at 121 °C for 45 minutes, then cooled, and 50 ml of spore suspension was added to each 500 g. The inoculated maize was then incubated at 28-30 $^0$C at intervals of shaking to allow growth of the fungi to be uniform everywhere.

### *Nomination for Experimental Diets and Structure of Treatments*

The experimental diets were prepared by blending maize grains inoculated with Aspergillus flavus with aflatoxin-free maize (Aflasafe) to create a basal feed containing 200 parts per billion (ppb) of aflatoxin per kilogram of diet. Diets were created and used experimentally to meet the nutrient demands of broilers at the starter, grower, and finisher stages. The starter diet had 3104.76kg/kg -1 metabolizable energy (ME) and 23.00 percent crude protein (CP); the grower diet had 3100kcal/kg -1 ME and 20.01 percent CP; and the finisher diet had 3150kcal/kg -1 ME and 19.20 percent CP. Five dietary treatments were established as follows:

- Diet 1 (Control): Basal diet without activated charcoal
- Diet 2: Basal diet + 200 ppb aflatoxin + 0.5% activated charcoal
- Diet 3: Basal diet + 200 ppb aflatoxin + 1.0% activated charcoal
- Diet 4: Basal diet + 200 ppb aflatoxin + 1.5% activated charcoal
- Diet 5: Basal diet + 200 ppb aflatoxin + 2.0% activated charcoal.

Weight and feeds were made weekly. The weights of the body were measured on a digital scale with a precision of 1~g. The amount of the feed fed in was calculated by subtracting the amount of unused feed. The calculation of performance indices was done as follows:

i. Weight Gain (WG) = Final body weight -Initial body weight.
ii. Average Daily Gain (ADG) =weightgain/days.
iii. Average Daily Feed Intake (ADFI)= total weight of feed fed: number of days.
iv. Feed Conversion ratio (FCR): = total feed eaten/total increment of weight.

### *Blood Laboratory Analyses and Collection*

At the end of week 6, Birds were chosen randomly from the 5 treatments to sample their blood. The selected birds were humanely euthanized using cervical dislocation. 5mL of blood was taken through the jugular vein with sterile syringes. Half of each of the samples was moved into EDTA tubes to get hematologic analysis,while the other half was moved into plain tubes to get serum separation. Hematological parameters examined were packed cell volume (PCV), level of hemoglobin (Hb), the number of red blood cells (RBCs), white blood cells (WBCs), and the number of differential leukocytes. Biochemical parameters that were analysed in serum were the following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, glucose, and creatinine.

***Tissue Collection and Histopathological Examination***

At the end of the experimental period, two birds were randomly selected from each replicate for tissue sampling. The liver and kidney were carefully excised and rinsed in physiological saline to remove residual blood. Tissue samples were then fixed in 10% neutral buffered formalin for preservation. Standard histological procedures involving dehydration, embedding, sectioning, and staining were carried out. The prepared sections were examined under a light microscope to evaluate possible pathological lesions associated with dietary aflatoxin exposure and the ameliorative effects of activated charcoal.

**Statistical Analysis**

All data were examined according to SPSS version 25.0. One-way analysis of variance (ANOVA) was conducted, to find out the impact of diet treatments. Separating differences between means was done with Duncan Multiple Range Test with significance level of $p< 0.05$. Correlations analyses were performed to determine associations between aflatoxin exposure, physiological indices and growth performance parameters.

**Results**

***Performance of broiler chickens fed aflatoxin feed with varied inclusion levels of activated charcoal***

The performance indices of broilers fed aflatoxin-contaminated diets supplemented with graded levels of activated charcoal are presented in Table 4.1. Birds on the control diet (T1) recorded significantly higher average daily feed intake (106.84 ± 3.39 g) compared with those on aflatoxin-containing treatments T2 (81.21 ± 5.58 g), T3 (65.48 ± 2.61 g), T4 (64.69 ± 4.55 g), and T5 (63.92 ± 4.55 g). Similarly, average daily weight gain was highest in T1 (48.17 ± 7.57 g) and markedly reduced across T2 (29.32 g), T3 (24.89 g), T4 (19.48 g), and T5 (20.53 g). Feed conversion ratio (FCR) was lowest and most efficient in T1 (2.25 ± 0.29), moderate in T2 and T3 (2.78 ± 0.10 and 2.78 ± 0.62, respectively), and poorest in T4 (3.44 ± 0.76), followed by T5 (3.14 ± 0.37). These findings indicate that aflatoxin exposure adversely affected feed intake, weight gain, and feed utilisation, with the greatest inefficiency observed in T4. However, statistical analysis revealed no significant differences among treatments ($P > 0.05$).

Table 1: Performance of broiler chickens fed aflatoxin feed with varied inclusion levels of activated charcoal

| *Parameter* | *T1* | *T2* | *T3* | *T4* | *T5* | *SEM* |
|---|---|---|---|---|---|---|
| ADFI (g/bird) | $106.84^{a}$ ± 3.39 | $81.21^{b}$ ±5.58 | $65.48^{c}$ ±6.25 | $64.69^{c}$ ± 2.61 | $63.92^{c}$ ±4.55 | 2.34 |
| ADWG (g/bird) | $48.17^{a}$ ±7.57 | $29.32^{b}$ ±3.08 | $24.89^{b}$ ±7.94 | $19.48^{b}$ ± 4.22 | $20.53^{b}$ ± 2.61 | 2.78 |
| FCR | $2.25^{a}$ ± 0.29 | $2.78^{ab}$ ± 0.10 | $2.78^{ab}$ ± 0.62 | $3.44^{b}$ ±0.76 | $3.14^{ab}$ ±0.37 | 0.24 |

$^{abc}$ Treatment means with different superscripts on the same row significantly differ ($P<0.05$), T1- T5 (Treatment 1-5), ADFI- Average daily feed intake, ADWG- Average daily body weight gain, FCR- Feed conversion ratio, g/bird- gram per day.

***Haematology of broiler chicken fed aflatoxin feed with varied inclusion levels of activated charcoal.***

The effects of graded levels of activated charcoal on the haematological parameters of broilers fed aflatoxin-contaminated diets are presented in Table 4.2. Packed Cell Volume (28.83–32.83%), Hemoglobin (9.27–10.62 g/dL), and Red Blood Cell counts (2.91–3.34 $\times 10^{6}$/µL) did not differ significantly among treatments ($P > 0.05$). White Blood Cell counts ranged from 15.36 to 17.83/µL, while platelet values varied between 13.52 and 16.80/µL. Lymphocyte percentages were highest in T5 (65.17%) and lowest in T4 (59.33%), whereas heterophils ranged from 28.00% to 31.17%. Monocyte values were lowest in T5 (2.67%) and highest in T1 (3.17%), while eosinophils ranged from 3.67% to 4.17%. Overall, activated charcoal inclusion did not significantly influence haematological indices. Basophils (BA) were low across the various treatments from 0.00 ± 0.00% (T3 and T5) to 0.50 ± 0.55% (T1). Mean corpuscular volume (MCV) ranged from 97.19 fL (T3) to 100.40 fL (T2), while mean corpuscular hemoglobin (MCH) ranged from 31.34 pg (T3) to 32.49 pg (T2). The heterophil to lymphocyte (H/L) ratio ranged from 0.43 (T5) to 0.56 (T4). The statistical evaluation of the measured haematological indices used a significance threshold of $P<0.05$.

***Serum biochemical indices of broiler chickens fed aflatoxin diets with varied inclusion levels of activated charcoal***

The effects of graded activated charcoal inclusion on serum biochemical parameters are presented in Table 4.3. Alanine aminotransferase (ALT), total protein (2.97–3.65 g/dL), albumin (0.87–1.27 g/dL), cholesterol (108.86–138.79 mg/dL), glucose (132.61–148.11 mg/dL), and calcium (8.94–9.47 mg/dL) were not significantly affected ($P > 0.05$) by dietary treatments. Although aspartate aminotransferase (AST) showed overall significance ($p < 0.05$), post-hoc analysis revealed no significant pairwise differences among treatments. Conversely, significant differences ($P < 0.05$) were observed in high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid, alkaline phosphatase (ALP), creatinine, and phosphorus. HDL was highest in T5 (77.52 mg/dL), while LDL peaked in T1 (61.77 mg/dL) and was lowest in T3 (38.92 mg/dL). Uric acid and creatinine increased progressively with higher charcoal inclusion, with peak values in T4. ALP was highest in T1 (58.94 IU/L) and lowest in T3 (40.82 IU/L), indicating differential biochemical responses to activated charcoal supplementation under aflatoxin challenge.

***Histological analysis of the kidney of broiler chicken fed aflatoxin feed with varying inclusion levels of activated charcoal***

Histological examination of kidney sections (Plate 4.1) revealed treatment-dependent alterations in broilers fed aflatoxin-contaminated diets supplemented with activated charcoal. Birds in the control group (T1) showed normal renal architecture with intact tubular and glomerular structures. Mild diffuse tubular degeneration was observed in T2 (0.5% charcoal), while T3 (1.0%) exhibited moderate periglomerular cellular infiltration, indicating localised inflammation. Birds in T4 (1.5%) showed mild to moderate cortical congestion. Notably, T5 (2.0%) presented no visible lesions, with renal morphology comparable to the control group, suggesting improved protective effects at higher charcoal inclusion.

***Histopathological report of liver in broiler chickens fed aflatoxin diets with varying inclusion levels of activated charcoal***

Histological sections of livers from broiler chickens that received some level of aflatoxin in their diets with different levels of activated charcoal are shown in Plate 4.5. The birds that were in the control diet, which were observed to be free of aflatoxin and charcoal in the (T1), had no visible lesions. Their hepatocytes were arranged in normal cords, and the sinusoids were intact. In contrast, sections from broilers on an aflatoxin diet with 0.5% activated charcoal (T2) displayed mild diffuse hydropic degeneration and necrosis of hepatocytes. This was characterised by cellular swelling and cytoplasmic breakdown. Chickens on a diet with 1.0% charcoal (T3) showed severe diffuse hepatic degeneration and necrosis, resulting in significant loss of hepatic structure.

Table 2: Haematology of broiler chicken fed aflatoxin feed with varied inclusion levels of activated charcoal.

| *Parameter* | *T1* | *T2* | *T3* | *T4* | *T5* | *SEM* |
|---|---|---|---|---|---|---|
| PCV (%) | $32.00 \pm 4.86^{ab}$ | $32.83 \pm 4.92^{a}$ | $32.17 \pm 3.43^{ab}$ | $28.83 \pm 3.31^{b}$ | $32.67 \pm 3.72^{ab}$ | 2.37 |
| H.B (g/dl) | 10.43 ± 1.47 | 10.62 ± 1.66 | 10.37 ± 1.13 | 9.27 ± 1.11 | 10.60 ± 1.14 | 0.76 |
| RBC ($x10^6$/uL) | 3.27 ± 0.33 | 3.27 ± 0.35 | 3.31 ± 0.26 | 2.91 ± 0.50 | 3.34 ± 0.25 | 0.2 |
| WBC ($x10^9$/L) | 17.83 ± 2.70 | 16.60 ± 1.72 | 16.98 ± 1.52 | 15.36 ± 1.38 | 17.38 ± 2.57 | 1.18 |
| PLATELET($x10^3$/L) | 16.10 ± 3.97 | 14.18 ± 2.24 | 13.52 ± 7.96 | 14.20 ± 2.47 | 16.80 ± 2.73 | 1.53 |
| LYM (%) | 62.50 ± 5.24 | 64.33 ± 5.20 | 62.33 ± 5.35 | 59.33 ± 7.20 | 65.17 ± 3.97 | 3.17 |
| HET (%) | 29.83 ± 6.59 | 28.67 ± 5.01 | 31.17 ± 5.85 | 33.33 ± 6.25 | 28.00 ± 3.41 | 3.2 |
| MON (%) | 3.17 ± 0.98 | 3.00 ± 0.89 | 2.83 ± 0.98 | 3.00 ± 0.63 | 2.67 ± 0.52 | 0.48 |
| EO (%) | 4.00 ± 2.00 | 3.67 ± 1.86 | 3.67 ± 1.63 | 4.00 ± 2.28 | 4.17 ± 0.98 | 1.04 |
| BA (%) | 0.50 ± 0.55 | 0.33 ± 0.52 | 0.00 ± 0.00 | 0.33 ± 0.52 | 0.00 ± 0.00 | 0.24 |
| MCV(fL) | 97.8 | 100.40 | 97.19 | 99.08 | 97.80 | - |
| MCH(pg) | 31.89 | 32.49 | 31.34 | 31.85 | 31.74 | - |
| H/L(Ratio) | 0.48 | 0.45 | 0.50 | 0.56 | 0.48 | - |

[abc] Treatment means with different superscripts on the same row significantly differs (P<0.05), T1- T5 (Treatment 1-5), SEM- standard error of means,PCV- packed cell volume, Hb- Hemoglobin, RBC- Red blood cell, WBC- white blood cell, LYM- lymphocytes, HET - heterophiles, MON- Monocytes, EO - Eosinophils, BA- Basophil

Table 3: Serum biochemical indices of broiler chickens fed aflatoxin diets with varied inclusion levels of activated charcoal

| *Parameter* | *T1* | *T2* | *T3* | *T4* | *T5* | *SEM* |
|---|---|---|---|---|---|---|
| AST (IU/L) | 86.33±27.95 | 82.18±11.20 | 89.54±20.84 | 101.92±22.68 | 107.30±20.97 | 12.37 |
| ALT (IU/L) | 4.43±2.21 | 2.93±0.81 | 5.15±2.01 | 4.43±2.47 | 6.48±3.46 | 1.36 |
| TP (g/dL) | 2.97±0.50 | 3.00±0.54 | 3.08±1.06 | 3.65±0.55 | 3.43±0.38 | 0.38 |
| ALB (g/dL) | 0.97±0.13 | 1.14±0.19 | 1.24±0.08 | 1.15±0.16 | 1.27±0.32 | 0.11 |
| CHOL (mg/dL) | 138.79±40.67 | 108.86±26.71 | 111.78±12.44 | 135.83±11.52 | 137.11±15.80 | 13.92 |
| GLUC (mg/dL) | 146.89±29.26 | 132.61±35.40 | 148.11±31.26 | 137.41±9.34 | 141.57±18.94 | 15.35 |
| HDL (mg/dL) | $68.08^{ab}$±7.83 | $68.51^{ab}$±7.93 | $62.21^{b}$±9.14 | $72.07^{ab}$±5.58 | $77.52^{a}$±9.11 | 4.63 |
| LDL (mg/dL) | $61.77^{a}$±7.37 | $46.76^{b}$±4.09 | $38.92^{b}$±6.18 | $45.67^{b}$±7.93 | $48.89^{b}$±6.08 | 3.73 |
| U.A. (mg/dL) | $3.80^{b}$±0.69 | $3.55^{b}$±0.62 | $4.29^{ab}$±0.41 | $4.79^{a}$±0.43 | $4.32^{ab}$±0.45 | 0.31 |
| ALP (IU/L) | $58.94^{a}$±2.47 | $45.01^{bc}$±4.98 | $40.82^{c}$±4.19 | $47.70^{bc}$±6.14 | $50.40^{b}$±4.32 | 2.64 |
| CRT | $0.14^{c}$±0.03 | $0.43^{b}$±0.11 | $0.63^{ab}$±0.13 | $0.79^{a}$±0.04 | $0.62^{ab}$±0.24 | 0.08 |
| PHOS (mg/dL) | $13.98^{ab}$±1.34 | $11.29^{b}$±1.98 | $11.85^{b}$±2.06 | $34.05^{a}$±14.55 | $27.72^{a}$±13.23 | 5.14 |
| CAL (mg/dL) | $26.52^{a}$±3.59 | $27.27^{a}$±2.82 | $26.50^{a}$±3.24 | $28.81^{a}$±3.46 | $28.31^{a}$±3.44 | 1.92 |

abc Treatment means with different superscripts on the same row significantly differs (P<0.05), T1- T5 (Treatment 1-5), SEM- standard error of means, AST- Aspartate amino transferase, ALT - Alanine amino transferase, TP - Total protein, CHOL -Choline, HDL - High density lipoprotein, LDL - Low density lipoprotein, U/A- Uric acid, ALP- Alkaline phosphatese, CRT -Creatinine, PHOS - Phosphorus, CAL -Calcium

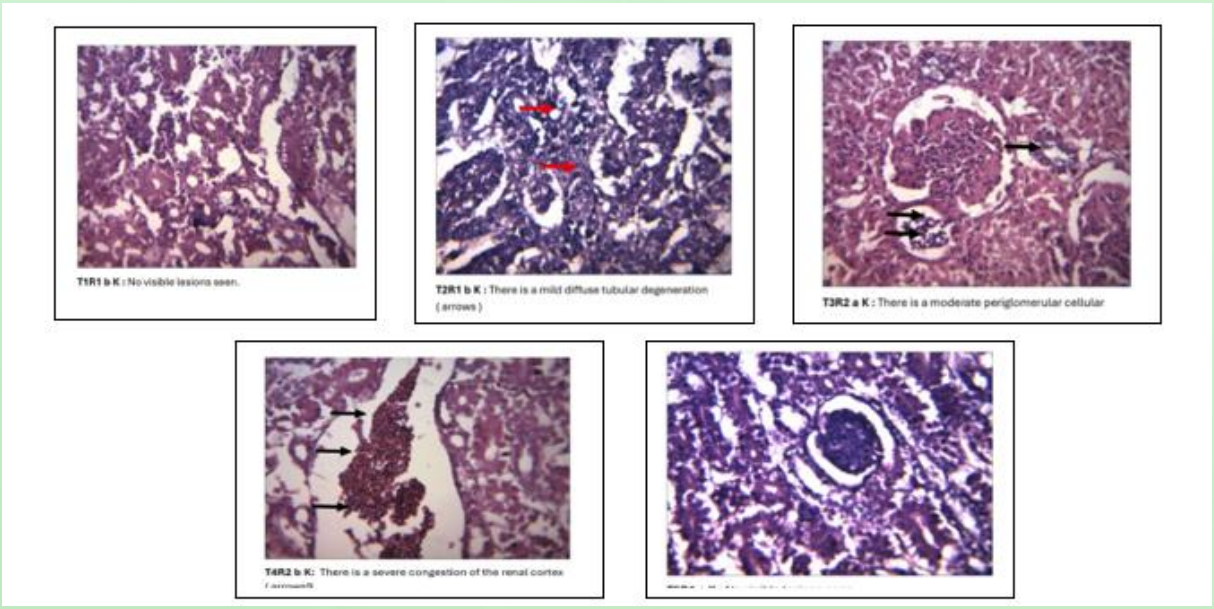



Plate 1 Histological section of the kidney of a broiler fed aflatoxin poultry feed

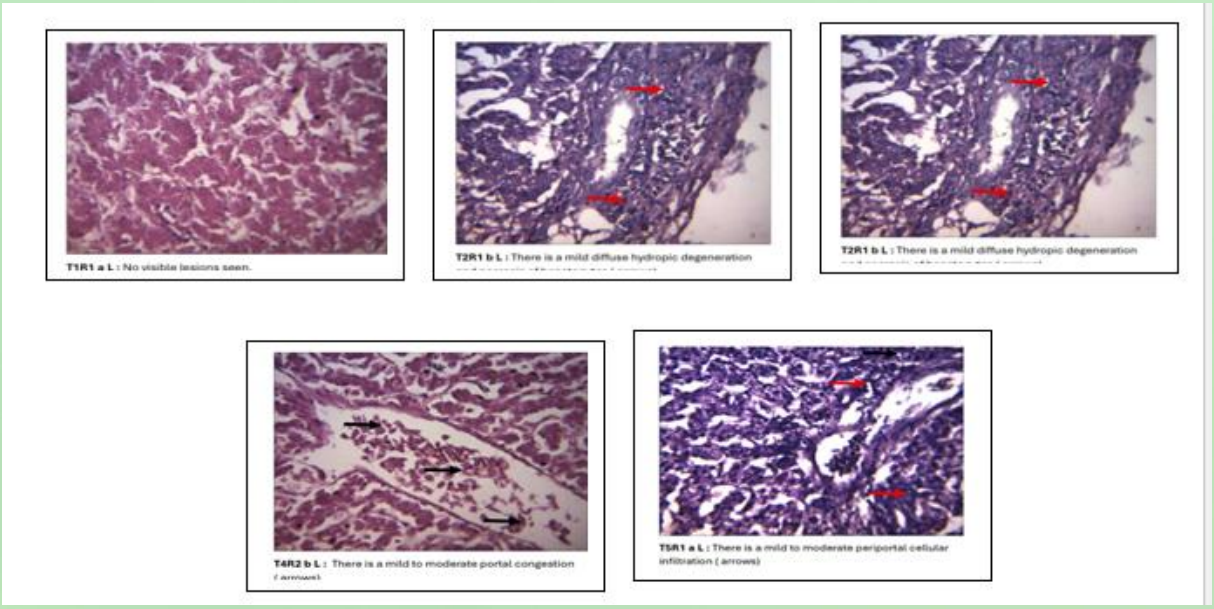



Plate 2: Histological section of the liver of a broiler fed aflatoxin poultry feed

## Discussion

### *Growth Performance*

The study supports the already known growth-depressive action of aflatoxin among broiler chickens. Birds kept on the control diet, free of toxins, had a better feed consumption, weight gain and feed efficiency which are signs of optimum nutrient efficiency in a nutritional context. On the other hand, aflatoxin exposure led to reduced feed intake and retarded gain ratio thus confirming earlier studies that aflatoxin damages intestinal integrity, interferes with nutrient absorption and mitigates metabolic efficiency (Yakout, 2024; Oloruntola *et al*., 2025). Furthermore, the high feed ratios of aflaxosin-challenged make additional witness to lower biological efficiency, which can be probably ascribed to decreased protein synthesis, as well as oxidative stress induced by the aflatoxin metabolites (Jobe *et al*., 2023). The dose-dependent non-linear protective response was discovered in activated charcoal supplementation. Proportional improvements in growth performance were not experienced with increased dosages but levels of inclusion were lower.

### *Haematological Indices*

The haematological parameters act as the indicators of the systemic toxicity and physiological stress that are sensitive. It was indicated by the absence of major differences in packed cell volume, haemoglobin concentration,

and erythrocyte counts that activated charcoal counteracted the extreme haematopoietic repression despite the presence of aflatoxin. It is known that aflatoxin interrupts the process of erythropoiesis by inhibiting protein synthesis and promoting the occurrence of oxidative damage (Fujii *et al*., 2021; Yuan *et al*., 2024). This means that the preservation of near physiological indices of red-cells in charcoal supplemented groups implies partial safeguarding against anaemic states. The differences in the leukocyte profiles also gave a bit more hints on the issue of immune modulation. The low counts of white blood cells in some treatments, are consistent with immunosuppression instigated by aflatoxin (Ekpo *et al*., 2024). An increase in lymphocyte proportions at increase in charcoal inclusion may be an indicator of a restoration of immune competence due to a reduction in toxin bioavailability. These finding are in line with those that attest that dietary adsorbent reduces aflatoxin-induced immune compromise (Jose Mendes *et al*., 2024; Kipkoech *et al*., 2025). Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) are erythrocyte indices that describe the average size of red blood cells and the average amount of hemoglobin present in each cell, respectively. In the present study, MCV and MCH values were relatively similar across treatments, indicating that dietary inclusion of activated charcoal did not adversely affect erythrocyte morphology or hemoglobin concentration in the red blood cells. Stable MCV and MCH values suggest that the birds maintained normal erythrocyte characteristics despite exposure to aflatoxin, possibly due to the toxin-binding properties of activated charcoal. Similar findings were reported by Adeyemi *et al*. (2023) and Kipkoech *et al*. (2025), who observed that dietary toxin binders helped maintain normal erythrocyte indices in broiler chickens exposed to mycotoxins.

***Biochemical Responses in the Serum***

Additional signs of organ specificity were serum biochemical indices. The constancy of alanine aminotransferase, total protein, albumin, glucose, and calcium in the treatments implies that hepatocellular integrity and primary metabolic events were not very disturbed. Other studies have also obtained comparable results in which the adsorbent supplementation inhibits excessive hepatic leakage of enzymes during aflatoxin challenge (Bhatti *et al*., 2022; Jobe *et al*., 2023). Nevertheless, the considerable change in aspartate aminotransferase indicates minor hepatic stress. It is believed that AST tends to be more sensitive compared to ALT to identify hepatocellular changes due to aflatoxin (Sang *et al*., 2023). Changes in lipid fractions, especially the corresponding higher levels of the high-density lipoprotein with higher charcoal addition can reflect better lipid metabolism of reduced hepatic load. On the other hand, high uric acid and creatinine levels in high inclusion levels indicate that the kidney may be strained, or may have a defective nitrogen metabolism. Similar reports have been carried out in previous studies, which have indicated mild renal loading and high toxin binders (Selle *et al*., 2021; Forni *et al*., 2022).

***Renal Histopathology***

Histological observation of renal tissues showed that progressive changes were observed with the increase of exposure to aflatoxins and to amounts of charcoal. The incomplete detoxification of the charcoal supplementation was manifested by mild tubular degeneration and inflammatory infiltration at the lower levels, which allowed charcoal-mediated nephrotoxicity to occur. Lesions of this type are typical of oxidative injury and vascular deviation that is caused by aflatoxins (Dabuo *et al*., 2022). It is important to note that the architecture of the kidneys of birds with the largest charcoal addition had similarities with the control album, which suggested that the protection effectiveness was significant at this scale. This finding indicates better adsorption of aflatoxin in the gastrointestinal tract hence averting systemic circulation and kidney deposition. The broilers have been reported to have similar protective renal outcomes when subjected to effective amounts of adsorbents (Bhatti *et al*., 2022).

***Hepatic Histopathology***

Liver being the most prominent organ in terms of aflatoxin metabolism showed different degrees of degeneration in the treatments. The condition of hydropic degeneration, necrosis, and vascular congestion in those groups challenged with aflatoxin is associated with deteriorated protein synthesis and lipid peroxidation (Moloi *et al*., 2023; Wang *et al.*, 2024). Suppression of lesion severity with increased charcoal addition, however, only indicated a mild degeneration change, which is a sign of partial hepatoprotection. The presence of mild lesions being persistent is an indication that the hepatic detoxification mechanisms are active even when the systemic toxicity levels have lowered. A dose responsive protective trend can be observed when hepatic and renal results are viewed as one. Less than optimal inclusion levels of charcoal (0.52 -1000) moderately mitigated, and higher inclusion (2.0) were associated with a renal intact state and a significant decrease in hepatic pathology. On the whole, the results indicate that activated charcoal has an effect of protection against aflatoxin-induced toxicity on broiler chicken in a dose-dependent manner. Maximal inclusion however is not simply proportional to performance improvement, thus, the importance of optimisation. The results provide a useful piece of knowledge regarding the application of practical feed safety interventions, especially in tropical production systems where aflatoxin contamination is still prevalent (Ochieng *et al*., 2021).

## Conclusion

This study evaluated the effect of activated charcoal on broiler chickens fed aflatoxin contaminated diets. Birds in the control group (T1) received a diet free of aflatoxin and activated charcoal, while birds in T2, T3, T4, and T5 received aflatoxin-contaminated diets supplemented with 0.5%, 1.0%, 1.5%, and 2.0% activated charcoal respectively. The results showed that aflatoxin caused negative effects on the performance and tissue structure of the birds. However, the inclusion of activated charcoal reduced these effects. Hematological parameters remained within normal physiological ranges across treatments, while serum biochemical indices showed some variations due to aflatoxin exposure. Histopathological examination revealed that birds fed lower levels of activated charcoal showed some kidney and liver lesions, whereas birds fed 2.0% activated charcoal (T5) showed improved tissue structure similar to the control group. Therefore, activated charcoal was effective in reducing the toxic effects of aflatoxin in broiler chickens, with 2.0% inclusion level showing the best protective effect.

## Recommendation

Activated charcoal should be included in broiler diets contaminated with aflatoxin to reduce its toxic effects. An inclusion level of 2.0% activated charcoal is recommended based on the results of this study. However it is recommended that broilers ration should include activated charcoal at optimum levels (1.0 -2.0%). Poultry farmers should ensure proper storage of feed ingredients to prevent aflatoxin contamination.

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# Effect of Abiotic Stress Conditions on Alkaloid Metabolism: A Bibliometric Analysis

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## Abstract

Alkaloids are nitrogen-containing secondary metabolites that play essential roles in plant defense, stress adaptation and pharmaceutical applications. Increasing climatic variability has intensified the exposure of plants to abiotic stresses such as drought, salinity, temperature extremes and ultraviolet (UV) radiation, thereby influencing alkaloid biosynthesis and accumulation. This study aimed to synthesize current knowledge on the physiological, biochemical and molecular mechanisms regulating alkaloid metabolism under abiotic stress. While, providing a comprehensive bibliometric assessment of global research trends in this field. A systematic bibliometric analysis was conducted using the Web of Science Core Collection database and the retrieved records were analyzed through VOSviewer to evaluate publication trends, leading contributors, collaboration networks and emerging research themes. The findings revealed that abiotic stresses significantly alter alkaloid metabolism through phytohormonal signaling, transcriptional regulation and metabolic reprogramming. Key stress-responsive pathways involving jasmonic acid, abscisic acid, reactive oxygen species and transcription factors such as AP2/ERF, WRKY and MYB were identified as major regulators of alkaloid biosynthesis. Bibliometric analysis demonstrated a substantial increase in research output over recent decades, with China and the United States emerging as the most productive countries. The Chinese Academy of Sciences, China Agricultural University and the University of Calgary were among the leading research institutions. Industrial Crops and Products, Molecules and Plant Physiology were identified as influential journals. While, Elsevier dominated as the leading publisher in this research domain. Keyword analysis highlighted a shift from fundamental alkaloid biosynthesis studies toward climate resilience, stress adaptation and multi-omics approaches. Future research should integrate genomics, transcriptomics, metabolomics, artificial intelligence and genome-editing technologies to accelerate the development of climate-resilient medicinal plants with stable alkaloid profiles under changing environmental conditions.

***Keywords:*** *Climate resilience, Secondary metabolite biosynthesis, Plant stress signaling, Multi-omics integration, Medicinal plant biotechnology and VOSviewer.*

## Introduction

Alkaloids are a structurally diverse group of naturally occurring nitrogen-containing secondary metabolites synthesized by plants, fungi and some microorganisms. These compounds include well-known examples such as nicotine, morphine, quinine and caffeine, each characterized by distinct heterocyclic nitrogen structures and significant pharmacological activities (Samanta & Manna, 2025; Ushie et al., 2023). In plants, alkaloids function primarily as chemical defense molecules that protect against herbivores, insects and pathogenic microorganisms through their toxic, deterrent or antimicrobial properties (Divekar et al., 2022). Beyond ecological significance, alkaloids possess considerable economic and medicinal value, serving as important sources of pharmaceuticals, stimulants, analgesics and anticancer agents (Hashim et al., 2025). Recent studies have demonstrated that alkaloid biosynthesis is highly responsive to environmental fluctuations, particularly abiotic stress conditions such as drought, salinity, extreme temperatures and ultraviolet (UV) radiation (Yeshi et al., 2022). Stress-induced metabolic reprogramming often alters carbon and nitrogen allocation within plants. Thereby, influencing secondary metabolite accumulation (Khan et al., 2026). Two major theoretical frameworks, namely the Carbon/Nutrient Balance Hypothesis (CNBH) and the Growth-Differentiation Balance Hypothesis (GDBH), explain these responses in FIGRE 1. The CNBH proposes that environmental stress modifies the relative availability of carbon and nutrients, leading to enhanced production of carbon-rich secondary metabolites under nutrient-limited conditions (Zhang, 2022). Conversely, the GDBH suggests that plants under moderate stress divert metabolic resources from growth processes toward differentiation and defense-related pathways, including alkaloid biosynthesis in FIGRE 1. These adaptive responses enable plants to enhance stress tolerance through antioxidant activity, osmotic regulation and protective signaling mechanisms (Choudhary et al., 2023; Mishra et al., 2023). The objective of this study was to comprehensively evaluate the effects of major abiotic stress factors, including drought, salinity, temperature extremes and UV radiation on alkaloid metabolism in plants. The paper also aimed to synthesize current knowledge on the physiological, biochemical and molecular mechanisms regulating stress-induced alkaloid biosynthesis. While, identifying emerging research trends, technological advances and future opportunities through bibliometric analysis.

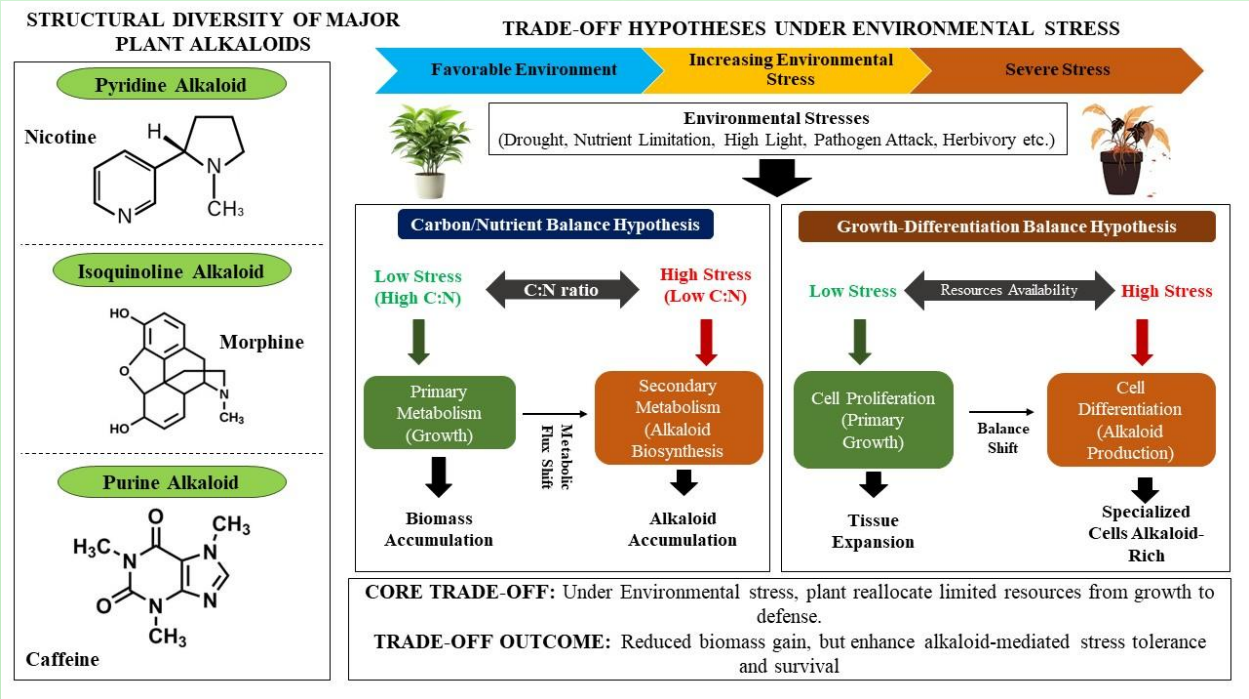



Figure 1. Structural diversity of major plant alkaloids and the trade-off hypotheses under environmental stress.

**Influence of Specific Abiotic Stresses on Alkaloid Metabolism**

Abiotic stress conditions profoundly influence plant secondary metabolism, particularly the biosynthesis and accumulation of alkaloids. Environmental stressors such as drought, salinity, temperature fluctuations and ultraviolet (UV) radiation trigger complex physiological and molecular responses that reprogram carbon and nitrogen allocation within plant tissues (Shoaib et al., 2024). Since alkaloids are nitrogen-containing defense compounds, their production is often closely associated with stress adaptation mechanisms. Under adverse environmental conditions, plants frequently divert metabolic resources from growth-related processes toward the synthesis of protective secondary metabolites, including alkaloids (Mishra et al., 2025; Salam et al., 2023). The extent and direction of these metabolic changes depend on stress intensity, duration, plant species and developmental stage.

***Drought and Water Deficit***

Drought stress is one of the most extensively studied abiotic factors affecting alkaloid metabolism. Water deficit generally reduces plant biomass and cellular expansion, leading to a phenomenon known as the "concentration effect," where alkaloid levels increase due to reduced dilution within plant tissues (He et al., 2026; Y. Sun et al., 2023). However, drought-induced alkaloid accumulation is not solely a passive consequence of reduced growth. It is also actively regulated through stress-responsive metabolic pathways (Arif et al., 2025).

Under drought conditions, plants experience oxidative stress, stomatal closure and disruption of primary metabolism, which collectively stimulate secondary metabolite production (Sattar et al., 2024). Several medicinal plants such as *Nicotiana tabacum*, *Catharanthus roseus* and *Datura stramonium*, exhibit enhanced alkaloid accumulation during moderate water stress (Amirifar et al., 2022). In tobacco plants, drought stress has been associated with increased nicotine biosynthesis due to elevated activity of putrescine N-methyltransferase (PMT), a key enzyme involved in pyrrolidine alkaloid formation (Yin et al., 2025). PMT catalyzes the methylation of putrescine, representing an important regulatory step in nicotine biosynthesis. Increased jasmonic acid signaling during drought further activates transcription factors that enhance alkaloid biosynthetic gene expression (Bhushan et al., 2023; Ma et al., 2026). Additionally, drought-induced accumulation of alkaloids may contribute to osmotic adjustment and antioxidant defense. Certain alkaloids stabilize cellular membranes and help scavenge reactive oxygen species (ROS), thereby protecting plant cells from oxidative damage (Haghpanah et al., 2024; Ikram et al., 2025). Nevertheless, severe or prolonged drought may eventually inhibit alkaloid production due to excessive impairment of photosynthesis and energy metabolism (Amirifar et al., 2022).

***Salinity Stress***

Salinity stress affects plants through osmotic imbalance, ion toxicity and nutrient disturbances, particularly alterations in nitrogen metabolism. Excessive sodium and chloride ions disrupt cellular homeostasis, impair enzyme activities and induce oxidative stress (Farhan et al., 2024). To cope with these adverse effects, plants often enhance the synthesis of protective secondary metabolites (Yeshi et al., 2022). Salt stress has been shown to

stimulate alkaloid accumulation in several medicinal species such as *Catharanthus roseus*, *Hyoscyamus muticus* and *Atropa belladonna* (Wen et al., 2023). In *Catharanthus roseus*, salinity enhances the accumulation of terpenoid indole alkaloids through the upregulation of tryptophan decarboxylase (TDC) and strictosidine synthase (STR), two key enzymes in alkaloid biosynthesis (Sharma et al., 2018). These metabolic adjustments are believed to strengthen antioxidant defenses and reduce oxidative injury caused by excessive ROS generation under saline conditions (Rao et al., 2025).

Furthermore, salinity-induced alkaloids may function in osmotic regulation by contributing to intracellular solute balance. Some alkaloids also interact with stress-responsive signaling pathways mediated by abscisic acid (ABA) and jasmonates, which coordinate adaptive metabolic responses (Akhtar et al., 2026). However, the relationship between salinity and alkaloid biosynthesis is highly species-specific. Moderate salt stress often enhances alkaloid production, whereas extreme salinity may suppress metabolic activity and reduce overall secondary metabolite accumulation due to impaired growth and cellular toxicity (Debbagh-Nour et al., 2026).

***Temperature Extremes***

Temperature is another critical environmental factor regulating alkaloid metabolism. Both heat stress and cold stress significantly affect enzymatic activities, precursor availability, membrane stability and gene expression associated with secondary metabolism, in Table 1.

***Heat Stress***

High temperatures can destabilize proteins and enzymes involved in alkaloid biosynthesis, resulting in altered metabolic fluxes. Heat stress often accelerates respiration and disrupts photosynthetic efficiency (Sharma et al., 2022). Thereby, reducing the availability of carbon skeletons required for secondary metabolite production. In some plant species, elevated temperatures decrease alkaloid accumulation due to degradation of biosynthetic precursors and suppression of enzyme activity (Yeshi et al., 2022). For example, excessive heat has been reported to reduce morphine accumulation in Papaver somniferum by affecting the activity of enzymes such as codeinone reductase (COR), which participates in benzylisoquinoline alkaloid biosynthesis (Tilkat et al., 2024). Heat stress also enhances ROS production, leading to oxidative damage that may impair metabolic pathways. Nevertheless, mild heat stress may occasionally stimulate alkaloid synthesis as part of an adaptive defense response, particularly when stress signaling pathways involving heat shock proteins and jasmonates are activated (Wu et al., 2026).

***Cold Stress***

Cold stress induces significant physiological and biochemical changes that often promote the accumulation of protective metabolites. Low temperatures slow down metabolic processes and may trigger the synthesis of specific alkaloids that function as cryoprotectants (He et al., 2023). These compounds help stabilize cellular membranes, preserve protein integrity and reduce oxidative damage during chilling or freezing conditions (Grgac et al., 2022). In certain medicinal plants, cold exposure enhances alkaloid accumulation through activation of stress-responsive transcription factors and secondary metabolic pathways (Alami et al., 2024). For instance, low temperatures can increase tropane alkaloid levels in species such as Datura and Hyoscyamus. Cold-induced alkaloids may also contribute to ROS scavenging and osmotic protection, thereby improving stress tolerance (Luo et al., 2025). However, prolonged freezing conditions can ultimately impair biosynthetic machinery and reduce overall metabolite production.

***Light Intensity and UV Radiation***

Light is an essential environmental regulator of plant metabolism, influencing photosynthesis, development and secondary metabolite biosynthesis. Variations in light intensity and exposure to ultraviolet radiation, particularly UV-B radiation (280-315 nm), strongly affect alkaloid production (Wang et al., 2025). UV-B radiation acts as an abiotic elicitor that stimulates defense-related metabolic pathways. Exposure to UV-B induces oxidative stress and DNA damage, leading to activation of signaling cascades involving reactive oxygen species, jasmonates and transcription factors (Mmbando, 2023). Consequently, plants enhance alkaloid biosynthesis as a protective mechanism against photo-oxidative stress (Ashraf et al., 2024).

In *Catharanthus roseus*, UV-B exposure increases the accumulation of vindoline and catharanthine through upregulation of genes associated with terpenoid indole alkaloid biosynthesis (Tang et al., 2022). Similarly, caffeine accumulation in tea and coffee plants has been linked to light intensity and UV exposure. Alkaloids produced under UV stress may function as UV-absorbing compounds, antioxidants or deterrents against stress-weakened herbivore attacks (Liu et al., 2023). However, excessive radiation can damage chloroplast structures and inhibit primary metabolism, ultimately reducing biosynthetic efficiency. Therefore, optimal levels of light and UV exposure are critical for maintaining balanced alkaloid production in medicinal plants (Alyas et al., 2023).

Table 1. Differential impacts of specific abiotic stresses on alkaloid accumulation, key enzymatic triggers and adaptive functions.

| *Representative Plant Species* | *Major Alkaloid Affected* | *Key Enzyme/Gene Regulated* | *Direction of Alkaloid Accumulation* | *Physiological/Adaptive Function* | *Proposed Mechanism Under Stress* | *References* |
|---|---|---|---|---|---|---|
| Abiotic Stress: Drought / Water Deficit | | | | | | |
| *Nicotiana tabacum* | Nicotine | Putrescine *N*-methyltransferase (PMT), Quinolinate phosphoribosyltransferase (QPT) | Increased | Antioxidant defense, herbivore deterrence, osmotic adjustment | Water deficit enhances jasmonic acid signaling, activating nicotine biosynthetic genes and concentrating alkaloids due to reduced biomass | (Selmar & Kleinwächter, 2013) |
| *Catharanthus roseus* | Vincristine, Vinblastine, Catharanthine | Tryptophan decarboxylase (TDC), Strictosidine synthase (STR) | Increased | ROS scavenging and stress adaptation | Drought induces terpenoid indole alkaloid pathway through ABA and JA-mediated transcriptional regulation | (Isah, 2019) |
| *Datura stramonium* | Tropane alkaloids (Atropine, Scopolamine) | Tropinone reductase I (TRI) | Moderate increase | Maintenance of cellular homeostasis | Enhanced nitrogen allocation toward secondary metabolism during stress | (Wink, 2010) |
| Abiotic Stress: Salinity Stress | | | | | | |
| *Catharanthus roseus* | Ajmalicine, Vindoline | TDC, STR | Increased | Oxidative stress protection and osmotic regulation | Salt stress induces ROS production and activates alkaloid biosynthesis through ABA-dependent pathways | (Isah, 2019; Khashan & Al-Athary, 2016) |
| *Hyoscyamus muticus* | Hyoscyamine | Hyoscyamine 6β-hydroxylase (H6H) | Increased | Ion homeostasis and ROS detoxification | Salinity alters nitrogen metabolism and stimulates tropane alkaloid synthesis | (Jangpangi et al., 2025) |
| *Atropa belladonna* | Scopolamine | Putrescine decarboxylase (PDC) | Slight increase | Osmotic balance and membrane protection | Accumulation linked with stress-induced nitrogen remobilization | (Facchini, 2001) |
| Abiotic Stress: Heat Stress | | | | | | |
| *Papaver somniferum* | Morphine, Codeine | Codeinone reductase (COR) | Decreased under severe heat | Protection against oxidative injury | High temperature destabilizes biosynthetic enzymes and reduces precursor availability | (Hasanuzzaman et al., 2021) |
| *Catharanthus roseus* | Catharanthine | Deacetylvindoline acetyltransferase (DAT) | Variable | Cellular defense under moderate heat | Mild heat may stimulate secondary metabolism, whereas severe heat suppresses biosynthetic activity | (Akula & Ravishankar, 2011) |
| *Rauvolfia serpentina* | Reserpine | Strictosidine β-glucosidase (SGD) | Reduced | Stress signaling regulation | Heat-induced metabolic imbalance suppresses alkaloid biosynthesis | (Yang et al., 2018) |
| Abiotic Stress: **Cold Stress** | | | | | | |
| *Datura inoxia* | Scopolamine | Tropinone reductase II (TRII) | Increased | Cryoprotection and ROS scavenging | Low temperature activates stress-responsive genes and membrane stabilization pathways | (Wink, 2010) |
| *Hyoscyamus niger* | Hyoscyamine | H6H | Increased | Maintenance of membrane integrity | Cold-induced oxidative signaling stimulates tropane alkaloid metabolism | (Isah, 2019) |
| *Camellia sinensis* | Caffeine | Caffeine synthase (CS) | Slight increase | Antioxidant and stress mitigation role | Cold stress enhances purine alkaloid accumulation for metabolic protection | (Ashihara et al., 2008) |
| Abiotic Stress: UV-B Radiation / High Light Intensity | | | | | | |
| *Catharanthus roseus* | Vindoline, Catharanthine | STR, Geraniol 10-hydroxylase (G10H) | Significant increase | UV shielding and antioxidant activity | UV-B acts as an elicitor, activating ROS-mediated signaling and secondary metabolism | (Akula & Ravishankar, 2011) |
| *Coffea arabica* | Caffeine | Xanthosine methyltransferase (XMT) | Increased | Photo-protection and defense enhancement | Increased light intensity stimulates purine alkaloid biosynthesis | (Mazzafera, 1999) |
| *Nicotiana tabacum* | Nicotine | PMT | Moderate increase | Photo-oxidative stress protection | UV exposure activates JA-responsive pathways involved in nicotine synthesis | (Shoji et al., 2010) |
| Abiotic Stress: Combined Stress Conditions | | | | | | |
| *Catharanthus roseus* | Terpenoid indole alkaloids | TDC, STR, ORCA3 transcription factor | Strong increase under moderate combined stress | Enhanced stress tolerance and metabolic plasticity | Crosstalk among ABA, JA, and ROS signaling pathways coordinately regulates alkaloid metabolism | (El-Sayed & Verpoorte, 2007) |
| *Nicotiana tabacum* | Nicotine | PMT, A622 gene | Variable depending on stress intensity | Integrated defense response | Combined drought and heat alter carbon–nitrogen balance affecting nicotine biosynthesis | (Shoji et al., 2010) |

**Physiological and Molecular Mechanisms Regulating Stress-Induced Alkaloid Biosynthesis.**

Plants respond to abiotic stress through highly coordinated physiological and molecular mechanisms that regulate secondary metabolite production such as alkaloid biosynthesis (Sana et al., 2025). Environmental stresses such as drought, salinity, extreme temperatures, and ultraviolet radiation trigger complex signaling networks involving phytohormones, transcription factors and intracellular transport systems (Rabeh et al., 2025). These adaptive responses enable plants to modulate alkaloid metabolism for enhanced survival under adverse environmental conditions (Leite Dias & D'Auria, 2025).

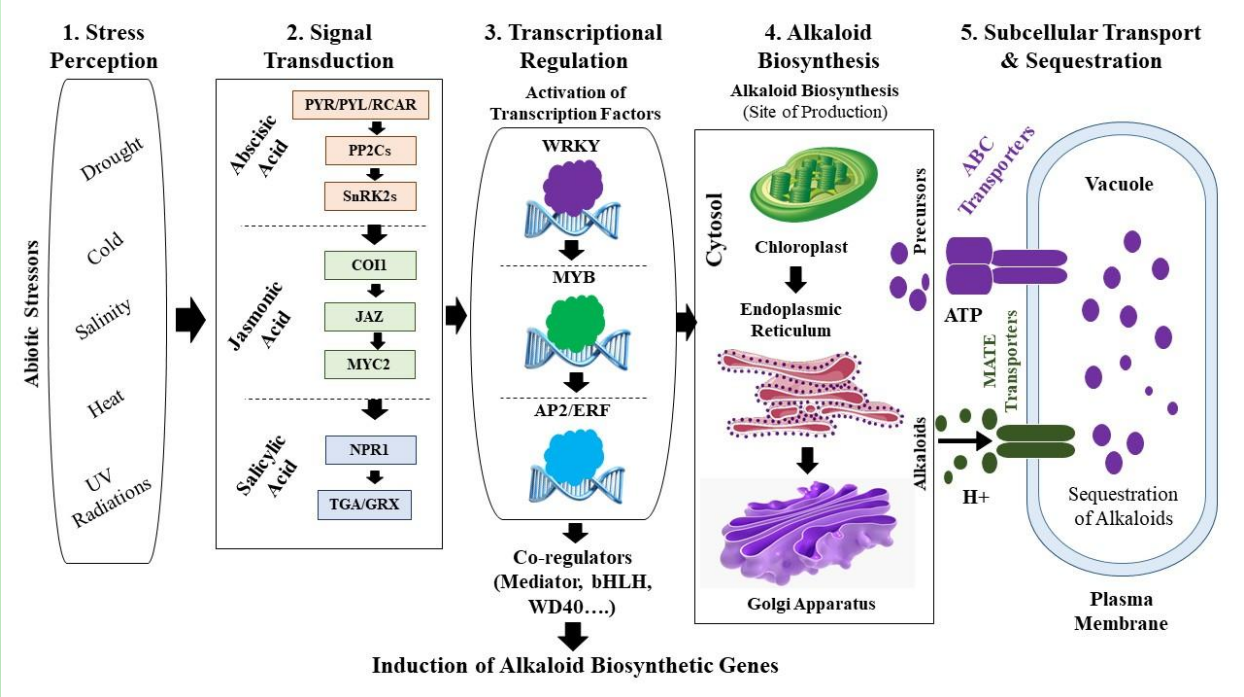



Figure 2. Molecular signaling cascade regulating stress-induced alkaloid biosynthesis and subcellular transport.

***Signal Transduction and Phytohormonal Regulation***

Phytohormones play central roles in mediating stress-responsive alkaloid biosynthesis, in **Figure 2.** Among these, jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) are considered major signaling molecules that coordinate plant defense and metabolic reprogramming during abiotic stress (Verma et al., 2025). Jasmonic acid is one of the most important elicitors of alkaloid biosynthesis. Under stress conditions, JA signaling activates genes associated with secondary metabolism, particularly those involved in nicotine, terpenoid indole alkaloid and tropane alkaloid pathways (Jeyasri et al., 2023). In *Nicotiana tabacum*, drought or wounding induces JA accumulation, which subsequently stimulates the expression of putrescine N-methyltransferase (PMT) and quinolinate phosphoribosyltransferase (QPT), key enzymes in nicotine biosynthesis (Lei et al., 2025). Similarly, in *Catharanthus roseus*, JA enhances the transcription of tryptophan decarboxylase (TDC) and strictosidine synthase (STR), leading to increased production of vinblastine and vincristine precursors (Guedes et al., 2024).

Abscisic acid is primarily associated with drought and salinity responses. Stress-induced ABA accumulation regulates stomatal closure, osmotic balance, and antioxidant defense while also influencing secondary metabolism (Sheuli et al., 2025). ABA interacts synergistically with JA signaling pathways to activate stress-responsive genes involved in alkaloid biosynthesis. Furthermore, salicylic acid contributes to stress tolerance by modulating reactive oxygen species (ROS) signaling and activating defense-related metabolic pathways (Lukan & Coll, 2022). SA-mediated signaling often enhances antioxidant alkaloid accumulation under oxidative stress conditions (Upadhyay et al., 2025).

***Role of Transcription Factors***

Stress-responsive transcription factors function as molecular switches that regulate alkaloid biosynthetic pathways. Among the most significant transcription factor families are WRKY, MYB and AP2/ERF proteins, which bind to promoter regions of biosynthetic genes and modulate their expression under stress conditions (Ma et al., 2026). The AP2/ERF family is particularly important in jasmonate-mediated alkaloid regulation. In tobacco, ERF transcription factors such as ORC1 and ERF189 activate nicotine biosynthetic genes, including PMT and A622, during drought and herbivory stress (Chen et al., 2026; Nie & Wang, 2023). In *Catharanthus roseus*, the AP2/ERF transcription factor ORCA3 positively regulates terpenoid indole alkaloid biosynthesis by activating TDC and STR genes in response to jasmonate signaling (Rai et al., 2022).

WRKY transcription factors also play crucial roles in stress-induced secondary metabolism. These proteins are involved in ROS-responsive signaling and defense regulation (Wu et al., 2022). Several WRKY proteins enhance alkaloid accumulation by activating genes associated with phenylpropanoid and alkaloid pathways during environmental stress (Rai et al., 2022). Likewise, MYB transcription factors regulate carbon allocation and secondary metabolic flux, influencing alkaloid synthesis under stress-induced metabolic reprogramming (Imran et al., 2025). The coordinated action of these transcription factors ensures rapid metabolic adaptation to environmental stress while balancing growth and defense responses.

***Subcellular Compartmentation and Alkaloid Transport***

Subcellular compartmentation is another critical aspect of alkaloid metabolism during stress conditions. Alkaloids are often synthesized in specific tissues such as roots or young leaves and subsequently transported to storage compartments including vacuoles, laticifers, or epidermal cells (S. Sun et al., 2023). Stress conditions significantly influence this transport process by regulating membrane transporters and intracellular trafficking systems (Ahmed et al., 2026). ATP-binding cassette (ABC) transporters and multidrug and toxic compound extrusion (MATE) proteins are major transport systems involved in alkaloid sequestration and translocation (Kar et al., 2022). In nicotine-producing plants, nicotine is synthesized in roots and transported through the xylem to leaves, where it accumulates in vacuoles. Under stress conditions, enhanced transporter activity increases alkaloid compartmentation to minimize cytotoxicity and maximize defensive efficiency (Xiao et al., 2025). Vacuolar sequestration also protects cellular organelles from alkaloid toxicity while maintaining intracellular osmotic balance during abiotic stress. Additionally, compartmentation facilitates rapid remobilization of nitrogen-containing alkaloids when stress conditions subside (Saleem et al., 2022). Therefore, stress-regulated alkaloid transport and storage represent essential adaptive strategies that contribute to plant survival and metabolic homeostasis (Gillani et al., 2026).

**Functional Role of Alkaloids in Stress Tolerance**

Alkaloids are multifunctional secondary metabolites that play crucial roles in plant adaptation and survival under adverse environmental conditions. Beyond their classical defensive functions against herbivores and pathogens, alkaloids actively contribute to abiotic stress tolerance through antioxidant protection, osmotic regulation and nitrogen storage (Divekar et al., 2022). Environmental stresses such as drought, salinity, heat, cold and ultraviolet radiation frequently disrupt cellular homeostasis and trigger oxidative damage, thereby necessitating protective metabolic responses. Under such conditions, plants enhance alkaloid biosynthesis as part of an integrated defense strategy aimed at maintaining physiological stability and cellular integrity (Shoaib et al., 2024).

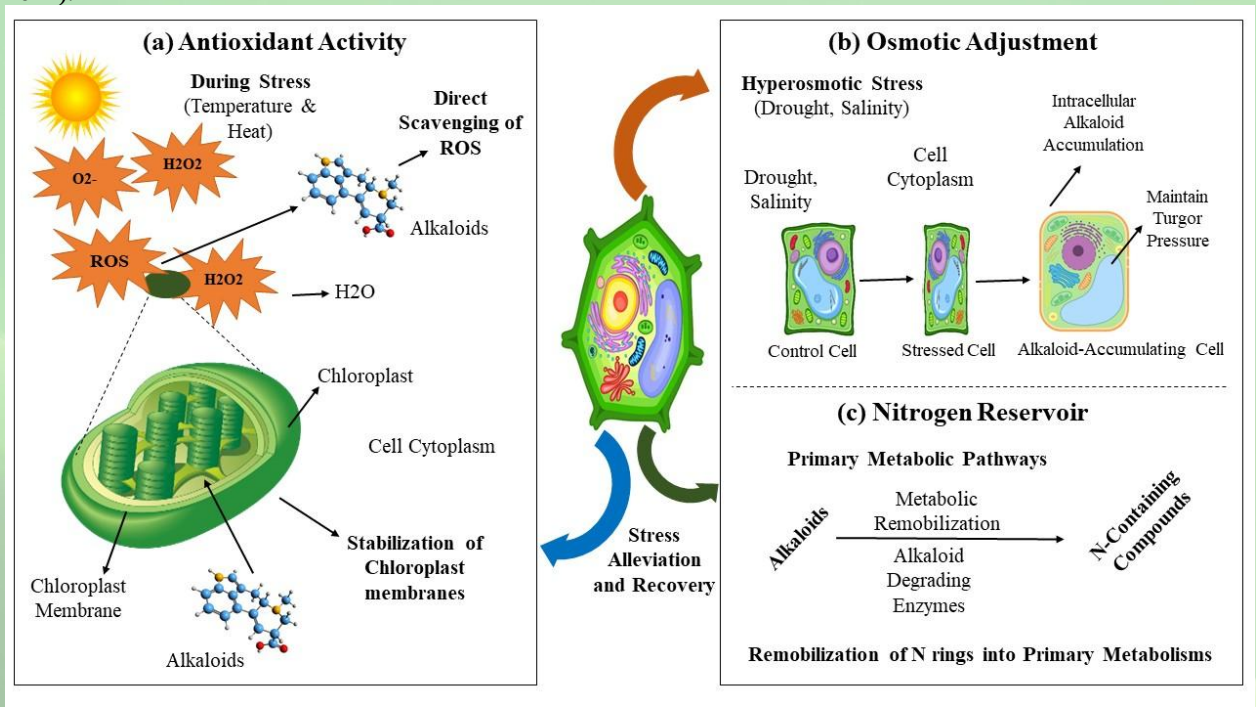



Figure 3. Tripartite protective mechanism of alkaloids during environmental stress.

***Antioxidant Activity and Reactive Oxygen Species (ROS) Scavenging***

One of the primary functional roles of alkaloids during abiotic stress is their involvement in antioxidant defense systems, in Figure 3. Environmental stresses commonly induce excessive production of reactive oxygen species (ROS), including superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (OH) (Jomova et al.,

2023). Although ROS serve as signaling molecules at moderate concentrations, their excessive accumulation damages proteins, lipids, nucleic acids and cellular membranes, ultimately impairing plant growth and metabolism (Kesawat et al., 2023). Several alkaloids possess strong antioxidant properties that help neutralize ROS and reduce oxidative injury. Nicotine, caffeine, berberine and various indole alkaloids have been reported to stabilize membrane structures and limit lipid peroxidation under stress conditions (Verma et al., 2026). *In Catharanthus roseus*, stress-induced accumulation of terpenoid indole alkaloids contributes to enhanced antioxidant capacity by scavenging free radicals generated during drought and salinity stress. Similarly, caffeine in tea and coffee plants functions as an antioxidant molecule that mitigates photo-oxidative stress caused by excessive light and UV radiation (Saleem et al., 2022). Alkaloids also interact with enzymatic antioxidant systems such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD), thereby strengthening the overall cellular defense network (Raza et al., 2022). Through these mechanisms, alkaloids help maintain redox homeostasis and protect vital metabolic processes during environmental stress.

***Osmotic Adjustment and Maintenance of Cellular Turgor***

Abiotic stresses such as drought and salinity reduce cellular water availability and disrupt osmotic balance. Under these conditions, plants accumulate compatible solutes and osmoprotective compounds to maintain cell turgor and prevent dehydration (Guru et al., 2025). Certain alkaloids contribute significantly to osmotic adjustment by acting as nitrogen-containing osmolytes that stabilize cellular structures and regulate intracellular osmotic potential (Haghpanah et al., 2024). Tropane alkaloids, nicotine and related compounds have been associated with improved osmotic regulation during water-deficit stress. These metabolites accumulate within vacuoles and cytoplasmic compartments, where they help retain water and preserve membrane stability (Chada et al., 2023). In saline environments, alkaloid accumulation may also counterbalance ionic toxicity caused by excessive sodium and chloride ions. This protective mechanism supports cellular hydration, enzyme functionality and metabolic continuity under stressful conditions (Gul et al., 2022).

Moreover, alkaloids can interact with membrane lipids and proteins to enhance membrane integrity during dehydration stress (Khan, 2025). Their accumulation often coincides with increased levels of abscisic acid (ABA), which coordinates osmotic stress responses and promotes stress acclimation. Consequently, alkaloid-mediated osmotic adjustment represents an important adaptive strategy that improves plant resilience under fluctuating environmental conditions (Zambelli et al., 2025).

***Alkaloids as Nitrogen Reservoirs***

Because alkaloids contain substantial amounts of nitrogen, they also function as temporary nitrogen storage compounds within plant tissues. Nitrogen is an essential macronutrient required for amino acids, proteins, nucleic acids and chlorophyll synthesis (Farhan et al., 2024). Yet, its availability is often limited during environmental stress. Under unfavorable conditions, plants may divert excess nitrogen into alkaloid biosynthesis as a means of conserving and safely storing nitrogen resources (Alami et al., 2024). This storage function is particularly important during stress recovery. When environmental conditions improve, nitrogen incorporated within alkaloid structures can be remobilized and redirected toward primary metabolism, growth and tissue repair (Salam et al., 2023). Such metabolic flexibility allows plants to maintain nutrient balance while simultaneously enhancing defense capacity during stress exposure. In addition, nitrogen-rich alkaloids may help regulate carbon-nitrogen balance during abiotic stress by preventing accumulation of toxic nitrogen intermediates (Li et al., 2025). This adaptive strategy supports metabolic homeostasis and enables plants to optimize resource allocation between growth and defense pathways. Therefore, alkaloids serve not only as protective metabolites but also as dynamic reservoirs that contribute to nutrient recycling and long-term stress adaptation (Prashar et al., 2025).

**Emerging Trends and Technologies in Alkaloid Research**

Rapid advances in plant biotechnology, systems biology and environmental analytics have significantly transformed the study of alkaloid metabolism under abiotic stress conditions (Khan et al., 2025). Traditional approaches focused mainly on biochemical characterization of individual pathways. Whereas, modern technologies now enable large-scale exploration of stress-responsive metabolic networks at genomic, transcriptomic, proteomic and metabolomic levels (Satrio et al., 2024). These emerging tools are improving our understanding of how environmental stress regulates alkaloid biosynthesis and are facilitating the development of climate-resilient medicinal plants with stable phytochemical profiles, in **Table 2**.

***Omics Integration and Systems Biology Approaches***

The integration of omics technologies, particularly transcriptomics and metabolomics, has become a powerful strategy for studying stress-induced alkaloid metabolism (Yan et al., 2024). Transcriptomics enables comprehensive analysis of gene expression patterns under environmental stress, helping researchers identify biosynthetic genes, transcription factors, signaling molecules and regulatory pathways involved in alkaloid production (Huang et al., 2024). Simultaneously, metabolomics provides detailed profiling of alkaloid composition and metabolic fluxes within plant tissues. Combined omics approaches allow researchers to map complex stress-responsive metabolic networks and uncover interactions between primary and secondary metabolism

(Varadharajan et al., 2025). In medicinal plants such as *Catharanthus roseus*, transcriptomic analyses have identified key stress-responsive genes including tryptophan decarboxylase (TDC), strictosidine synthase (STR) and AP2/ERF transcription factors that regulate terpenoid indole alkaloid biosynthesis during drought and salinity stress (Gupta et al., 2023). Metabolomic studies further reveal dynamic changes in alkaloid accumulation associated with environmental fluctuations.

Multi-omics integration also enhances understanding of signaling pathways mediated by jasmonic acid, salicylic acid and abscisic acid under stress conditions. Advanced bioinformatics and network analysis tools are increasingly used to correlate transcriptomic data with metabolite profiles, enabling prediction of regulatory hubs and metabolic bottlenecks (Sanches et al., 2024). Such systems-level approaches provide valuable insights into stress adaptation mechanisms and facilitate targeted improvement of medicinal plant productivity (Lv & Fan, 2026). Moreover, next-generation sequencing (NGS), RNA sequencing (RNA-Seq) and mass spectrometry-based metabolomics have accelerated the discovery of novel alkaloid biosynthetic genes and unknown intermediate metabolites (Singh et al., 2025). These technologies are particularly valuable for non-model medicinal species lacking complete genomic information.

***Metabolic Engineering and Climate-Smart Medicinal Plants***

Metabolic engineering has emerged as a promising approach for enhancing alkaloid production and developing "climate-smart" medicinal plants capable of maintaining stable bioactive compound profiles under environmental stress (Das et al., 2023). Conventional cultivation of medicinal plants is highly sensitive to climatic variability, which often causes fluctuations in alkaloid content and pharmaceutical quality. Modern genetic engineering techniques aim to overcome these limitations by manipulating biosynthetic pathways and stress-responsive regulatory networks (Khalid et al., 2026). One major strategy involves overexpression of rate-limiting enzymes such as PMT, TDC and STR to enhance alkaloid biosynthesis. Genetic modification of transcription factors, particularly AP2/ERF and WRKY families, has also been used to improve stress-responsive secondary metabolism (Su et al., 2025). For example, overexpression of ORCA3 in *Catharanthus roseus* significantly enhances terpenoid indole alkaloid accumulation under stress conditions (Rai et al., 2022).

The development of CRISPR/Cas9 genome-editing technology has further revolutionized alkaloid engineering. CRISPR-based approaches enable precise editing of biosynthetic genes, transporter proteins and regulatory elements associated with alkaloid metabolism (Rynjah et al., 2025). These technologies offer opportunities to improve stress tolerance, optimize carbon-nitrogen allocation and stabilize alkaloid yield despite drought, salinity or temperature fluctuations (Ullah et al., 2026). Additionally, synthetic biology approaches are increasingly used to reconstruct alkaloid biosynthetic pathways in microbial hosts such as yeast and bacteria for sustainable production of pharmaceutically important compounds (Qin et al., 2025). Such innovations may reduce dependence on environmentally sensitive medicinal plant cultivation while ensuring consistent alkaloid supply.

***Geospatial Influence and Environmental Chemotyping***

Geographical and climatic conditions strongly influence alkaloid composition in wild and cultivated medicinal plants. Variations in altitude, temperature, precipitation, soil characteristics, solar radiation and seasonal patterns create distinct environmental pressures that shape plant metabolic responses (Yadav et al., 2024). Consequently, medicinal plants collected from different regions often exhibit unique chemical fingerprints or chemotypes characterized by quantitative and qualitative differences in alkaloid profiles (Noviana et al., 2022). Geospatial analysis combined with environmental monitoring has become an important tool for understanding regional variability in medicinal plant chemistry (Nagavi et al., 2024). Climatic factors such as drought frequency, UV radiation intensity and temperature extremes can significantly alter secondary metabolite accumulation by regulating stress-responsive biosynthetic pathways (Khan, 2025). For example, high-altitude medicinal plants frequently accumulate greater concentrations of protective alkaloids due to increased UV exposure and low-temperature stress (Kumar et al., 2026).

Modern geographic information systems (GIS), remote sensing technologies and ecological modeling are increasingly applied to predict alkaloid distribution patterns across different climatic zones (Tiwari et al., 2023). These approaches help identify optimal cultivation regions for medicinal plants with superior phytochemical quality. Furthermore, geospatial metabolomics can support conservation strategies for wild medicinal species threatened by climate change and habitat degradation (Borthakur et al., 2026). Understanding environmental chemotyping is also important for pharmaceutical standardization because climatic variability directly affects the consistency, efficacy and safety of plant-derived bioactive compounds (Alum et al., 2025). Therefore, integrating geospatial analytics with metabolomic and genomic studies represents a promising direction for future medicinal plant research and sustainable phyto-pharmaceutical production (Tekade & Saraf, 2026).

Table 2. Applications of multi-omics integration and metabolic engineering in optimizing alkaloid profiles.

| Approach | Medicinal Plant Species | Target Alkaloid(s) | Omics/Engineering Strategy | Key Genes/Pathways Identified | Stresses | Major Outcomes and Applications | References |
|---|---|---|---|---|---|---|---|
| Transcriptomics Integration | *Catharanthus roseus* | Vinblastine, Vincristine, Catharanthine | RNA-Seq analysis under drought and salinity stress | *TDC, STR, DAT, ORCA3* | Drought, salinity | Identification of stress-responsive terpenoid indole alkaloid (TIA) pathways and regulatory transcription factors | (Khalkho et al., 2024; Ngara & Shavrukov, 2026) |
| Metabolomics Profiling | *Nicotiana tabacum* | Nicotine | LC-MS/MS and GC-MS metabolic fingerprinting | Nicotine biosynthetic pathway | Drought and UV stress | Quantification of stress-induced nicotine accumulation and metabolic flux reprogramming | (Shoji et al., 2010) |
| Integrated Transcriptomics & Metabolomics | *Papaver somniferum* | Morphine, Codeine, Thebaine | Combined RNA-Seq and metabolite network analysis | *COR, SalAT, T6ODM* | Heat and drought stress | Mapping of stress-regulated benzylisoquinoline alkaloid pathways and identification of metabolic bottlenecks | (Facchini, 2001) |
| Proteomics-Assisted Metabolic Mapping | *Rauvolfia serpentina* | Reserpine | Stress-responsive proteome analysis | Secondary metabolite regulatory proteins | Temperature stress | Identification of enzyme stability mechanisms controlling alkaloid biosynthesis | (Yang et al., 2018) |
| CRISPR/Cas9 Genome Editing | *Catharanthus roseus* | Vinblastine precursors | CRISPR-mediated editing of regulatory genes | AP2/ERF transcription factors, *ORCA3* | Multiple abiotic stresses | Enhanced alkaloid biosynthesis and improved stress resilience in engineered lines | (Giri et al., 2025) |
| Overexpression of Rate-Limiting Enzymes | *Nicotiana tabacum* | Nicotine | Genetic overexpression of biosynthetic enzymes | Putrescine *N*-methyltransferase (*PMT*), *QPT* | Drought and herbivory stress | Increased nicotine accumulation and enhanced defense capability | (Shoji et al., 2010) |
| Transcription Factor Engineering | *Catharanthus roseus* | Terpenoid indole alkaloids | Overexpression of transcription factors | *ORCA3*, WRKY, MYB proteins | Salinity and drought | Improved activation of alkaloid biosynthetic pathways under stress | (El-Sayed & Verpoorte, 2007) |
| Synthetic Biology Platforms | Yeast (*Saccharomyces cerevisiae*) engineered with plant genes | Morphinan alkaloids | Reconstruction of alkaloid biosynthetic pathways in microbial systems | Multi-enzyme benzylisoquinoline pathways | Climate-independent production | Sustainable production of pharmaceutical alkaloids independent of environmental variability | (Galanie et al., 2015) |
| Metabolite Network Modeling | *Hyoscyamus niger* | Hyoscyamine, Scopolamine | Computational metabolomic network analysis | Tropane alkaloid biosynthetic pathways | Salinity and cold stress | Prediction of pathway regulation and optimization of alkaloid yield | (Ashihara et al., 2008) |
| Epigenomics and Stress Memory Studies | *Arabidopsis thaliana* (model system for medicinal plant stress studies) | Indirect alkaloid-related secondary metabolism | DNA methylation and chromatin remodeling analysis | Stress-responsive epigenetic regulators | Recurrent drought stress | Identification of heritable stress-adaptive metabolic responses | (Selmar & Kleinwächter, 2013) |
| Geo-Metabolomics and Environmental Chemotyping | *Camellia sinensis* | Caffeine | GIS-linked metabolomics and climatic profiling | Purine alkaloid pathways | Altitude, UV radiation, temperature variation | Identification of regional alkaloid chemotypes and optimal cultivation zones | (Ashihara et al., 2008) |
| Remote Sensing-Assisted Precision Cultivation | *Cannabis sativa* | Cannabinoid alkaloid-like compounds | Integration of environmental sensors with metabolomic data | Secondary metabolite stress pathways | Heat and drought stress | Development of climate-smart cultivation systems with stable metabolite profiles | (Chandra et al., 2017) |
| Transporter Engineering | *Nicotiana tabacum* | Nicotine | Manipulation of vacuolar transport proteins | ABC and MATE transporters | Combined stress conditions | Improved alkaloid sequestration and reduced cellular toxicity | (Yazaki, 2005) |
| Multi-Omics Assisted Breeding | *Withania somnifera* | Withanolide alkaloids | Integration of genomics, transcriptomics and metabolomics | Stress-responsive biosynthetic genes | Drought and salinity | Selection of elite climate-resilient medicinal genotypes with stable bioactive yields | Kumar et al., 2022 |
| Machine Learning and AI-Assisted Metabolic Prediction | Multiple medicinal plant species | Diverse alkaloids | AI-based predictive metabolic modeling | Integrated stress-metabolic networks | Variable climatic stress | Prediction of stress-responsive alkaloid accumulation and optimization strategies | (Yang et al., 2018) |

## Materials and Methods (Bibliometric Approach)

Two of the most comprehensive and internationally recognized transdisciplinary citation databases the Web of Science Core Collection (WoS) and Scopus, were used as the primary data sources for this bibliometric analysis. These databases were selected due to their extensive coverage of peer-reviewed literature in environmental sciences, plant biology and physiology and agricultural sciences. Thereby ensuring a robust and representative dataset for research on alfalfa production systems. The Clarivate Analytics Web of Science (WoS) is one of the most widely used platforms for academic citation searches and bibliometric analyses and constitutes one of the largest scientific databases worldwide. It encompasses a broad range of high-impact scholarly publications (Pranckutė, 2021). Publication records were retrieved from the WoS Core Collection. This including the indices such as Science Citation Index Expanded (SCI-EXPANDED, 1975-2026) with 82 papers, Conference Proceedings Citation Index-Science (CPCI-S, 1975-2026) with 4 papers, Book Citation Index-Science (BKCI-S, 1975-2026) with 4 papers and the Emerging Sources Citation Index (ESCI, 1975-2026) with 8 papers. Data collection was completed on May 10, 2026, to minimize potential database update bias.

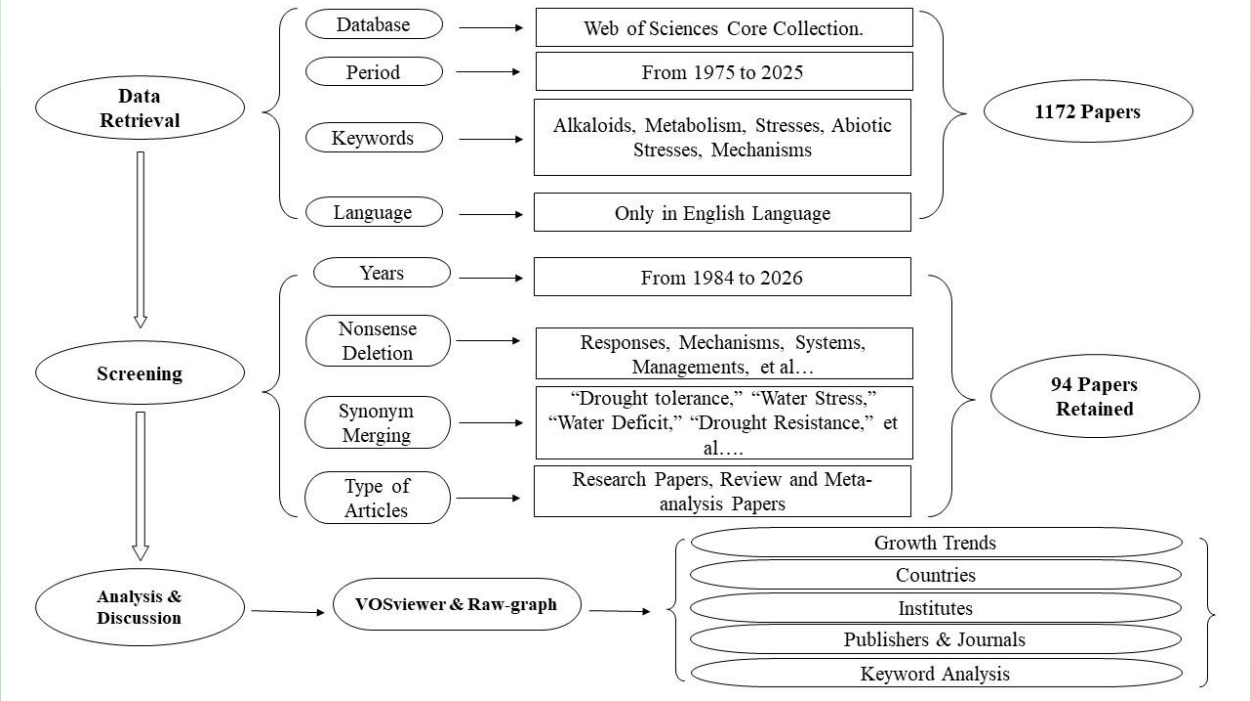



Figure 4. PRISMA flowchart for article selection based on WoS criteria.

The search was conducted using the topic-based keyword alkaloid metabolites, biosynthesis and abiotic stresses in plants. In total, 94 publications related to the title of the study "Effect of Abiotic Stress Conditions on Alkaloid Metabolism" were identified. All records were exported in "full record and cited references" format and saved as RIS, EndNote, and tab-delimited files. The retrieved data were subsequently imported into VOSviewer for further bibliometric visualization and network analysis in Figure 4.

### *VOSviewer*

The datasets obtained from this search were analyzed and visualized using VOSviewer, a user-friendly and freely available bibliometric visualization software. This tool was selected due to its wide range of analytical capabilities, its ability to process large volumes of data, and its capacity to generate intuitive visual representations that facilitate data interpretation (McAllister et al., 2022; Van Eck & Waltman, 2010). In the present study, VOSviewer was employed to examine the level of international collaboration among authors, institutions, and countries, as well as to identify research trends through keyword analysis. The software was also used to perform cluster and network analyses. All analyses were conducted using the default parameters provided by VOSviewer (Suryantoro et al., 2023). In the network visualizations, items are represented by circles, with the size of each circle reflecting the relative weight or importance of the item. Different colors indicate clusters of related elements. The spatial distance between items represents the strength of their relationships, with items positioned closer together indicating stronger associations.

**Results of the bibliometric analysis**

***Type of document and publication language***

A total of 1172 papers on effect of abiotic stress conditions on alkaloid metabolism were published between 1975 and 2026 and identified through keyword-based searches. The publications were indexed in several Web of Science databases, including the Science Citation Index Expanded (SCI-EXPANDED; 904 records), Conference Proceedings Citation Index–Science (CPCI-S; 860 records), Book Citation Index-Science (BKCI-S; 167 records), Emerging Sources Citation Index (ESCI; 63 records), Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH; 14 records), Social Sciences Citation Index (SSCI; 7 records), Arts & Humanities Citation Index (A&HCI; 2 records) and Book Citation Index-Social Sciences & Humanities (BKCI-SSH; 2 records). Regarding publication language, the vast majority of the papers (91) were published in English. A limited number of publications were written in other languages, including Chinese, French and German (1 paper in each).

***Publications Trend***

An integrated meta-analysis of the chronological data reveals a distinct evolutionary trajectory within this research field, characterized by a foundational surge in publication volume that subsequently catalyzed long-term citation impact, in Figure 5. The field achieved its absolute historical peak in production during 2010, yielding the maximum number of annual publications. This is driven heavily by core pioneering institutions like the University of Calgary and foundational researchers such as Facchini. In contrast, the peak for scientific impact lag-shifted significantly, with the maximum number of citations recorded years later in 2015. This temporal gap directly illustrates a classic bibliometric citation delay model. The immense volume of high-quality primary literature established during the 2010 production boom required a multi-year gestation period to be thoroughly absorbed, integrated and cited by the global scientific community. Following this 2015 citation zenith, the overlay trends show that while the total volume of new papers from the original core nucleus tapered off. It successfully sparked a decentralized wave of modern, highly collaborative studies between 2020 and 2025. Consequently, the publication trends act as a leading indicator of field maturity, transitioning from high-volume institutional output to widespread, global citation diffusion. The 94 papers analyzed from 1975 to 2026 had an h-index of 30 and received a total of 3281 citations during the study period, with an average of 36.73 citations per publication.

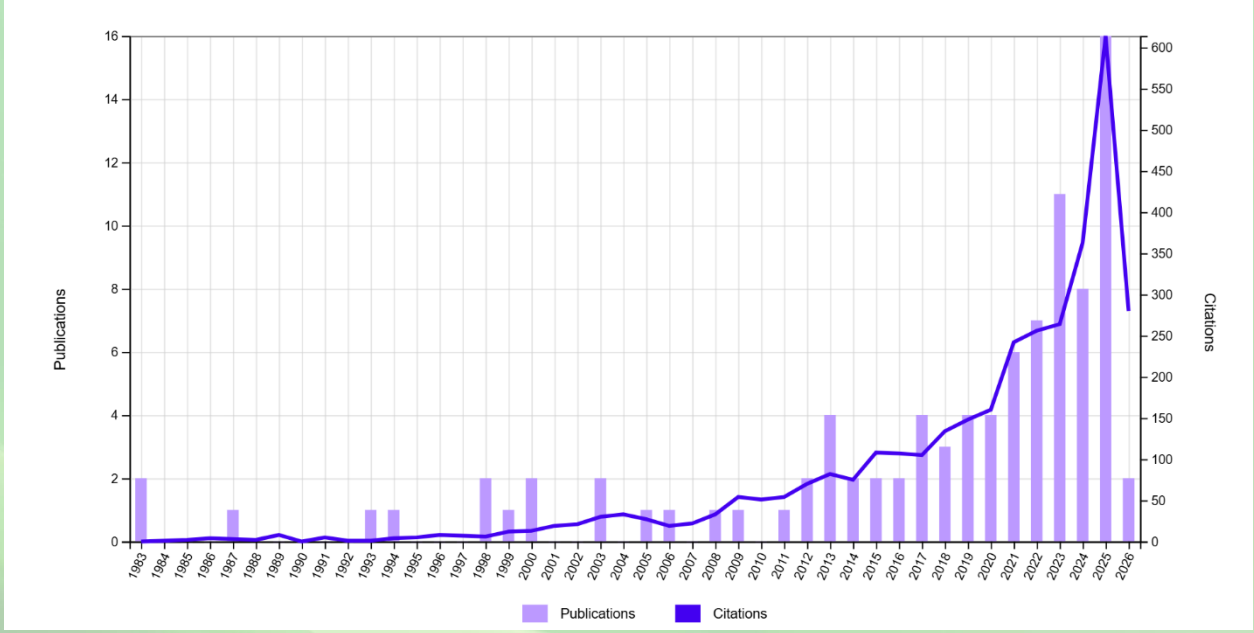



Figure 5. Scholarly article publication trends concerning the effect of abiotic stress conditions on alkaloid metabolism between 1992 and 2025.

***Web of Science Categories, Research Areas and Sustainable Development Goals***

A bibliometric meta-analysis of literature published between 1975 and 2026 reveals critical trends regarding the "Effect of Abiotic Stress Conditions on Alkaloid Metabolism", in **Figure 6**. Within the broader disciplinary landscape, the dataset spans 21 distinct research areas and 31 Web of Science (WoS) categories, heavily anchored in plant biology and chemistry. Plant Sciences stands as the predominant domain with 57 publications, followed closely by Biochemistry & Molecular Biology (15 papers in research areas; 14 in WoS categories). Other foundational disciplines driving this meta-analysis include Agriculture (9 papers), Chemistry (9 papers) and Environmental Sciences & Ecology (9 papers), alongside targeted contributions from Biotechnology & Applied Microbiology (5 papers) and Pharmacology & Pharmacy (4 papers). When mapped against global sustainability

frameworks, this scientific output aligns dynamically with 6 core Sustainable Development Goals (SDGs), reflecting a total of 112 strategic alignments. Research is overwhelmingly concentrated in SDG 03: Good Health and Well-Being (35 papers) and SDG 13: Climate Action (34 papers), illustrating how understanding stress-induced alkaloid variations directly connects to both medicinal resource discovery and climate resilience. Additionally, SDG 02: Zero Hunger accounts for 30 papers, highlighting the profound impact of abiotic stress on agricultural productivity and food security. The ecological dimension is captured by SDG 15: Life on Land (11 papers), with marginal representations in SDG 06 (Clean Water and Sanitation) and SDG 12 (Responsible Consumption and Production), each holding a single paper. Together, these metrics map a highly specialized yet interdisciplinary research cluster bridging environmental pressure, plant biochemistry and sustainable health solutions.

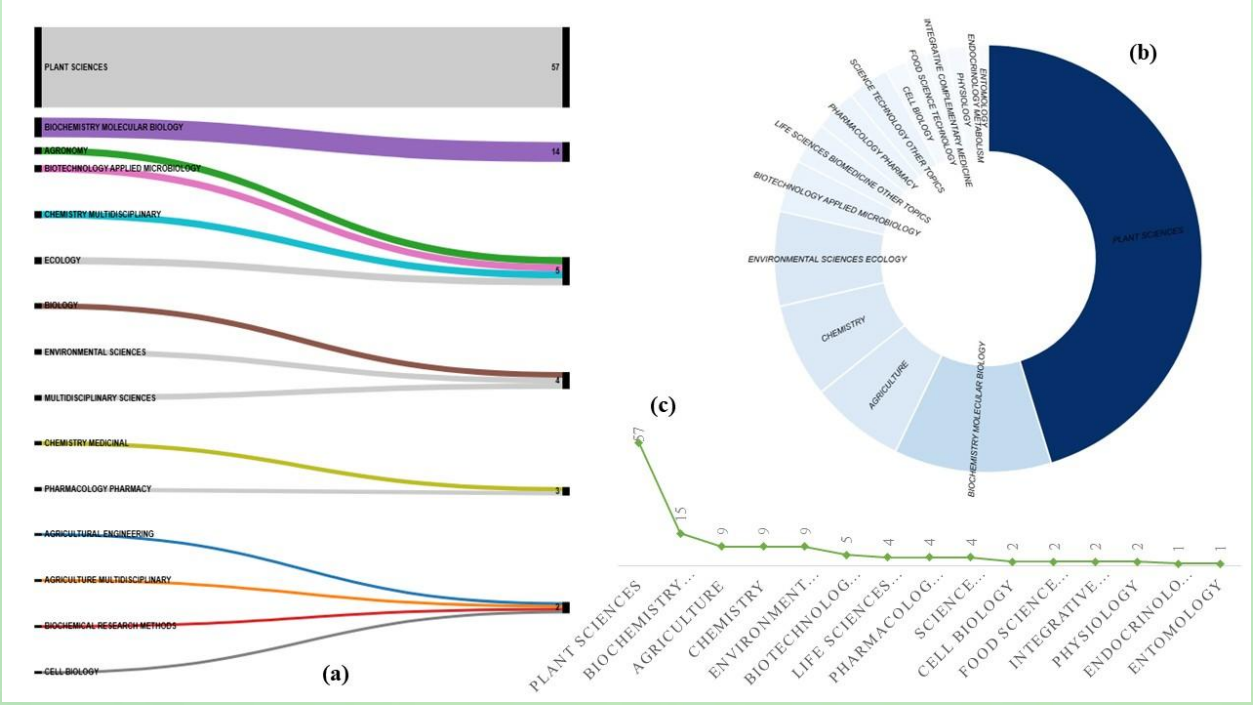



Figure 6. The most important Web of Sciences Categories, Research and SDGs are demonstrated in this field.

***Core Journals and Publishers***

These are the most prominent publishing avenues and intellectual hubs driving research on the Effect of Abiotic Stress Conditions on Alkaloid Metabolism, in **Figure 7, 8 and 9**. When analyzing the core data across the top-performing journals, distinct leaders emerge based on total publication volume and overall citation impact. *Industrial Crops and Products* stands out as a premier venue, striking an exceptional balance by topping both productivity and impact metrics, which emphasizes its role in translating stress-induced chemical shifts into industrial and agricultural applications. Following closely in publication frequency are authoritative plant science and molecular biology journals, including *Molecules*, *Phytochemistry*, *Journal of Agricultural and Food Chemistry*, *Plant Physiology and Biochemistry*. These platforms consistently document how environmental stressors like drought, salinity and temperature fluctuations trigger complex metabolic pathways to alter secondary metabolite profiles. However, when evaluating the intellectual weight through citation counts, journals like *Plant Physiology*, *Frontiers in Plant Science* and the *Journal of Experimental Botany* command significant scientific influence. Despite publishing fewer total papers relative to the high-volume leaders. These high-impact journals accumulate substantial citations, proving that they serve as foundational references for mechanistic insights into plant stress responses. Collectively, this journal ecosystem reveals a highly integrated research network where high-volume chemical analysis perfectly complements high-citation physiological studies, shaping the global understanding of how crops adapt to a changing climate.

Similarly, a wide range of publishers contribute to publications across different research fields. Among these publishers, the field is supported by emerging contributions from Taylor & Francis, Frontiers Media and Wiley. Higher-volume output is driven by MDPI and Springer Nature. While, Elsevier clearly dominates as the premier publishing house for this domain, in Figure 10.

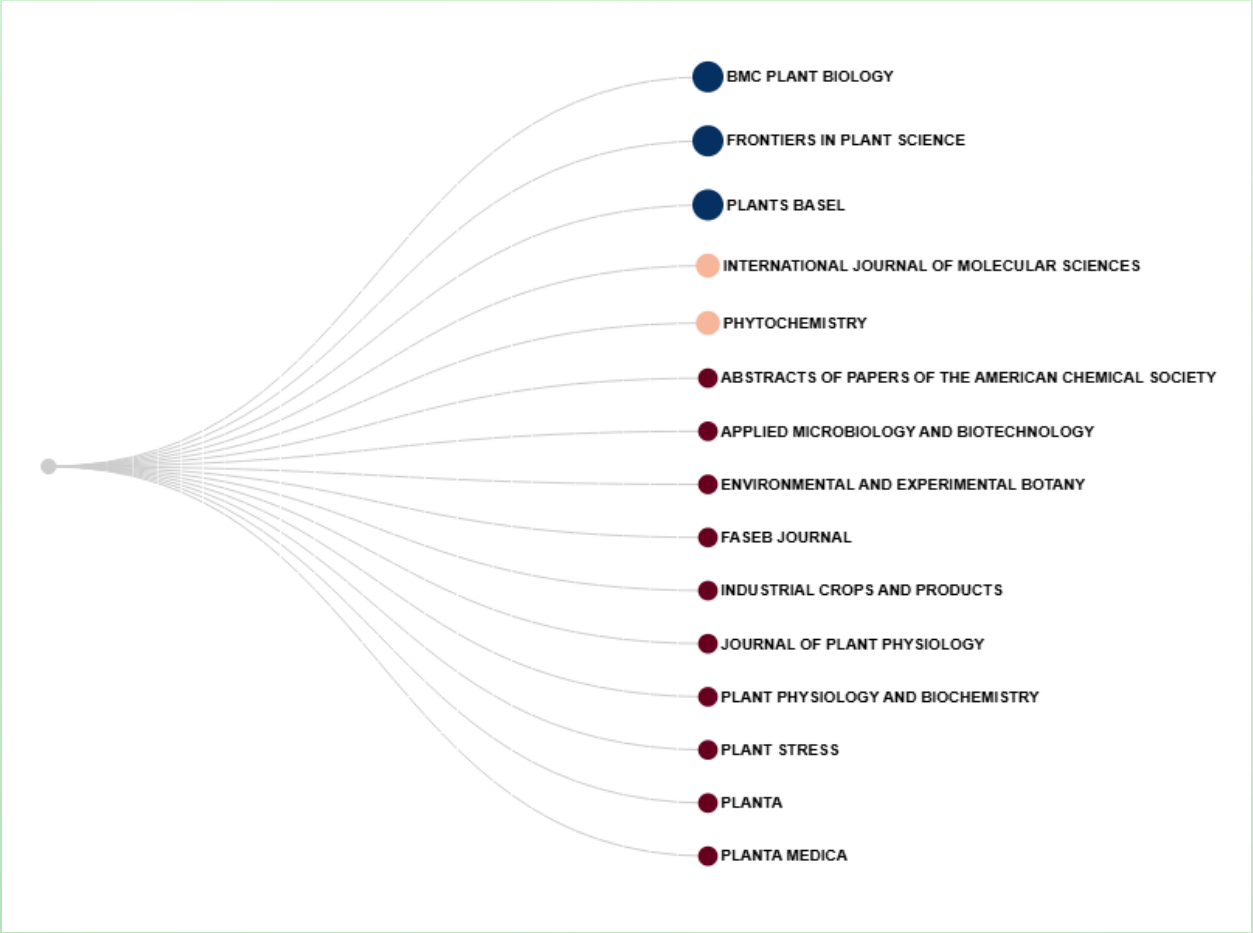



Table 7. List of the 15 most important journals pertaining to plant alkaloid metabolites.

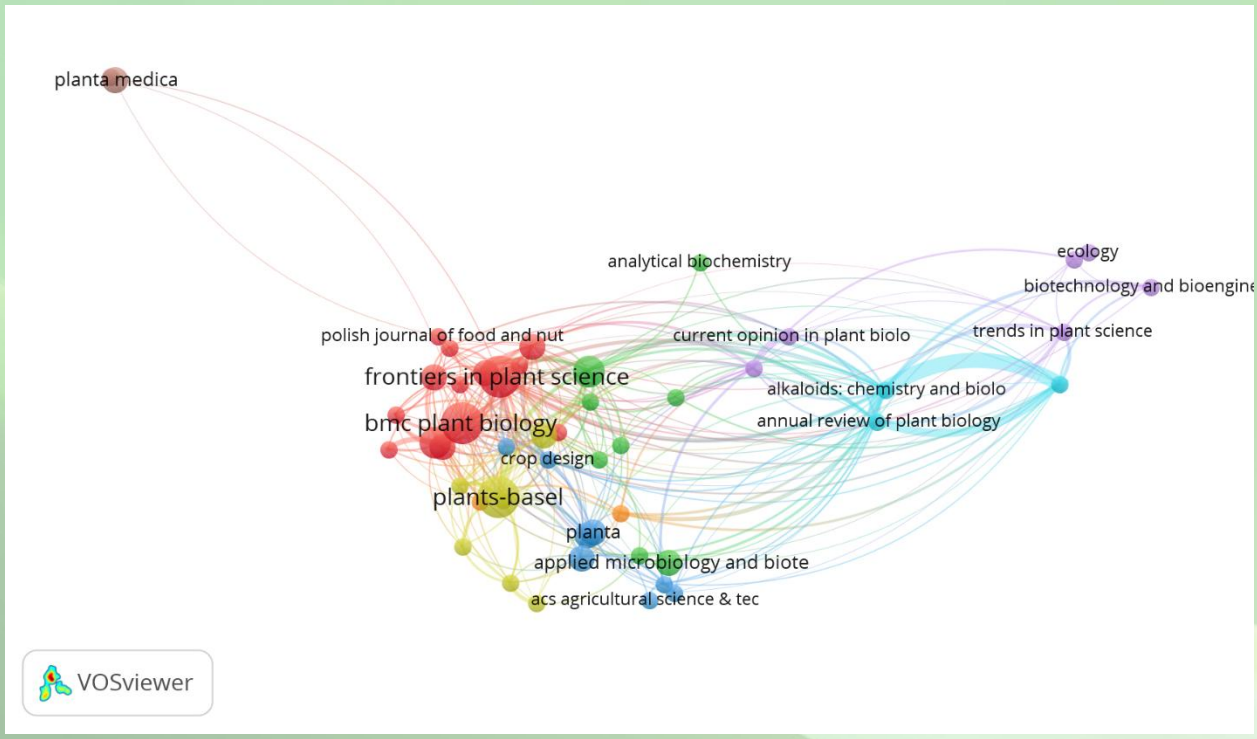



Figure 8. Most highly rated journals in which the most papers were cited with an average citation.

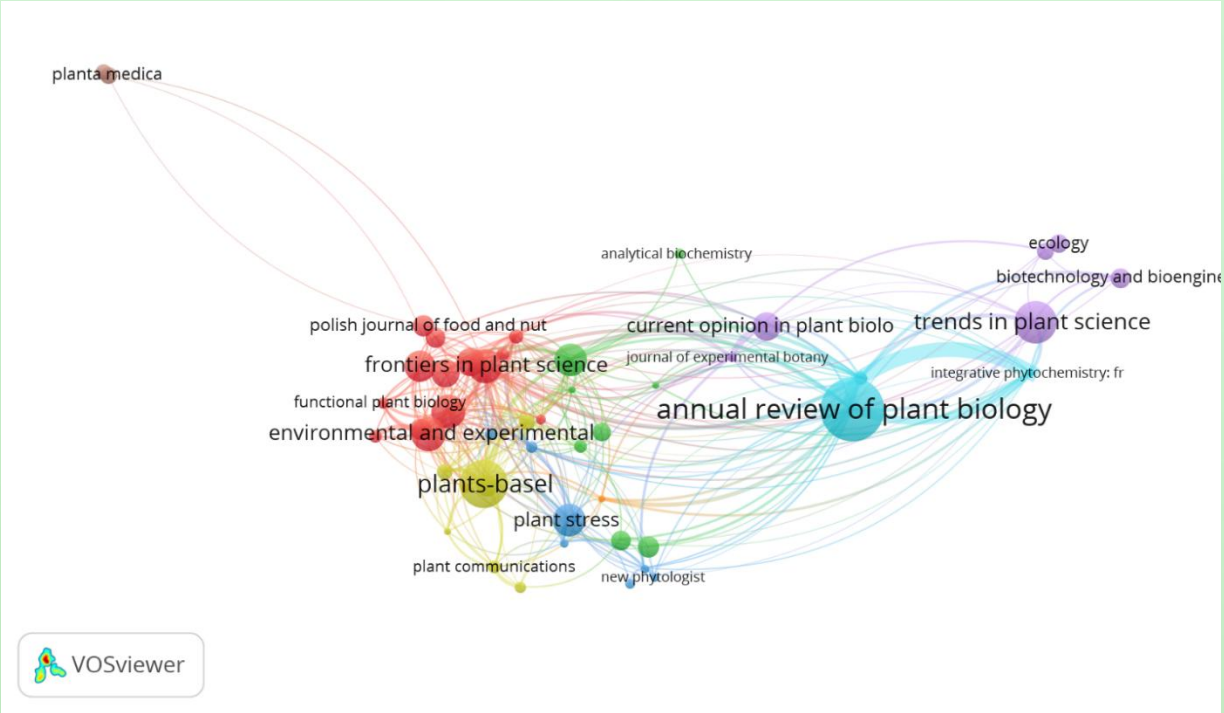



Figure 9. Most highly rated journals in which the most papers were cited with an average citation.

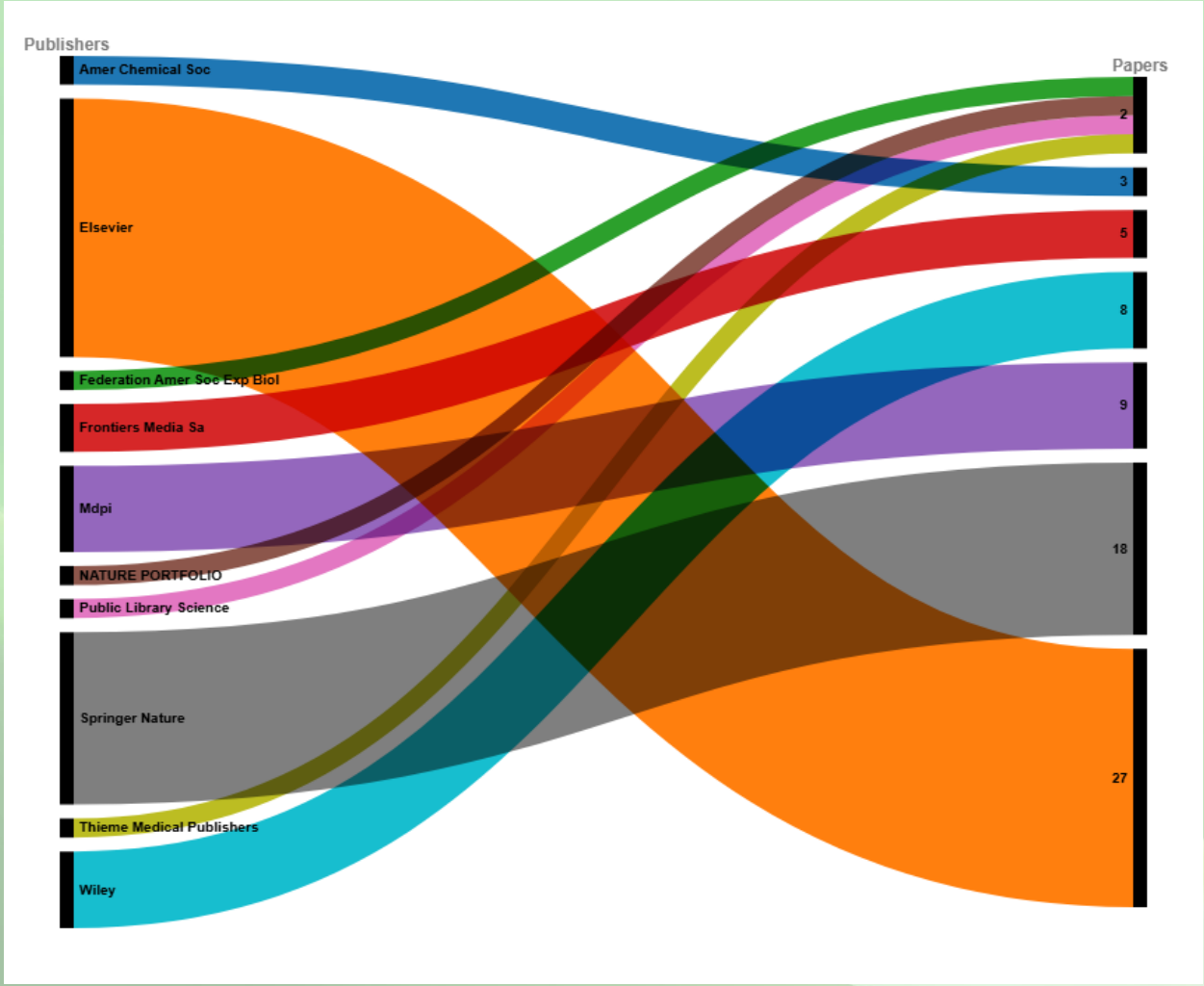



Figure 10. Top 10 publishers associated with the effect of abiotic stress conditions on alkaloid metabolism in plants.

***Countries co-authorship analysis***

A distinct geographical distribution in research exploring this field. Globally, China and the USA emerge as the primary titans of productivity out of the 34 countries, driving the vast majority of publications and establishing the foundational frameworks for how environmental pressures dictate plant chemical defense mechanisms, in Figure 11 and 12. Beyond these two dominant superpowers, significant research clusters thrive across Asia, Europe and the Middle East. Countries such as Iran, Germany, India, Japan and Brazil contribute substantial literature, illustrating a shared global priority to safeguard crop resilience against climate variations. Furthermore, the active participation of nations like Egypt, Turkey and South Korea underscores a diversified, transcontinental effort. This international research network effectively links regional agricultural challenges ranging from arid-zone salinity to temperature extremes with advanced global secondary metabolite research.

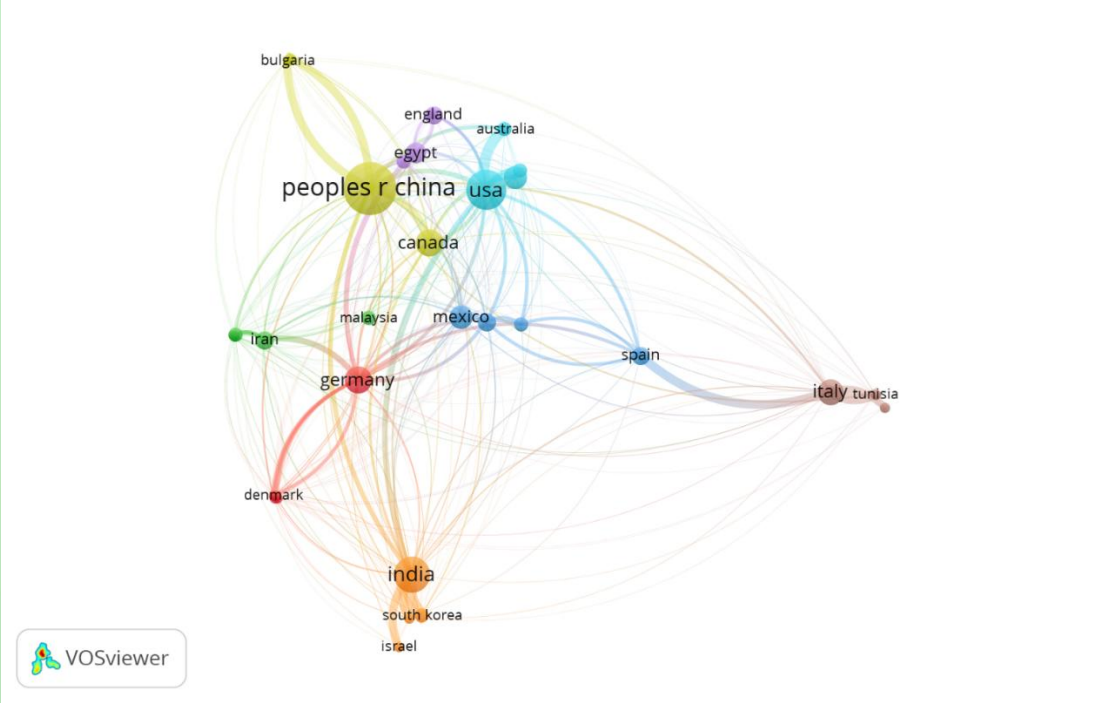



Figure 11. Countries working on Plant Alkaloid Metabolites on the bases of papers.

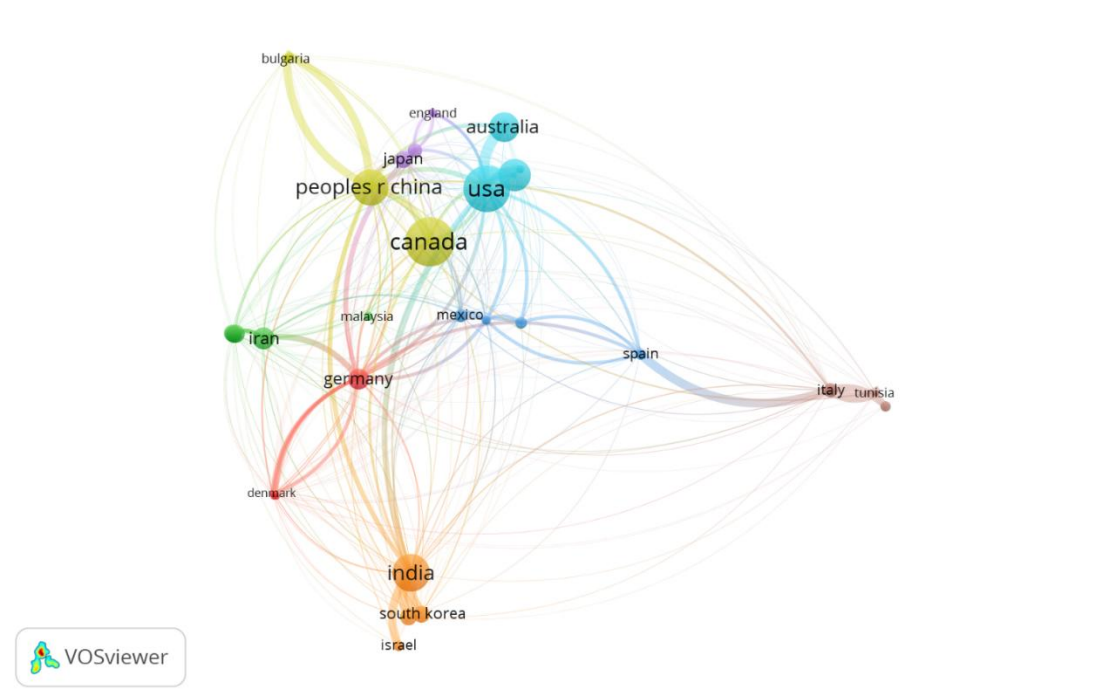



Figure 12. Countries working on Plant Alkaloid Metabolites with minimum 5 citations per paper.

***Authors and co-authorship analysis***

The researcher network highlights key contributors within this specialized scientific field, structured by publication volume, citation impact and chronological evolution. At the foundational core of this network stands Facchini, Peter J., who dominates the landscape as the top researcher, characterized by the largest node size and an intense heat signature indicating maximum citations and extensive collaborative links, in **Figure 13 and 14**. Moving upwards in an ascending analysis of the research cluster, peripheral and emerging scholars such as *Berni, Roberto*, *Lucini, Luigi* and *Cabrita, Ana R. J.* represent highly localized nodes. Each contributing minimal thresholds such as 1 paper and 5 citations acting as independent or newly integrated entry points into the field. Intermediates like *Parida, Asish Kumar*, *Jalmi, Siddhi Kashinath* and *Chaudhary, Talha* bridge the gap, showcasing growing citation metrics and tighter integration with the central hub. Chronologically, the overlay visualization reveals a clear temporal shift: legacy contributions centered around Facchini (predominantly pre-2015) have paved the way for recent. Modern developments (2020-2025) led by peripheral authors like *Gao, Yang-yang*, indicating a vibrant, evolving field that continues to expand outwards from its foundational authors.

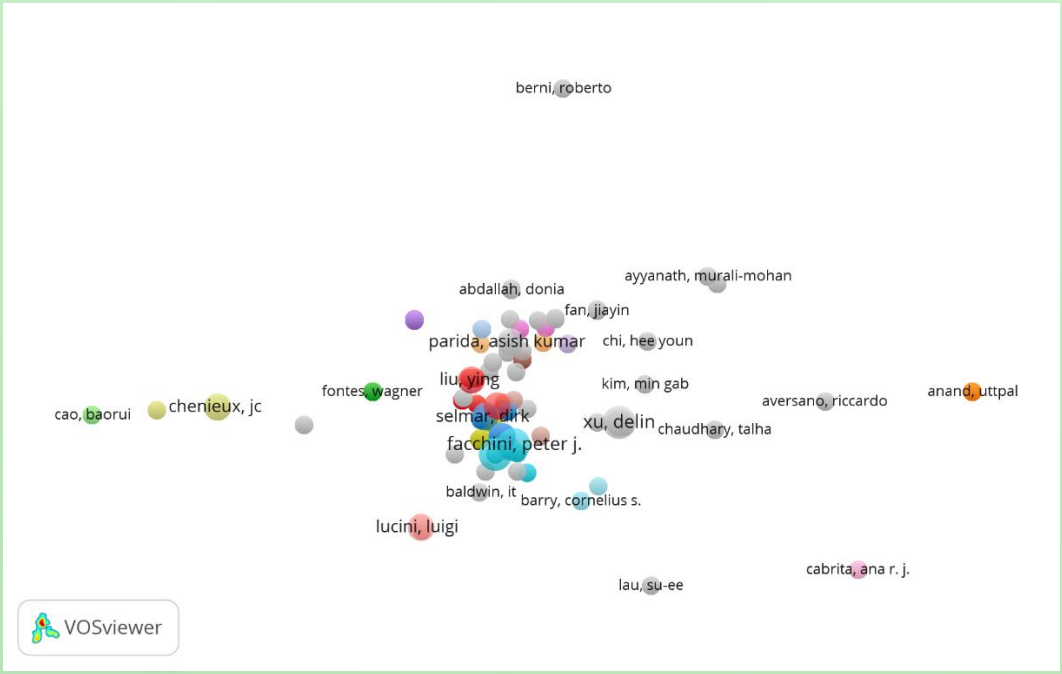



Figure 13. Network visualization map of the most prominent researchers throughout the world with more than 1 PAPER per author.

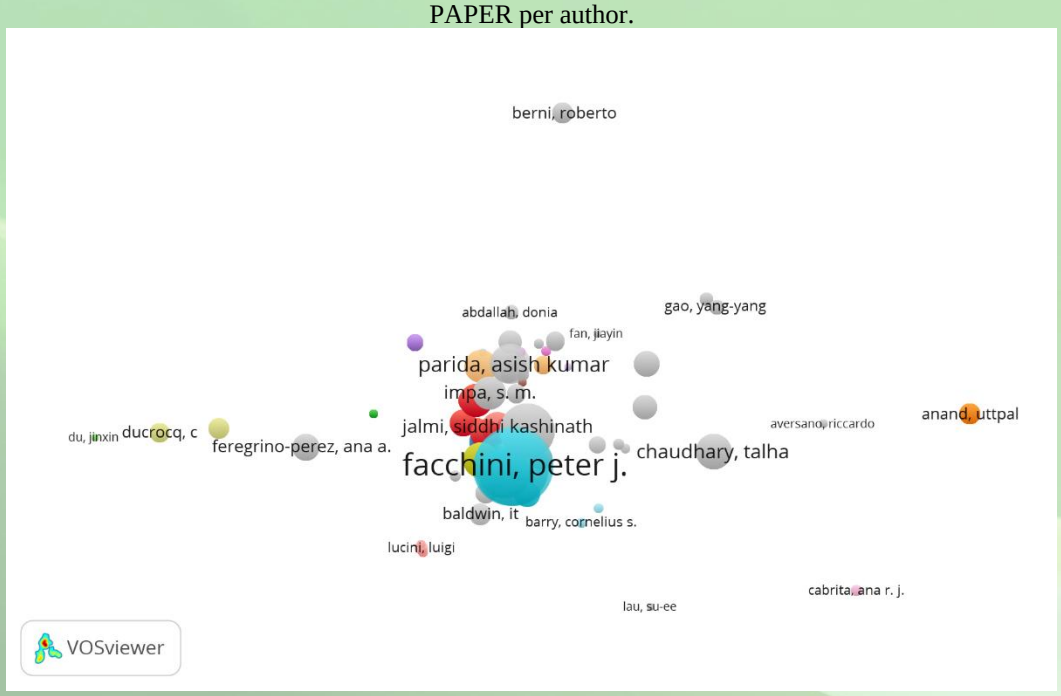



Figure 14. Network visualization map of the most prominent researchers throughout the world with more than 5 citations per author.

***Organizations co-authorship analysis***

The institutional landscape driving research on the effect of abiotic stress conditions on alkaloid metabolism in plants, showcasing a powerful concentration of scientific output across elite global organizations, in Figure 15 and 16. Analyzing the contributing research institutes reveals that the Chinese Academy of Sciences (CAS) stands out as the global leader in productivity out of 200 organizations, anchoring a massive portion of the literature. This heavy contribution underscores China's strategic academic focus on dissecting how environmental constraints alter plant secondary metabolites. The institutional network expands through highly productive clusters, prominently featuring the University of California System and the United States Department of Agriculture (USDA), both of which serve as major Western pillars linking metabolic stress responses to agricultural resilience. Domestically, the academic momentum within China is further driven by specialized institutions like China Agricultural University, Zhejiang University and the Chinese Academy of Agricultural Sciences, establishing a dense, collaborative ecosystem focused on crop adaptability. On the global stage, foundational contributions from the University of London and Institut National de la Recherche Agronomique (INRA) add crucial European expertise in plant physiology and biochemical pathways. Together, these top organizations form an interconnected, intercontinental research network. Their collective publication volume demonstrates that decoding the intricate relationship between abiotic stress and alkaloid metabolism has become a highly prioritized, resource-intensive global scientific endeavor bridging advanced molecular biology with sustainable agricultural practices.

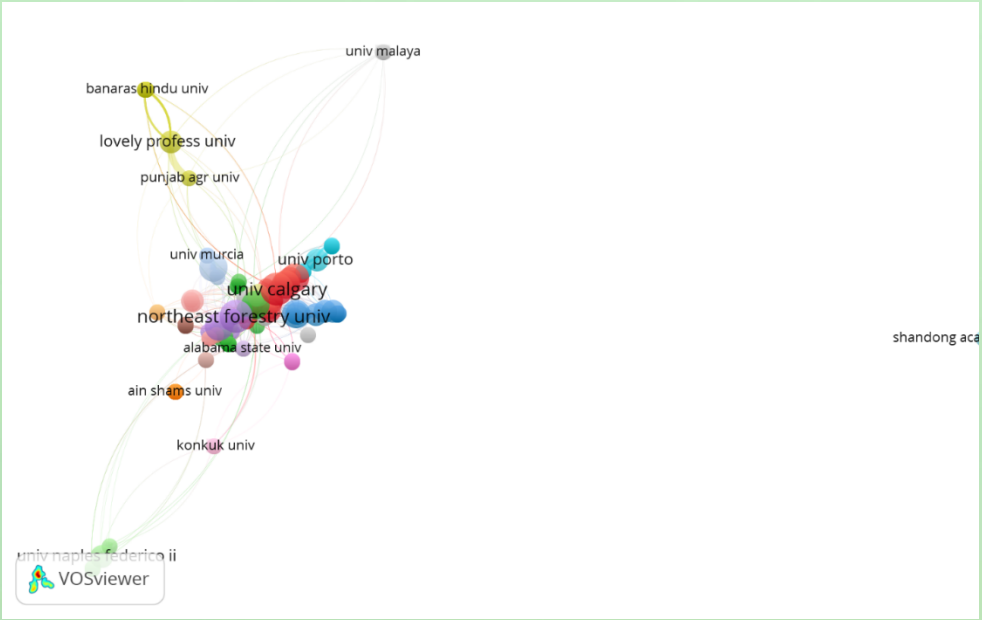



Figure 15. Research Institutes working on Alkaloid Metabolites on Paper bases.

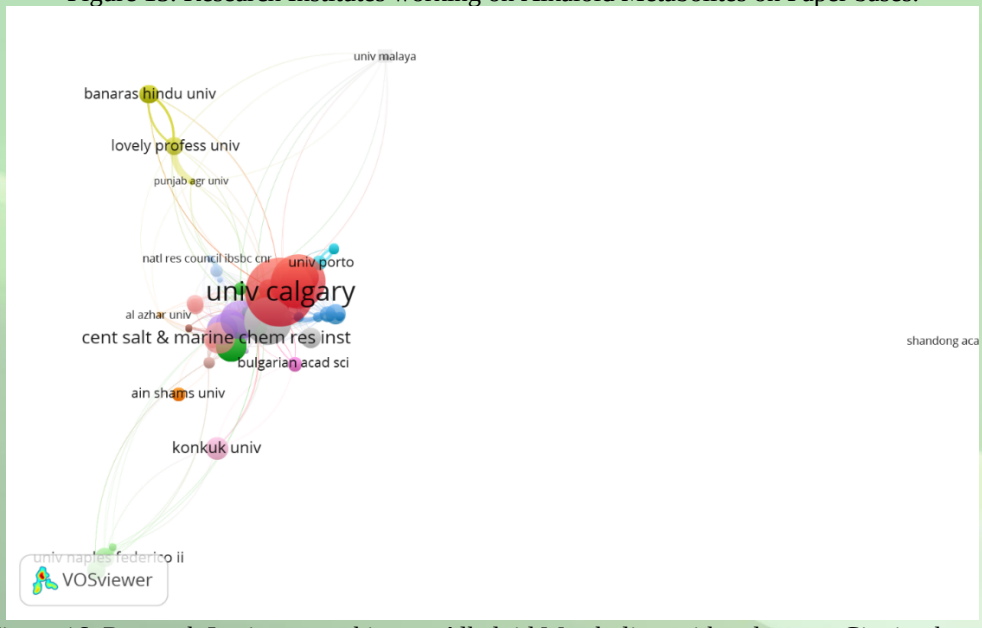



Figure 16. Research Institutes working on Alkaloid Metabolites with at least on Citation bases.

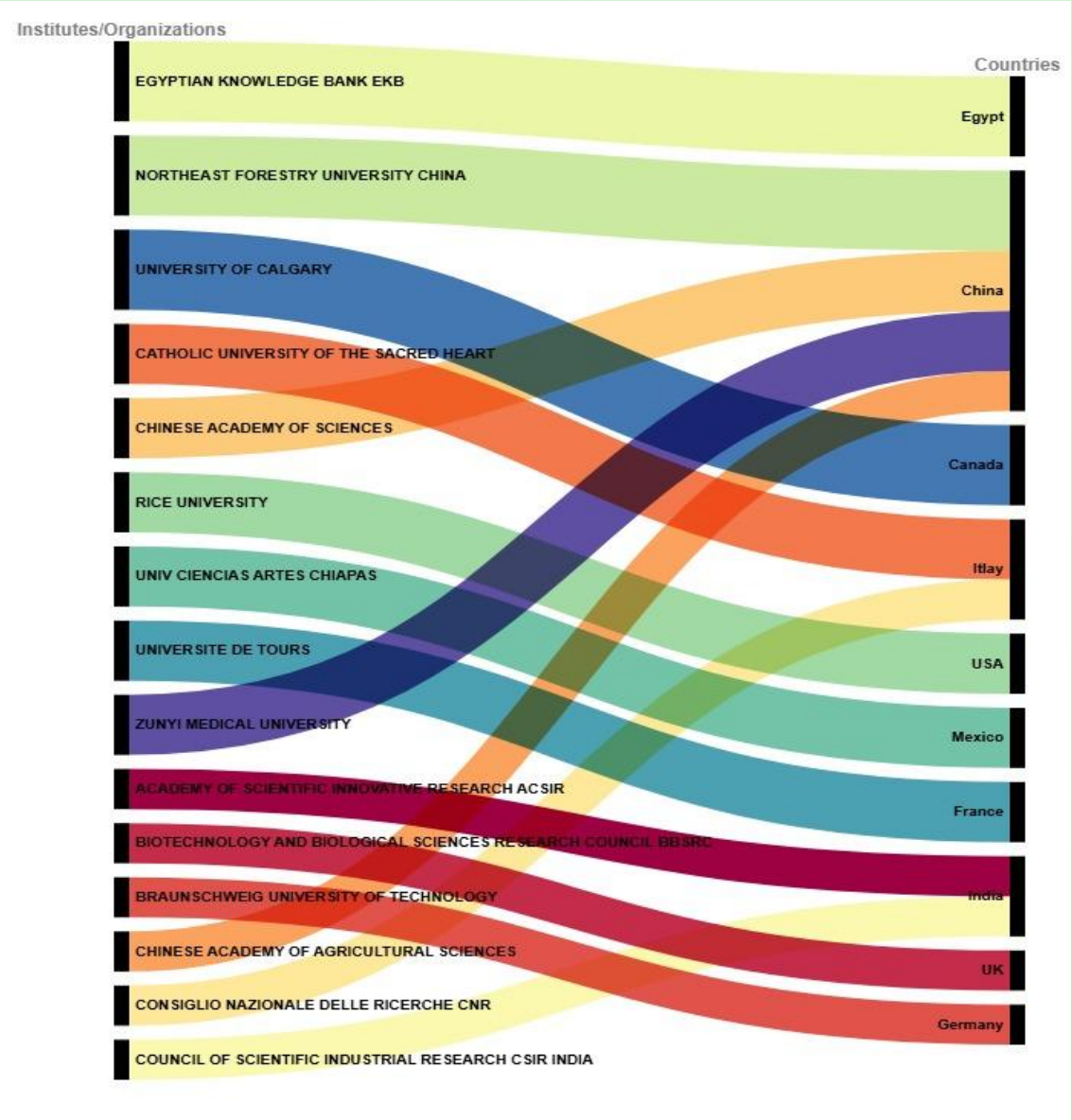



Figure 17. Top Research Institutes working on this topic.

The scientific domain deeply focused on plant specialized metabolism, benzylisoquinoline alkaloid biochemistry and synthetic biology is anchored by the University of Calgary, which stands as the absolute apex contributor with 23 published papers. *China Agricultural University* leads the intermediate tier with 6 papers, followed closely by a dense cluster consisting of the *Banaras Hindu University*, the *Chinese Academy of Agricultural Sciences* and *Southwest University*, each contributing 5 papers. Rounding out the baseline of the top 10 network are the *University of Porto*, *Università Cattolica del Sacro Cuore*, the *University of Natural Resources and Life Sciences Vienna* and *Purdue University* with 4 papers apiece, terminating at the *University of Delhi* with 3 papers. This distribution emphasizes a healthy research pipeline where a highly specialized primary nucleus drives the core bibliometric weight, in Figure 17. Supported, globally by Asian, European and American institutions expanding the field's peripheral scope.

***All keywords co-occurrence analysis***

The network of all-keyword co-occurrences in a specific botanical or plant biochemistry research domain, highlighting thematic clusters, research intensity and chronological evolution. Image 6 (the network visualization) reveals distinct color-coded clusters that represent specialized sub-fields of study. Central to this network are heavily weighted nodes such as biosynthesis, flavonoids, and abiotic stress, which exhibit the highest density of interconnections, underscoring their roles as the foundational pillars of this research. These core terms link outwards to specific physiological responses, such as water-stress, plant defense, and herbivory, as well as model organisms like arabidopsis-thaliana. Figure 18 further emphasizes this structural focus, showing a prominent center concentrated around the biosynthesis and flavonoids cluster, indicating a substantial volume of publications and intense scholarly focus in this core area. This chronological view reveals that earlier research focused on

foundational topics like *periwinkle*, *tropane alkaloids* and *plant-cell cultures*. In contrast, more recent research frontiers have shifted prominently toward abiotic stress management, specific enzyme characterization and specialized pathways like *caffeine biosynthetic-pathway*. This is illustrating a dynamic research transition from basic secondary metabolite identification to climate-resilient crop stress responses, in Figure 18 and 19.

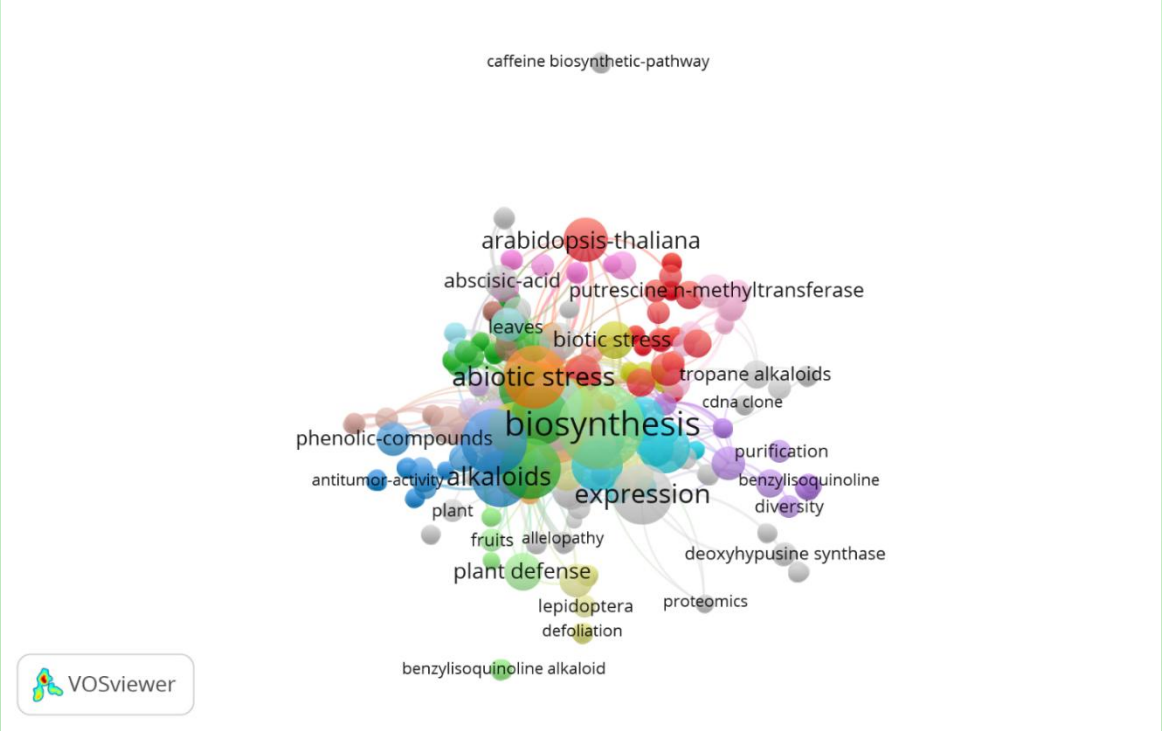



Figure 18. Co-occurrence network visualization diagram of the most frequently occurring keywords in full counting, pertaining to the effect of abiotic stress conditions on alkaloid metabolism in plants. Clusters of related terms are represented by nodes that share the same color.

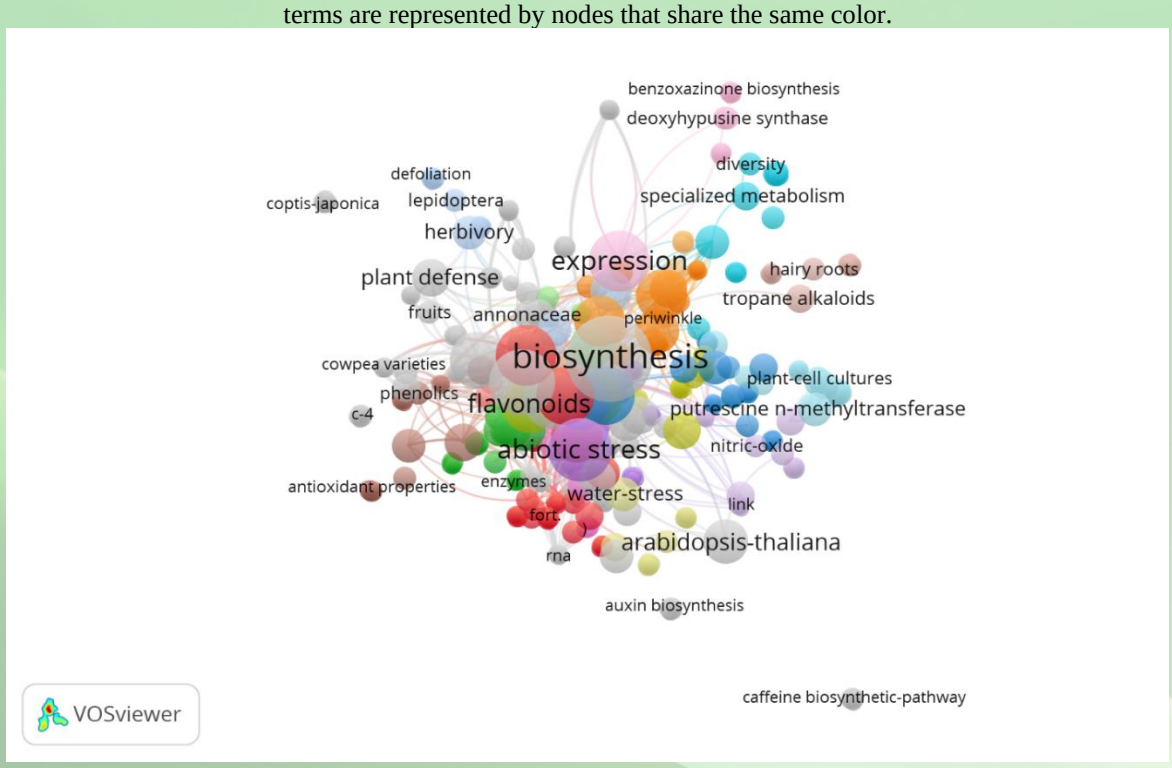



Figure 16. Co-occurrence network visualization diagram of the most frequently occurring keywords in binary counting pertaining to effect of abiotic stress conditions on alkaloid metabolism in plants, represented by clusters that share the same color.

## Discussion

The findings of this study demonstrate that abiotic stresses profoundly influence alkaloid metabolism through complex physiological, biochemical and molecular mechanisms. Drought, salinity, temperature extremes, and UV radiation consistently emerged as major environmental drivers capable of altering alkaloid biosynthesis and accumulation across diverse medicinal and crop species. These observations support previous reports indicating that environmental stress redirects carbon and nitrogen resources from primary growth processes toward the production of defensive secondary metabolites (Akula & Ravishankar, 2011; Yeshi et al., 2022). The enhanced accumulation of alkaloids under moderate stress conditions aligns with the Carbon/Nutrient Balance Hypothesis and the Growth-Differentiation Balance Hypothesis, which predict increased investment in defense-related compounds when growth becomes constrained by environmental limitations (Zhang, 2022). The reviewed literature further highlights the central role of stress-responsive signaling pathways involving jasmonic acid (JA), abscisic acid (ABA) and reactive oxygen species (ROS) in regulating alkaloid biosynthesis. Key biosynthetic enzymes, including putrescine N-methyltransferase (PMT), tryptophan decarboxylase (TDC) and strictosidine synthase (STR), were frequently upregulated under drought and salinity stress, resulting in enhanced production of nicotine, tropane alkaloids and terpenoid indole alkaloids (Guedes et al., 2024; Jeyasri et al., 2023). These metabolites contribute not only to plant defense but also to antioxidant protection, osmotic adjustment and nitrogen storage, thereby enhancing stress resilience (Divekar et al., 2022; Haghpanah et al., 2024).

The bibliometric analysis revealed a substantial increase in research output over the last two decades, reflecting growing scientific interest in understanding stress-induced metabolic reprogramming. China and the United States were identified as major contributors. While, Plant Sciences and Biochemistry & Molecular Biology represented the dominant research areas. Emerging technologies such as transcriptomics, metabolomics, CRISPR/Cas9 genome editing and artificial intelligence-assisted metabolic prediction are accelerating discoveries in alkaloid research and facilitating the development of climate-smart medicinal crops (Huang et al., 2024; Khalid et al., 2026). Future studies should focus on integrating multi-omics datasets with field-scale environmental monitoring to improve the predictability and stability of alkaloid production under changing climatic conditions.

## Conclusion

Abiotic stress conditions, including drought, salinity, temperature extremes, and ultraviolet radiation, play a pivotal role in regulating alkaloid metabolism in plants. These environmental factors trigger complex physiological and molecular responses that reprogram carbon and nitrogen allocation, resulting in significant alterations in alkaloid biosynthesis and accumulation. Alkaloids not only function as defensive compounds but also contribute to stress tolerance through antioxidant activity, osmotic adjustment, and nitrogen storage. The evidence synthesized in this study highlights the critical involvement of phytohormones, transcription factors and stress-responsive genes in mediating these adaptive responses. Bibliometric analysis revealed a rapidly expanding research landscape, with increasing emphasis on multi-omics technologies, metabolic engineering, CRISPR/Cas9-based approaches and climate-smart crop development. Future research should focus on integrating genomics, metabolomics and environmental monitoring to better predict stress-induced metabolic changes and optimize alkaloid production. Such advances will support the development of resilient medicinal plants and sustainable phytopharmaceutical production under changing climatic conditions.

## Data Availability Statement

All the data sources can be provided on demands.

## Ethics Statement

The authors have nothing to report.

## Consent

The authors have nothing to report.

## Author Contributions

Muhammad Khizar Hayat and Muhammed Said Yolcu did a significant contribution to this work. In the conception, study design, execution and interpretation. Both of these authors took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published. Both of these authors are agreed on the conference to which the article has been submitted and agree to be accountable for all aspects of the work.

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# Technology in Dairy Cattle Farming: Automated Milking Systems, Internet of Things, and Artificial Intelligence

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## Abstract

Global milk production has reached 965.7 million tons as of 2023. Dairy farming is dealing with multiple pressures at the same time. These primarily include labor shortages, tightening animal welfare regulations, and environmental targets. In the table of identified goals, advanced technologies such as automatic milking systems (AMS), Internet of Things (IoT) sensor networks, and artificial intelligence (AI) stand out in this field. AMS has been reported in many studies to increase milk yield per cow by 5–15% and to raise the milking frequency. The impact on milk quality, however, varies more with management conditions than with technology. While IoT enables real-time herd tracking, infrastructure inadequacies and data fragmentation in rural areas render much of this potential ineffective. AI is rapidly advancing in disease detection, production forecasting, and genomic breeding, but the shortcoming in this field does not seem to be capacity, but rather the inability of closed platforms to communicate with each other. The welfare aspect of all this is still under-researched for both animals and farmers. For the improvement of this sector, long-term sustainability assessments, open data standards, and innovations to make these technologies accessible to small producers are needed. If the sector remains under the dominance of large enterprises, while some of the industry's problems are solved, the deepening of inequality will continue.

***Keywords:*** *Automated milking systems, Artificial intelligence, Internet of Things, Dairy cattle, Precision livestock farming, Sustainability.*

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# Reproductive behavior in peafowl

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## Abstract

The reproductive behavior of peafowl is one of the most striking examples of sexual selection rituals, unlike those observed in other animals. The long, brightly colored, and patterned tail feathers of male peacocks are not only a physical characteristic but also a significant interaction tool in the selection of female mates. During the breeding season, male peacocks perform a spectacular courtship dance, fanning and vibrating their brightly colored and magnificent tail feathers to attract females. Female birds choose their mates based on this visual spectacle and vocalizations. This behavior affects not only the reproductive success of individuals but also the genetic structure and evolutionary development of the species. During this period, when females actively participate in mate selection, significant competition arises among males, and the process concludes with the selection of the highest-performing males. This study examines the fundamental characteristics of reproductive behavior in peafowl, courtship mechanisms, female selection, and the evolutionary significance of these behaviors.

***Keywords:*** *Peafowl, Breeding Behavior, Courtship, Tail Feather, Ocelli*

## Tavus kuşlarında üreme davranışı

### Özet

Tavus kuşlarının üreme davranışı, diğer hayvanlarda gözlenmeyen ve cinsel seçilim ritüelleri içerisinde en dikkat çekici örneklerden bir tanesidir. Erkek tavus kuşlarının sahip oldukları uzun, parlak renkli ve desenli kuyruk tüyleri fiziksel bir özellik olmanın yanı sıra, dişi bireylerin eş seçiminde rol oynayan önemli bir etkileşim aracıdır. Üreme mevsimi boyunca erkek tavus kuşları, dişileri etkilemek için parlak renkli ve muhteşem kuyruk tüylerini bir yelpaze gibi açarak ve titreştirerek gösterişli bir kur dansı sergiler. Dişi kuşlar bu görsel şölene ve seslere göre eşlerini seçerler. Bu davranış biçimi, sadece bireylerin üreme başarısını değil, türün genetik yapısını ve evrimsel gelişimini de etkiler. Dişi bireylerin eş seçiminde aktif rol oynadığı bu süre içerisinde, erkekler arasında önemli bir rekabet oluşur ve en yüksek performansa sahip erkeklerin seçilmesiyle süreç son bulur. Bu çalışma, tavus kuşlarında üreme davranışının temel özelliklerini, kur mekanizmalarını, dişi seçimini ve bu davranışların evrimsel önemini ele almaktadır.

***Anahtar kelimeler:*** *Tavus Kuşu, Üreme Davranışı, Kur Yapma, Kuyruk Tüyü, Tüy Deseni*

### Giriş

Tavus kuşları dünyadaki bütün kuşlar arasında görsel özellikleri bakımından en dikkat çekici kuşlar arasında yer almaktadır. Türe ait erkeklerin sahip olduğu uzun ve gösterişli renkli kuyruk tüyleri, sadece görsel bir özellik olmayıp, bireyin üreme başarısını doğrudan etkileyen önemli bir biyolojik yapıdır. Bu nedenle tavus kuşları, bilim insanları tarafından davranış ekolojisi ve evrimsel biyoloji alanlarında cinsel seçilim mekanizmalarını araştırmak için sıklıkla kullanılan türler arasında yer alır (Darwin, 1859; Jalme, 2005).

Bu çalışma, tavus kuşlarında üreme davranışlarının temel özelliklerini, kur mekanizmalarını ve dişilerde seçim süreçlerini inceleyerek, üreme davranışlarının evrimsel ve ekolojik kökenini açıklamayı hedeflemiştir.

### Üreme Davranışının Genel Özellikleri

Tavus kuşlarında üreme davranışı, görsel ve işitsel başta olmak üzere birçok duyusal sinyallerin bir arada kullanıldığı karmaşık bir davranış sürecidir. Bu durum yalnızca çiftleşme ile sınırlandırılamayacak kadar çok sayıda aşamaya sahip bir süreçtir. Çiftleşme öncesi gerçekleşen bir kur etkileşimi, dişiler tarafından yapılan eş seçimi ve erkekler arası rekabet davranışlarını da barındıran kompleks bir yapıya sahiptir. Özellikle erkeklerin sergilediği görsel şölen ve dişilerin bu gösterilere verdiği davranışsal tepkiler, sistemin temel taşları olarak kabul edilmektedir (Jalme, 2005; Petrie ve ark., 1991).

Üreme döneminde erkek tavus kuşları sabahın erken saatlerinden başlayarak gün boyu yüksek ve tiz bir sesle öterler. Her iki cinsiyette bu dönemde ötme sesini bir iletişim aracı olarak kullanır. Dişiler potansiyel eşlerini bulmak, erkekler ise dişi ziyaretçilerini çekmek için öterler (Hernowo ve ark., 2011; Dakin ve Montgomerie, 1014a).

Genellikle Nisan-Ağustos ayları arasında erkek tavus kuşları, dişilerin dikkatini çekebilmek adına "lek" sistemi olarak tanımlanan toplu gösterilerin yapıldığı belirli alanlarda bir araya gelerek muhteşem gösteriler sergilerler. Bu alanlar birden fazla erkeğin aynı anda gösteri yapmasına imkan sağladığı için önemli bir rekabet ortamı oluşturarak erkekler arasındaki kalite farklılıklarını ön plana çıkarır. Dişiler bu alanlarda erkekleri gözlemleyerek karşılaştırmalı değerlendirmeler yapabilmekte ve kuyruk uzunluğuna ve göz beneği sayısına bakarak kolaylıkla eş seçimini yapabilmektedir (Dakin ve Montgomerie, 2011; Petrie ve ark., 1991).

Erkeklerde kur davranışının en belirgin göstergesi, üst kuyruk tüylerinin geniş bir yelpaze gibi açılmasıdır (Şekil 1). Bu tüyler üzerinde "ocelli" olarak isimlendirilen göz benzeri oluşumlar bulunur (Şekil 2).



Şekil 1. Tavus kuşlarında kuyruk görüntüsü



Şekil 2. Tavus kuşlarında tüylerin görüntüsü

Kuyruk uzunluğu ve bu oluşumların sayısı dişilerin görsel olarak etkileyen en önemli sinyaller arasındadır (Manning, 1987). Gösteri esnasında erkekler tüylerini titreterek açar. Dişi, gösteri yapan erkeğin yanına veya arkasına doğru yaklaştığında, erkek belirli aralıklarla birincil kanatlarını çırpar, bu arka taraf gösterisidir. Dişi erkeğin önüne doğru hareket ettiğinde ise erkek doğrudan ona döner ve parıldayan tüylerini periyodik olarak çırparak çıngırdama benzeri bir ses çıkarır, bu ön taraf gösterisidir. Ardından erkek dişiden uzaklaşır (Petrie ve ark., 1992). Bu sinyaller hem görsel hem işitsel kombine bir iletişim sistemi oluşturur ve dişilerin dikkatini çeker, aynı zamanda dikkatlerinin bir süre erkeklerde kalmasına da yardımcı olur (Yorzinski ve ark., 2013; Petrie ve ark., 1992).

Dişi tavus kuşları üreme sürecinde pasif bir izleyici değildir, aksine aktif bir seçici olarak rol oynar. Toplanma alanındaki farklı erkeklerin görsel gösterilerini izler, karşılaştırır ve seçimini yapmak için birden fazla gösteriyi gözlemler. Sonunda bu gösterileri değerlendirir, bir veya birkaç farklı erkekle çiftleşir (Petrie ve ark., 1992). Bu durum, dişiler tarafından cinsel seçilim isteği doğrultusunda baskıyı artırır ve erkekler arasında önemli bir performans rekabeti oluşturur. Sonuçta üreme başarısı fiziksel özelliklere bağlı olmaktan çıkar, aynı zamanda davranışsal performansa da bağlı hale gelir (Jalme, 2005; Petrie ve ark., 1991).

**Üreme Davranışını Etkileyen Faktörler**

Tavus kuşlarındaki üreme davranışı, bireylerin genetik özelliklerinin yanı sıra bir dizi farklı olayı da içeren çok boyutlu bir süreçtir. Üreme davranışının ortaya çıkışı ve başarısı; çevre koşulları, besin kaynakları, sosyal yapı ve bireyin fizyolojik durumu gibi birçok faktör tarafından şekillendirilmektedir. Bundan dolayı üreme davranışı, hem biyolojik hem de ekolojik faktörlerin birlikte belirlediği dinamik bir sistem olarak kabul edilmektedir (Dakin ve Montgomerie, 2014a; Hewig ve ark., 2008; Loyau ve ark., 2007; Loyau ve ark., 2005)

Bu süreçte dişilerin tercihini etkileyen temel faktörler arasında erkeklerin kuyruklarının kalitesi, gösterişi, genişliği, uzunluğu, tüy rengi (mavi-yeşil göz beneği rengi), benek sayısı ve yoğunluğu, tüy simetrisi, renk yoğunluğu, gösteri süresi ve genel fiziksel kondisyonu sayılabilir. Bu durum, tür içinde sürekli bir seçilme baskısı oluşturarak daha kaliteli erkeklerin üreme başarısını artırır. Koşullara bağlı eş seçimi, cinsel seçilimin gücünü ve yönünü etkilediği düşünülmektedir (Dahlin, 2018; Dakin ve Montgomerie, 2014a; Dakin ve Montgomerie, 2014b; Dakin ve Montgomerie, 2013; Yorzinski ve ark., 2013; Dakin ve Montgomerie, 2011; Hewig ve ark., 2008; Takahashi ve ark., 2008; Loyau ve ark., 2005; Yasmin ve Yahya, 1996; Petrie ve Halliday, 1994; Patrie ve Williams, 1993; Petrie ve ark., 1991; Manning, 1987).

Kuyruk uzunluğu ve tarsus uzunluğu, lek içerisindeki bölge oluşturma aşamasında oldukça belirleyici rol oynar (Loyau ve ark., 2005). Kuyruk uzunluğu erkekler olgunlaşıncaya kadar yaşla birlikte arttığından, dişiler kuyruk genişliğini dikkate alarak erkeklerin yaşını belirleyebilir ve yalnızca olgun erkeklerle çiftleşme gerçekleştirebilir (Yasmin ve Yahya, 1996; Petrie, 1993; Manning, 1989). Buda dişilerin daha güçlü ve olgun erkekleri tercih ettiğini göstermektedir.

Diğer taraftan çevresel koşullar da üreme davranışını doğrudan etkileyen önemli unsurlardır. Besin kaynaklarının yakınlığı ve bolluğu, yaşam alanının kalitesi ve stres düzeyi erkeğin gelişimini ve enerjisini değiştireceğinden performansını etkiler. Bu durum hem erkeklerin gösteri performansını (gösteri süresi, ritmi ve tekrarlanabilirlik) hem de dişilerin seçim davranışını değiştirebilir. (Loyau ve ark., 2007; Cotton ve ark., 2006; Jennions ve Petrie 1997; Tomlinson ve O'Donald 1996). Eş seçimi ve değerlendirmesindeki bu koşullara bağlı esnekliğin, cinsel seçilimin gücünü ve yönünü etkilediği düşünülmektedir. Bu sebeplerden dolayı tavus kuşlarında üreme davranışı, sadece genetik değil aynı zamanda ekolojik bir süreç olarak değerlendirilmelidir.

Yapılan gözlemler ve bilimsel çalışmalar, erkek tavus kuşlarının gösteri performansı ve kalitesinin kuyruk uzunluğu ve büyüklüğü ile ilişkilendirmiştir. Dişilerin seçim tercihlerinin ve çiftleşme başarısının da bu özelliklerle doğrudan ilişkili olduğu ortaya konmuştur. Cinsel seçilim teorisine göre dişilerin bu özelliği tercih etmesi nedeniyle erkekler hayatta kalma açısından dezavantajlı olsa bile üreme başarısını artırdığı için evrimsel süreçte bu özelliklerini koruduğu gözlenmiştir (Darwin 1859).

**Sonuç**

Tavus kuşlarında üreme davranışı, çevre koşullarına bağlı olarak şekillenen, görsel ve işitsel sinyallerin yoğun bir şekilde kullanıldığı komplike bir süreçtir. Erkeklerin yaptığı kuyruk gösterileri ile dişilerin aktif eş seçimi temeline dayanan bir davranış biçimidir. Dünyada cinsel seçilim davranışlarının doğadaki en gösterişli örneklerinden biri olarak değerlendirilmektedir.

Erkek tavus kuşlarının gösteri kalitesi ile dişilerin eş seçim tercihleri arasında önemli bir ilişki olduğu gözlenmiştir. Bu durum, yalnızca bireylerin üreme başarısını değil, türün genetik çeşitliliğini ve evrimsel uyumunu da etkilemektedir. Bu nedenle tavus kuşlarında üreme davranışı, sadece biyolojik bir olay olmayıp; ekolojik, davranışsal ve evrimsel boyutları olan komplike bir sistemdir.

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# Effect Of Healthcare Access on The Productivity of Rural Farming Households in Yewa South Local Government, Ogun State, Nigeria

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## Abstract

In rural farming communities, poor health and inadequate access to quality healthcare services remain major constraints to sustained agricultural productivity. This study investigates the effect of healthcare access on the productivity of rural farmers in Yewa South Local Government Area, Ogun State, Nigeria. The study adopts a multistage sampling technique to select 120 respondents, with data collected through structured questionnaires. Both descriptive statistics and regression analysis were employed to analyze the data. Findings reveal that average age, education, household size, farm size and experience were 50years, 7 years, 4, 3ha and 17years respectively. Access to healthcare services has a statistically significant impact on farm productivity. Specifically, variables such as availability of a health facility ($p<0.010$) distance to the health facility ($p<0.005$) travel time to reach the health facility ($p<0.010$), age of the farmer ($p<0.001$), household size ($p<0.005$) and farming experience ($p<0.001$) were found to influence productivity levels. Farmers with good access to healthcare indicated fewer sick days, higher work efficiency, and better farm output, while those with limited access experienced more frequent illness-related disruptions. The study concludes that agricultural productivity among rural farming households in Ogun State is profoundly shaped by an intricate interplay of internal socioeconomic capacity and external healthcare access constraints. The study recommends that government and relevant stakeholders must prioritize improving the structural and functional quality of public rural health facilities by recruiting adequate personnel, procuring modern equipment, and ensuring a stable supply of essential medications.

***Keywords:*** *Healthcare access, rural farming, productivity, agricultural output, regression*

## Introduction

In determining the health status and consequently the productivity of farmers, access to healthcare services plays a critical role, Akinyele & Omonona (2020). Health is a fundamental determinant of labor productivity. Farmers in good health can work more efficiently, manage their farms better, and adopt improved agricultural practices. Conversely, poor health can lead to reduced work capacity, increased absenteeism, and higher medical expenses, all of which negatively impact agricultural productivity, Titus et al., (2015); Apata et al., (2026). In rural Nigeria, access to quality healthcare services is often limited due to factors such as inadequate healthcare facilities, shortage of medical personnel, high costs of services, and long distances to healthcare centers (Aregbeshola & Khan, 2018). These barriers lead to untreated illnesses and a decline in the productive capacity of farmers. The interplay between health and agricultural productivity underscores the importance of understanding how healthcare access affect the productivity of these households. Improving healthcare access could lead to better health outcomes, which in turn may enhance agricultural productivity and contribute to poverty reduction in rural communities (Ajani & Ugwu, 2021).

Recent discussions emphasize that while expanding health insurance is essential, it is insufficient to generate substantial improvements in health outcomes or meaningful poverty reduction without concurrent investments in healthcare infrastructure and workforce development (Obikeze et al., 2023). A phased strategy that prioritizes strengthening healthcare facilities and increasing the availability of healthcare professionals in rural areas is recommended (World Bank, 2022). However, the productivity of farming in rural areas is often hindered by various challenges, among which health-related issues are paramount.

Agricultural productivity is a key driver of economic growth in Nigeria, particularly in rural areas where farming is the main livelihood. However, the productivity of rural farming is often constrained by poor health outcomes resulting from inadequate healthcare access (Ajani & Ugwu, 2021). Health-related challenges, such as

high disease prevalence, malnutrition, and lack of preventive care, significantly reduce labor efficiency and output among farmers. The inability of farmers to access timely and quality healthcare services leads to frequent absenteeism, lower work capacity, and increased medical expenditures, which ultimately affects their agricultural productivity and income levels, Lawrence, (2024). Despite the recognized link between health and productivity, many rural farmers in Nigeria continue to face barriers to healthcare access. Studies indicate that a large proportion of farmers do not utilize healthcare services due to issues such as high medical costs, inadequate healthcare facilities, shortage of medical personnel, poor transportation infrastructure, and long distances to health centers (Fayehun et al., 2022). In many cases, farmers resort to self-medication or traditional medicine, which may not effectively address their health conditions, further worsening their productivity and quality of life.

In Yewa South Local Government, Ogun State, the problem of healthcare inaccessibility remains a pressing issue, yet there is limited empirical evidence on how it directly affects the productivity of rural farming households. While previous studies (Titus et al., 2015; Ajani & Ugwu, 2021; Fayehun et al., 2022; Obikeze et al., 2023; Lawrence, 2024) have focused on general rural healthcare challenges, there is a lack of specific research examining the relationship between healthcare utilization and agricultural productivity in this region. Understanding this relationship is essential for formulating policies that enhance healthcare access and, in turn, improve the livelihoods of farming communities. The main objective of this study is to examine the effect of healthcare access on the productivity of rural farming households in Yewa South Local Government, Ogun State. Specifically, describe the socioeconomic characteristics of rural farming households in the study area, assess the level of healthcare access among rural farming households and analyze the effect of healthcare utilization on agricultural productivity among rural and farmers.

**Theoretical Framework**

Grossman's Health Production Function (Grossman, 1972) conceptualizes health as a form of capital that individuals invest in and maintain over time to improve their well-being and productivity. According to this theory, individuals allocate their resources such as time and income toward producing and maintaining their health. Investments in health, such as accessing healthcare services and adopting preventive practices, enhance an individual's ability to work effectively, increasing labor productivity. For rural farming households, health is particularly important as their work involves physical labor. Poor health reduces their capacity to engage in farming activities, leading to lower yields and income. In this context, the availability of healthcare services become critical for maintaining the health capital of farmers and ensuring optimal productivity. Grossman's model highlights the role of external factors such as healthcare infrastructure, affordability, and health knowledge in determining health outcomes and, by extension, agricultural productivity (Smith & Goldman, 2020).

The Human Capital Theory, developed by Becker (1964), emphasizes the value of investing in human capital, such as education, training, and health, to improve productivity and economic outcomes. In this framework, health is considered a critical component of human capital. Good health enables individuals to work more efficiently, adapt to new technologies, and increase their earning potential. In the context of rural farming households, healthcare access are forms of human capital investment. Farmers with access to quality healthcare are less likely to suffer from chronic illnesses, absenteeism, or reduced work capacity, enabling them to achieve higher productivity levels. The Human Capital Theory also underscores the importance of education and income in facilitating healthcare access, as these factors influence a farmer's ability to recognize the value of health services and afford them, Ajani & Ugwu, (2021).

**Empirical Framework**

Several studies have established a strong link between healthcare utilization and agricultural productivity. Poor health often results in reduced workdays, diminished capacity to manage farms, and lower crop yields. These included Obikeze et al., (2023) who found that households with better access to healthcare services recorded a 20% higher agricultural output compared to those without access. The study emphasized that frequent illnesses among household members not only decreased labor availability but also led to financial strain due to increased medical expenses. Moreover, findings from Ethiopia revealed that farmers who utilized preventive healthcare services, such as vaccinations and regular health check-ups, had better physical endurance and were more likely to adopt modern farming techniques, leading to increased productivity (Gebremariam et al., 2022).

Healthcare access among rural farmers in Nigeria according to Aregbeshola & Khan, (2018) has been a subject of interest in recent years due to its direct influence on agricultural productivity and rural livelihoods. Ajani & Ugwu (2021) investigated the relationship between healthcare access and productivity among farming households in southeastern Nigeria. Their findings revealed that poor access to healthcare significantly reduced labor efficiency, with many farmers resorting to self-medication due to the unavailability of nearby health facilities. Similarly, Titus et al., (2015) found that healthcare costs were a major determinant of service utilization among rural farmers in Ogun State, with only 26% of farming households utilizing modern healthcare services. In

addition, similar challenges have been reported across Sub-Saharan Africa. Studies in Ghana and Kenya by Agyei-Baffour, Oppong & Banura (2019) for example, emphasize the role of health insurance schemes in improving healthcare access for rural populations. In Ghana, the National Health Insurance Scheme (NHIS) was found to increase the likelihood of healthcare utilization by 48%, with significant improvements in health outcomes among farming households. In Kenya, mobile health initiatives, such as SMS-based health alerts and community health workers equipped with digital tools, have helped bridge the gap in healthcare access for rural farmers. These interventions were associated with reduced absenteeism and improved productivity among farmers (Were et al., 2020).

## Methodology

The study was carried out at Yewa South Local Government Area (LGA) is located in Ogun State, Southwestern Nigeria. It serves as the administrative capital of Ogun State, with Abeokuta as its central town. The LGA is bordered by Abeokuta North LGA to the North, Odeda LGA to the East, and Ewekoro LGA to the South. It lies between latitude 7°09′N and longitude 3°20′E, covering an estimated land area of 71 square kilometers. Yewa South experiences a tropical climate, characterized by distinct wet and dry seasons. The rainy season occurs from April to October, while the dry season spans November to March. The region receives an annual rainfall of 1200–1600 mm, with average temperatures ranging from 25°C to 30°C. The topography is dominated by rocky outcrops and well-drained loamy soils, making it suitable for various agricultural activities, including crop cultivation and livestock rearing. The LGA is predominantly agrarian, with a significant proportion of the population engaged in farming as their primary occupation. Major crops cultivated include cassava, maize, yam, and vegetables, while some residents rear livestock such as poultry and goats. The presence of fertile soils and a favorable climate supports agricultural productivity in the area. In addition to farming, residents are involved in small-scale trading, craftsmanship, and civil service, reflecting a diverse economic base, Aminu & Asogba (2020).




Figure 1: Map of Ogun State, Nigeria, Source: Aboaba et al., (2023)

### *Sampling Technique*

The study used a multistage sampling technique to select respondents for this study, ensuring a representative sample of rural farming households in Yewa South (LGA). The study adopts a multistage sampling technique to select 120 respondents. The first stage of the sampling process involved the purposive selection of Yewa South LGA because of the LGA is driven primarily by agriculture. At the second stage, 50% of the ten (10) wards (Ilaro I, Ilaro II, Ilaro III, Iwoye, Idogo, Owode I, Owode II, Ilobi/Erinja, Oke-Odan and Ajilete) were selected. Third stage involved the random selection of two communities each from the five wards earlier selected. The final stage involve the random and proportionate selection of twelve (12) farmers from each community selected making a total of 120 respondent. To ensure fair representation, the sample size was proportionally distributed among the selected communities based on the population of farming households in each area.

### *Model Specification*

The Cobb-Douglas production function is used to evaluate the effect of healthcare utilization on agricultural productivity. The functional form is specified as:

$Y = AK^{\alpha}L^{\beta}H^{\gamma}e^{\varepsilon}$

Where:

$Y$: Agricultural productivity (measured as total farm output in kilograms).
$A$: Total factor productivity (constant).
$K$: Capital input (e.g., value of farming tools and inputs).
$L$: Labor input (measured in man-days of farm labor).
$H$: Healthcare utilization (frequency of visits to healthcare facilities or expenditure on healthcare).$\alpha$: Elasticities of productivity with respect to capital, labor, and healthcare utilization.
$e^{\varepsilon}$: Error term.

***Independent Variables in the Model***

The model considers the following independent variables: Age of Farmer (years) ($X_1$), Education Level ($X_2$), Income Level (Naira) ($X_3$), Household Size ($X_4$), Farm Size (hectares) ($X_5$), Access to Healthcare Facilities ($X_6$) – Dummy variable (1 = accessible, 0 = not accessible). Distance to Healthcare Facilities (km) ($X_7$), Cost of Healthcare (Naira) ($X_8$) – Average cost of healthcare services (Naira)

**Results and Discussion**

***Socioeconomic Characteristics of the Respondents***

This section presents and discusses the socio-economic characteristics of rural farming households in Ogun State. The study revealed that rural farming households in Ogun State are predominantly male-headed, with males accounting for the larger proportion of respondents, while most household heads were within their economically active age. This suggests that farming activities and major household decisions, including healthcare utilization, are largely controlled by men in the study area. The findings agree with the studies of Adewunmi & Lawal (2021) and Oladeji & Adebayo (2016) who reported that men play dominant roles in agricultural and household resource management in rural Nigeria. The study further showed that the respondents generally possessed low levels of formal education (average of 7 years in school), which may negatively affect their understanding of healthcare information and their ability to make informed production decisions. This finding corroborates the work of Imai, Gaiha & Thapa (2015); Apata et al., (2026), who emphasized that education improves health-seeking behaviour and agricultural productivity among rural farmers. Household size was moderate, indicating a balance between family labour supply and household consumption needs, which aligns with the findings of Imai, Gaiha & Thapa (2015) that household size significantly affects healthcare utilization and farm productivity.

The study also revealed a high level of membership in farmers' associations among respondents, indicating strong social organization and collaboration within the farming communities. Membership in these associations provided several benefits such as access to farmland, technical assistance, subsidized inputs, and loan facilities, all of which contribute positively to agricultural productivity and livelihood improvement. This finding is consistent with the studies of Awoyemi, Obayelu, & Opaluwa (2011) who reported that cooperative membership enhances access to credit facilities, healthcare services, and productive resources. This finding agrees with Ugal & Ukwayi (2014), who observed that small farm holdings are common among rural farmers in southwestern Nigeria, and Ajayi & Akinrinola (2017), who noted that farming experience positively influences productivity and innovation adoption.

The findings further showed that most respondents had access to healthcare facilities within their communities, although a considerable proportion still lacked nearby healthcare services. The availability of healthcare facilities suggests improved opportunities for disease prevention and treatment, which are important for sustaining labour productivity among rural farming households. This result supports the findings of Chukwuma, Wosu & Mbachu (2015), who noted that inadequate healthcare access can increase morbidity and reduce labour efficiency among rural dwellers. The study also established that most healthcare facilities available in the study area were government-owned, indicating the dominant role of public healthcare infrastructure in rural healthcare delivery. This finding aligns with Aboaba et al., (2023), who reported that government health facilities are often more accessible and affordable for low-income rural households. However, the limited presence of private healthcare facilities may indicate inadequate private sector investment in rural healthcare delivery, as noted by Ugal & Ukwayi (2014). Overall, the study suggests that improved healthcare access is essential for enhancing the productivity and welfare of rural farming households.

***Critical Issues on Healthcare by Rural Farming Households in the Study Area***

The study identified several critical healthcare challenges affecting rural farming households and their productivity in the study area. The findings revealed that although a majority of respondents had relatively fair access to healthcare facilities, a considerable proportion still traveled long distances and spent extended periods reaching healthcare centers.

Table 1: Socioeconomic Characteristics of the Respondents (n = 120)

| *Sex* | *F* | *%* | *Membership* | *F* | *%* |
|---|---|---|---|---|---|
| Male | 83 | 69.17 | Yes | 97 | 80.87 |
| Female | 37 | 30.83 | No | 23 | 19.13 |
| Age | | | Benefits Derived | | |
| 21–40 | 18 | 15.00 | Access to farmland | 46 | 38.33 |
| 41–60 | 93 | 77.50 | Provision of technical assistance | 23 | 19.17 |
| 61-80 | 9 | 7.50 | Loan facility | 19 | 15.83 |
| Mean | 50 | | Subsidized inputs | 17 | 14.17 |
| S. D. | 8.58 | | Information on input source from farm activities | 6 | 5.00 |
| Education | | | Selling farm output at high price | 6 | 5.00 |
| No Formal | 44 | 36.67 | More improved agronomic practices | 3 | 2.5 |
| Primary | 13 | 10.83 | Farm Experience | | |
| Secondary | 30 | 25.00 | <5 | 4 | 3.33 |
| Tertiary | 33 | 27.50 | 6-10 | 10 | 8.33 |
| Mean | 7 | | 11-15 | 37 | 30.33 |
| Household size | | | 16-20 | 52 | 43.33 |
| <= 5 | 116 | 96.67 | >20 | 17 | 14.17 |
| 6 – 10 | 4 | 3.33 | Mean | 17 | |
| Mean | 4 | | S.D | 4.89 | |
| S. D. | 1.05 | | Availability of Healthcare Services | | |
| Farm Size | | | Yes | 85 | 70.34 |
| ≤5 | 112 | 93.33 | No | 35 | 29.66 |
| 6–10 | 7 | 5.83 | Type of Facility | | |
| 11–15 | 24 | 20.00 | Government owned | 90 | 75 |
| 16–20 | 1 | 0.83 | Private | 20 | 16.67 |
| Mean | 3 | | Mission/Religious | 10 | 8.33 |

Source: Field Survey, 2025; F = Frequency; % = Percentage

Most respondents relied on motorcycles as their primary means of transportation, reflecting the poor transportation infrastructure commonly found in rural communities. The study further showed that while over half of the respondents only occasionally required medical attention, some households frequently experienced health-related problems associated with farming activities and rural environmental conditions. These findings support Obikeze, Adepoju & Mohammed (2023), who emphasized that inadequate healthcare infrastructure negatively affects rural productivity and welfare. Similarly, Ogunyemi, Olagunju & Ojo (2021) noted that long travel times discourage healthcare utilization among low-income rural households, while Were, Sabai & Mutiso (2020) advocated for improved rural road networks and mobile healthcare outreach services to enhance healthcare access. The findings also align with Okafor & Anozie (2017). and Ibrahim & Oloniniyi (2019), who linked frequent health problems among rural farmers to occupational and environmental risks associated with agricultural activities.

The study further revealed that although the majority of respondents utilized formal healthcare facilities during illness episodes, a substantial proportion still depended on alternative healthcare sources such as traditional healers, patent medicine stores, and self-medication. This indicates the persistence of affordability, accessibility, and trust-related barriers within the formal healthcare system. The findings agree with Hagen et al., (2022) who observed that healthcare-seeking behaviour in rural Nigeria is strongly influenced by cost, quality of care, and cultural beliefs. The widespread use of patent medicine vendors and traditional healers also supports the findings of Onyeneho & Igwe (2015) who highlighted the important role of informal healthcare providers in rural communities. In addition, respondents generally rated the quality of healthcare services as fair to good, although concerns regarding inadequate equipment, poor staffing, and limited emergency services remained evident. This finding corroborates the studies of Combary & Traore (2020) who stressed the need for improved funding, staffing, and comprehensive healthcare delivery in rural Nigeria.

***Challenges in accessing Healthcare Facilities by the Rural Households***

The study identified several challenges limiting rural households' access to healthcare facilities in the study area. The major challenge reported by respondents was lack of money, accounting for 38.33%, indicating that financial constraints remain the most significant barrier to healthcare utilization among rural farming households. This finding suggests that low income levels and high healthcare costs may discourage households from seeking timely medical attention, thereby negatively affecting their health status and productivity. Distance to healthcare facilities was also identified as a major constraint by 24.17% of the respondents, highlighting the problem of inadequate healthcare infrastructure and poor spatial distribution of health facilities in rural communities. In addition, issues such as long waiting times (12.50%), lack of trust in healthcare providers (15.00%), and lack of

time (10.00%) further reduced healthcare utilization among respondents. These findings are consistent with the studies of Ganiyu & Oladipo (2015) and Ogunyemi, Olagunju & Ojo (2021), who reported that poverty, distance, and poor service delivery are major barriers to healthcare access in rural Nigeria. Similarly, Imai, Gaiha & Thapa (2015) observed that lack of trust in healthcare providers and prolonged waiting periods discourage rural dwellers from utilizing formal healthcare services, thereby increasing dependence on informal healthcare options.

Table 2: Issues on Healthcare facilities by the Respondents (n =120)

| *Distance to facility* | *F* | *%* | *Health behavior* | *F* | *%* |
|---|---|---|---|---|---|
| <10 | 60 | 50 | Yes | 78 | 65 |
| 11-20 | 37 | 30.83 | No | 42 | 35 |
| 21-30 | 2 | 1.67 | Care source | | |
| >30 | 21 | 17.50 | Traditional healer | 23 | 19.17 |
| Mode of transport | | | Patent medicine store | 85 | 70.83 |
| Walk | 15 | 12.50 | Self-medication | 12 | 10.00 |
| Bicycle | 2 | 1.67 | Rate of quality healthcare | | |
| Motorcycle | 90 | 75.00 | Very good | 18 | 15.00 |
| Car/Taxi | 13 | 10.83 | Good | 38 | 31.67 |
| Travel time | | | Fair | 55 | 45.83 |
| <10 | 34 | 28.33 | Poor | 9 | 7.50 |
| 11-20 | 36 | 30.00 | Healthcare services | | |
| 21-30 | 16 | 13.33 | General consultation | 60 | 50.00 |
| 30 | 34 | 28.33 | Maternity care | 31 | 25.83 |
| Medical care (Frequency) | | | Child immunization | 15 | 12.50 |
| Never | 4 | 3.33 | Treatment of common diseases | 9 | 7.50 |
| Rarely | 25 | 20.83 | Emergency services | 5 | 4.17 |
| Sometimes | 70 | 58.33 | | | |
| Often | 20 | 16.67 | | | |
| Very often | 1 | 0.84 | | | |

Source: Field Survey, 2025; F = Frequency; % = Percentage

Table 3: Distribution of respondents by challenges in accessing Healthcare services

| | *Frequency* | *Percentage* |
|---|---|---|
| Lack of money | 46 | 38.33 |
| Distance to facility | 29 | 24.17 |
| Long waiting times | 15 | 12.50 |
| Lack of trust in healthcare providers | 18 | 15.00 |
| No time | 12 | 10.00 |
| Total | 120 | 100 |

Source: Field Survey, 2025

**Regression Results**

Out of the linear, quadratic, semi-log, and Cobb-Douglas functional forms, the Cobb-Douglas specification was selected as the best fit for the data in examining the effect of healthcare utilization on agricultural productivity among rural and farmers. In agricultural production economics, the Cobb-Douglas model is highly favored because its log-linear transformation allows the estimated coefficients to be directly interpreted as elasticities or constant percentage shifts. This form inherently respects the law of diminishing marginal returns and successfully captures the non-linear dynamics of rural production systems far better than rigid linear or flexible quadratic forms. The diagnostic statistics extracted from the analysis confirm that the estimated model possesses exceptional explanatory power and statistical reliability. The model yielded an overall F-statistic of 679.57, which is highly significant with a probability value of 0.0000. This completely rejects the null hypothesis that all explanatory variables simultaneously have no effect, confirming that the regressors collectively exert a highly significant influence on household productivity. Furthermore, the model achieved a Coefficient of Determination ($R^2$) of 98.86% and an Adjusted $R^2$ of 98.72%, indicating that over 98% of the total variation in the productivity of rural farming households is explained by the independent variables included in the model. A low Root Mean Squared Error (Root MSE) of 0.92465 further establishes the tight fit of the model, confirming that the standard deviation of the residuals is remarkably minimal.

The central focus of this empirical exercise lies in the healthcare access variables, which are captured through three indicators: availability of a health facility, distance to the facility, and travel time required to reach it. The availability of a health facility has a coefficient of -0.157, which is statistically significant at the 10% level (p =

0.072). The negative sign suggests an unexpected inverse relationship where the mere physical presence of a facility correlates with lower productivity. This phenomenon is often discussed in rural development literature; as Aminu & Asogba (2020) noted, the simple physical presence of an infrastructure does not equal functional quality. If local health clinics are plagued by severe understaffing, long wait times, or a chronic lack of essential medications, farming households may lose valuable labor time waiting for inadequate care, which ultimately detracts from their agricultural output.

Conversely, the distance to the health facility carries a positive coefficient of 0.016, which is statistically significant at the 10% level (p = 0.034). Similarly, the travel time to reach the health facility has a positive coefficient of 0.003 and is significant at the 10% level (p = 0.058). While conventional economic theory suggests that increased distance and travel times act as barriers that drain productivity due to delayed care (Aboaba et al., 2023; Combary & Traore, 2020), these minor positive coefficients within a Cobb-Douglas framework can indicate a behavioral or residential sorting effect. Farmers living further away from rural health hubs may deliberately invest more heavily in preventative health measures, traditional remedies, or informal labor-sharing arrangements to insulate their agricultural activities from health shocks. This allows them to maintain stable productivity despite geographic disadvantages.

The linear age of the farmer has a positive coefficient of 0.010 and is highly significant at the 1% level (p = 0.000). In rural farming arrangements, advanced age is deeply tied to accumulated wisdom, better management skills, and localized environmental adaptation. This allows older farmers to optimize resource allocation and maintain high productivity despite the physical challenges of aging. Conversely, household size exhibits a negative coefficient of -0.253, which is statistically significant at the 5% level (p = 0.018). This negative relationship points to the economic pressures of a high dependency ratio. A larger household size often means more young children or elderly dependents who consume household resources but do not actively contribute to farm labor. This burden dilutes per-capita resource pooling, shrinking the disposable capital available to purchase high-quality agricultural inputs or seek timely, premium medical interventions (Aminu & Asogba, 2020). The most powerful driver in the entire model is farming experience, which carries a substantial positive coefficient of 1.523 and is highly significant at the 1% level (p = 0.000). This underscores that hands-on, long-term experience gives farmers superior mastery over crop cycles, risk mitigation, and climate variations, making them exceptionally efficient.

Table 4: Regression Results

| *Variable* | *Coefficient* | *Std. Error* | *p-value* | *mfx* |
|---|---|---|---|---|
| Gender | 0.226 | 0.218 | 0.304 | 0.301 |
| Age | 0.010 | 0.00014 | 0.000*** | 0.000 |
| Education | -0.0056 | 0.0146 | 0.700 | 0.699 |
| Household size | -0.253 | 0.105 | 0.018** | 0.016 |
| Association membership | 0.087 | 0.262 | 0.740 | 0.739 |
| Farm size | -0.058 | 0.269 | 0.831 | 0.830 |
| Farming Experience | 1.523 | 0.333 | 0.000*** | 0.000 |
| Availability of health facility | -0.157 | 0.276 | 0.072* | 0.570 |
| Distance to health facility | 0.016 | 0.025 | 0.034* | 0.533 |
| Time to reach health facility | 0.003 | 0.0097 | 0.058* | 0.757 |
| Constant | 23.51 | 2.38 | 0.000 | |
| Number of Observation | 120 | | | |
| F(11, 86) | 679.57 | | | |
| Prob > F | 0.0000 | | | |
| $R^2$ | 98.86 | | | |
| Adj. $R^2$ | 98.72 | | | |
| Root MSE | 0.92465 | | | |

***; **, and * Represents 1%; 5% and 10% Respectfully, mfx = Marginal Effects after Regression

**Conclusion and Recommendations**

The study concludes that agricultural productivity among rural farming households in Ogun State is profoundly shaped by an intricate interplay of internal socioeconomic capacity and external healthcare access constraints. Empirically, the Cobb-Douglas functional form provides an exceptionally reliable fit for this relationship, demonstrating that internal human capital drivers—specifically a farmer's linear age and long-term farming experience—exert the most powerful positive influences on household yield. Conversely, expanded family sizes act as a significant drag on productivity, primarily due to heightened financial pressures and consumption demands from dependent household members. Crucially, while geographic distance and transit times do not severely depress output on their own, the mere physical presence of a medical center unexpectedly correlates with lower productivity when functional quality is compromised. To address these critical challenges, the study recommends

that government and relevant stakeholders must prioritize improving the structural and functional quality of public rural health facilities by recruiting adequate personnel, procuring modern equipment, and ensuring a stable supply of essential medications. Financial barriers must be aggressively dismantled through the introduction of subsidized health insurance schemes targeted specifically at smallholder farmers to eliminate the burden of out-of-pocket medical costs. Furthermore, relevant local authorities should invest heavily in rural infrastructure by expanding and grading road networks to minimize travel times and lower transportation costs for remote households. Public health agencies should also design targeted educational programs and localized mobile outreach services to overcome low formal education levels, thereby improving health literacy and safety practices among agricultural workers. Agricultural extension agents should actively collaborate with well-organized farmers' associations to build on their strong existing social networks and deliver specialized training on mitigating occupational health hazards. Finally, policymakers must intentionally integrate rural healthcare investments directly into wider agricultural development frameworks to guarantee that a healthy, resilient labor force can consistently maximize farm productivity.

## Declarations

### *Ethical Approval Certificate*

The ethical approval was received from the Directorate of Research, Innovation and Linkage Programme, through University Ethical and Legal Committee (ELRC), Federal University, Oye-Ekiti, for this study.

### *Author Contribution Statement*

Matthew Olufemi Adio: The lead Author and contributed significantly to the concept and design of the study, data collection, analysis and interpretation, manuscript drafting, critical revision, addition of intellectual content of the manuscript, serves as the corresponding author and approved the submission of the manuscript. Dan E. Oyoboh and Oluwaseun Adebayo Ojeleye: contributed significantly to the design and concept of the study and data collection instrument, oversaw the research process, as well as approved the submission of the manuscript. Elijah Kehinde Olayanju and Jeremiah Taiwo Olayanju: contributed significantly to the manuscript editing, critical revision, and approved the submission of the manuscript. Nathaniel Olufemi Akanbi and Nurudeen Ayoyinka Jatto: manuscript drafting, critical revision, approved the submission of the manuscript. Abisola Adenike Ogunkunle and Medinat Adeola Abdulaleem: Contributed significantly to the critical revision of the manuscript, approved the submission of the manuscript.

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This work was not funded.

### *Conflict of Interest*

The authors declare no competing interests.

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# Investigation of the Antiapoptotic Effects of Tofacitinib in C6 Glioma Cells Exposed to Oxidative Stress

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## Abstract

This study aimed to investigate the potential antiapoptotic and cell-protective effects of tofacitinib in C6 glioma cells damaged by oxidative stress. The C6 glioma cell line was used in this study. Hydrogen peroxide was applied to the cells to induce oxidative stress. The cells were divided into 6 groups to receive control, $H_2O_2$, 100 nM tofacitinib, 50 nM tofacitinib, 25 nM tofacitinib, and 12.5 nM tofacitinib. The protective effect of tofacitinib application on cell viability and its effects on apoptotic processes were evaluated. Cell viability analyses were performed under a microscope, and caspase-3 expression was examined using immunofluorescence. Simultaneously, oxidative stress response was evaluated by measuring total oxidant status (TAS) and total oxidant status (TOS) levels. Statistical analyses were performed using one-way ANOVA and Tukey post hoc test, with a $p<0.05$ value considered significant. While $H_2O_2$ applied to the cells caused oxidative damage and apoptosis in the cell line, it was observed that cell viability was maintained and caspase-3 expression decreased in the groups treated with tofacitinib. Furthermore, a decrease in TOS levels and an increase in TAS levels were detected. These findings suggest that tofacitinib may reduce oxidative stress-related damage and have an anti-apoptotic effect.Tofacitinib was observed to suppress stress in c6 glioma cells subjected to hydrogen peroxide stress, preserving viability, and this is thought to be effective via an anti-apoptotic pathway. The findings indicate that tofacitinib may have neuroprotective effects in addition to its primary anti-inflammatory use.

***Keywords:*** *Tofacitinib, C6 glioma, apoptosis, oxidative stress*

## Oksidatif Strese Maruz Birakilan C6 Glioma Hücrelerinde Tofacitinib'in Antiapoptotik Etkilerinin İncelenmesi

## Özet

Bu çalışmada, oksidatif stres ile hasar oluşturulan C6 glioma hücrelerinde tofacitinib'in olası antiapoptotik ve hücre koruyucu etkilerinin araştırılması amaçlandı. Çalışmada c6 glioma hücre hattı kullanılmıştır. Oksidatif stres oluşturmak amacıyla hücrelere hidrojen peroksit uygulandı. Hücreler kontrol, $H_2O_2$, 100nM tofacitinib, 50 nM tofacitinib, 25 nM tofacitinib ve 12.5 nM tofacitinib uygulamak üzere 6 gruba ayrıldı. Tofacitinib uygulamasının hücre canlılığına koruyuculuk etkisi ve apoptotik süreçlaer üzerindeki etkileri değerlendirildi. Hücre canlılığı analizleri mikroskopta incelendikten sonra caspase-3 ekspresyonu immünfloresan yöntem ile incelendi. Aynı zamanda toplam oksidan status (TAS) ve toplam oksidan status (TOS) düzeyleri ölçülerek oksidatif stres yanıtı değerlendirildi. İstatistiksel analizlerde one-way ANOVA ve Tukey post hoc testi kullanıldı ve $p<0.05$ değeri anlamlı kabul edildi. Hücrelere uygulanan $H_2O_2$ hücre hattında oksidatif hasara ve apoptotik sebebiyet verirken tofacitinib uygulanan gruplarda hücre canlılığının devam ettiği ve caspase-3 ekspresyonunun azaldığı gözlemlenmiştir. Ayrıca TOS düzeylerinde azalma, TAS düzeylerinde ise artış saptanmıştır. Bu bulgular eşliğinde tofacitinibin oksidatif strese bağlı hasarı azaltabileceği ve antiapoptotik etki oluşturabileceğini göstermiştir. Tofacitinib'in hidrojen peroksitle strese bırakılan c6 glioma hücrelerinde canlılığı koruyarak stresi baskıladığı görülmüş bununda antiapoptotik yoldan etkili olabileceği düşünülmüştür. Elde edilen bulgular tofacitinibin kendi endikasyonu olan antiinflamatuvar kullanımına ek olarak nöroproktektif etkilerininde olabileceği görülmüştür.

***Anahtar kelimeler:*** *Tofacitinib, C6 glioma, apoptoz, oksidatif stress.*

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# Use of Natural Antioxidants in Edible Films and Coatings

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## Abstract

Today, the intensive use of chemical fertilizers causes various environmental problems. Inoculation of microbial preparations as an alternative to chemical fertilizers to plants can be an effective method for sustainable production efforts. Silicon application shows potential to increase nutrient uptake by roots and nutrient availability in the rhizosphere through complex mechanisms, can increase P availability in the soil. In this study, the effects of silicon application and inoculation of phosphorus solubilizing bacteria and mycorrhiza on sunflower plant growth and phosphorus utilization efficiency were investigated. Sunflower Seed, *Glomus etunicatum*, mycorrhiza complex and with P bacteria were inoculated. Si application was also made at doses of 0-30-60 kg/da. The experiment was terminated when 50% of the flowers bloomed. According to the results, average plant height, flower wet and dry weights showed significant differences with Si treatments, Si with mycorrhiza and P bacteria treatments. 30 Si kg/ha dose had more positive effects on plant growth. The triple interaction had a positive effect on improving the P utilization efficiency of sunflower.

***Keywords:*** *Sunflower, Mycorrhiza, Silicon, Phosphorus, Phosphorus bacteria, P utilization efficiency*

**Ayçiçeğinde (*Helianthus annuus*) Fosfor Kullanım Verimliliğini ve Gövde Direncini Artırmada Silisyum, Mikoriza ve Fosfor Bakterilerinin Etkisi**

## Özet

The creation of edible films and coatings containing natural antioxidants has drawn more attention due to the rising demand for safe, sustainable, and minimally processed foods. These biodegradable films are made of lipids, proteins, and polysaccharides, act as barriers to improve food quality and increase shelf life. Natural antioxidants made from plant extracts, essential oils, herbs, and spices, are becoming more popular than synthetic antioxidants because of their safety, biocompatibility, and consumer acceptability. High concentration of polyphenols, flavones, glycosides, tannins, and flavonoids, are derived from number of herbal extracts, which acts as good antioxidants. These antioxidants aid in lowering lipid oxidation, postponing microbiological deterioration, maintaining sensory qualities, and preserving food items' nutritional value when added to edible films and coatings. All things considered, adding natural antioxidants to edible films and coatings is a sustainable and practical way to enhance food preservation and lessen dependency on synthetic packaging materials.

***Anahtar Kelimeler:*** *Food preservation, Edible films, Coating, Lipid oxidation, Antioxidant*

## Introduction

The short shelf life of food goods, which results from oxidation processes like oxidative rancidity, enzymatic browning, and degradation, is the biggest obstacle facing the food sector (Soliva-Fortuny & Martín-Belloso, 2003). One of the primary reasons of food deterioration is lipid oxidation. When lipids in food oxidize, rancidity-related off-flavors appear, making the product unfit for human ingestion. Toxic aldehyde production and nutritional quality loss due to polyunsaturated fatty acid (PUFA) breakdown are additional adverse impacts (Guillen & Goicoechea, 2008). A number of techniques have been used to lessen lipid oxidation, including the direct addition of antioxidants to food or the development of an appropriate packing technology, such as vacuum or modified-atmosphere packaging in conjunction with high-barrier packaging materials (Lopez de Dicastillo et al., 2010).

Food packaging now plays a more active role in promoting and safeguarding food products. Different creative ways of intelligent packaging are used to prolong the shelf life, preserve, enhance, or monitor food quality and safety. An edible film or coating is any type of substance that is used for food packaging to extend its shelf life and may be eaten with the food. Thin layers of material that are safe to eat and serve as a barrier against various agents (such as moisture, oxygen, and water vapor) make up edible films or coatings. They aid in enhancing the fresh and processed foods' quality and prolonging their shelf life. These films and coatings' functional qualities can be improved by adding active substances, such as antioxidants, which may make them useful for food preservation (Sánchez-González et al., 2011).

**Edible films and coatings**

An edible film is a prepared, thin layer of edible material that may be applied to or in between food components, whereas an edible coating is a thin layer of edible material created as a coating on a food product (McHugh, 2000). In addition to being similar to active packaging technology, edible film and coating technology may help prevent oxidative food deterioration. Reducing the rate of oxygen transport and the potential to include antioxidant chemicals in the edible film or coating matrix are the primary mechanisms of action. This vehicle has the benefit of tight interaction between food and coating in the latter scenario. The use of edible coatings or edible films in food applications is dependent on a number of factors, including cost, availability, functional characteristics, mechanical properties and optical properties (Ganiari et al., 2017).

The type of material used as a structural matrix (composition, molecular weight), the conditions under which films are produced (solvent type, pH, component concentration, and temperature), and the kind and concentration of additives (plasticizers, cross-linking agents, antimicrobials, antioxidants, or emulsifiers) all affect these characteristics (Rojas-Graü et al., 2009). In this technique, plasticizers, colors, scents, and antibacterial or antioxidant agents can be added. To create freestanding films, the film solution is subsequently cast and dried at the appropriate temperature and relative humidity (Bourtoom, 2008). Antioxidants are added to the films using a variety of technical techniques. The majority of them include either co-extrusion of the antioxidant with the film-forming plastic material (Granda-Restrepo et al., 2009) or direct mixing of an antioxidant agent with the plastic ingredients (LaCoste et al., 2005). Edible films or coatings are very likely to contain active chemicals including colorants, tastes, nutrients, spices, antioxidants, and anti-browning agents that can improve the food's organoleptic qualities and nutritional value or prolong its shelf life. The fact that these films and coatings may be eaten with the food, reducing the need for extra external packaging, is another significant aspect (Ganiari et al., 2017).

**Natural antioxidants**

Any substance that has the ability to postpone, slow down, or stop the development of rancidity in food or other oxidation-related flavor deterioration is considered a food antioxidant. The natural antioxidants, includes tocopherol, plant extracts, essential oils, or their components (Tovar et al., 2005). The potential utilization of food industry waste as a source of antioxidant agents is also pertinent (Barbosa-Pereira et al., 2013).

High quantities of phenolic compounds with potent H-donating activity are frequently found in spices and herbs, which are utilized in food for their flavor and in medical combinations for their physiological benefits. To stop oxidation, these substances can be collected and added to food systems. The Lamiaceae family of herbs, which includes rosemary, oregano, thyme, marjoram, savory, sage, and others, has been extensively studied. Tea, a member of the Theaceae family, is another herb that has been extensively studied as a natural antioxidant. Herbs and spices include antioxidant components that can be extracted and concentrated as oleoresins, extracts, or essential oils. Extracts from plant by-products, such as barley husks, pomegranate peel, olive leaves, etc., have shown good antioxidant properties in addition to herbs and spices (Marcos et al., 2014; Qin et al., 2015; Yuan et al., 2015).

**Antioxidant activity of enriched edible packaging films**

Devoid of employing the enhanced films to cover food, several studies have concentrated on their antioxidant qualities. It is evident that the greatest research has been done on chitosan films. Chitosan's inherent antibacterial and antioxidant qualities, together with its superior film-forming capacity, have made it a desirable material for food packaging (Abdollahi et al., 2012). The film's capacity to liberate the confined active polyphenols is linked to its antioxidant action. According to Peng et al. (2013), increasing the concentration of tea extracts in chitosan film led to a quicker release of the antioxidant compounds and, as a result, higher radical scavenging activity. Lopez de Dicastillo et al. (2015) also noted this with various film materials and additives. Martucci et al. (2015) measured the DPPH radical scavenging activity in gelatin films after adding oregano and lavender EOs (2000–6000 ppm). The findings indicated that the film was more effective against the DPPH radical when 6000 ppm of oregano EO was added, however the film's ability to scavenge radicals was minimal when lavender EO was added. These findings may be explained by the various activities of each plant's EO components or by interactions between the EO components and the gelatin matrix.

**Applications of enriched edible films as antioxidant**

The best antioxidants for fish and meat products are extracts of oregano and rosemary. More precisely, the oxidation of smoked sardines has been successfully delayed by adding aqueous extracts of both herbs to edible films composed of gelatin or gelatin-chitosan (Gomez-Estaca et al., 2007). Similar to this, smoked eel fillets were shielded by the ethanol extract of rosemary added to the CMC (carboxymethylcellulose) coating, which also

slowed the build-up of primary and secondary oxidation products (Choulitoudi et al., 2017). Polyphenols, which have a strong antioxidant potential and may be employed in films, could derived from borage seeds and leaves (Fabra et al., 2011). Films containing extract from borage leaves exhibited superior antioxidant qualities compared to those containing α-tocopherol (Gomez-Estaca et al., 2009). High concentration of polyphenols, flavones, glycosides, tannins, and flavonoids, a number of herbal extracts, derived from green tea (Wu et al., 2013), ginkgo leaves, ginger, and grape seeds (Li et al., 2014).

Biopolymers with antioxidant qualities can be created by adding these extracts to biodegradable films. For instance, it has been discovered that adding extracts from grape seeds, ginkgo leaves, or green tea reduced oxidation. Gingko leaf extracts were shown to have the greatest DPPH radical scavenging activity when added to films (Li et al., 2014). Due to its high antioxidant content, which includes phenolic acid and flavonoids, quince seed mucilage also provided films with high antioxidant activity by evaluating DPPH radical scavenging activity (Jouki et al., 2013). To prevent the oxidation of meat's lipids and pigments, antioxidants like vitamin C and E can be added to edible films and coatings (Kenawi et al., 2011).

## Conclusion

It is concluded from this review that edible films used for packaging and preservation of perishable food for longer time storage and transport purpose. As food especially meat could be damaged due to oxidation of lipids in it, so this process could be delayed by adding antioxidants in packaging materials. These antioxidants could retard or slow the process of food oxidation and preserve the food for longest time.

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# Phytotherapeutic Agents and Musculoskeletal Health: A Bibliometric Analysis of Global Research Trends (2000–2025)

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## Abstract

Musculoskeletal disorders rank among the leading causes of disability worldwide, and interest in phytotherapeutic approaches to their management has grown substantially over the past two decades. This study aimed to characterise the global scientific output on this topic using bibliometric methods. A comprehensive literature search was conducted in the Scopus database on 31 May 2026, using a Boolean string combining phytotherapy-related terms (phytotherapy, "herbal medicine", ethnopharmacology, "medicinal plant", phytomedicine, "traditional medicine") with musculoskeletal condition terms (musculoskeletal, osteoarthritis, "rheumatoid arthritis", "low back pain", fibromyalgia, rehabilitation), restricted to original research articles in English published between 2000 and 2025. A total of 3,032 articles were retrieved from 1,052 journals, accumulating 76,827 citations (mean: 25.34; H-index: 107). Annual output grew from 20 articles in 2000 to 262 in 2025. China was the most productive country (n=804), followed by India (n=403) and South Korea (n=348); Turkey ranked 20th with 36 publications. The Journal of Ethnopharmacology dominated journal rankings (n=295). Ha In-Hyuk (South Korea) was the most prolific author (n=43). Ginger (n=176), curcumin/turmeric (n=147), and nettle (n=92) were the most frequently studied phytotherapeutic agents. Keyword analysis identified four thematic clusters: Korean medicine and acupuncture; traditional Chinese medicine and complementary medicine; pain and osteoarthritis; and ethnobotany and traditional knowledge. Research on phytotherapy and musculoskeletal health has grown steadily but remains geographically concentrated in East Asia. Turkey, despite its rich endemic flora, has made a limited contribution to this body of literature, representing a significant opportunity for future research.

***Keywords:*** *phytotherapy, musculoskeletal health, bibliometric analysis, herbal medicine, ethnopharmacology, VOSviewer*

# Fitoterapi Ajanları ve Kas-İskelet Sağlığı: Küresel Araştırma Eğilimlerinin Bibliyometrik Analizi (2000–2025)

## Özet

Kas-iskelet sistemi hastalıkları dünya genelinde engelliliğin önde gelen nedenlerinden biri olup bu alandaki fitoterapi yaklaşımlarına olan ilgi son yirmi yılda önemli ölçüde artmıştır. Bu çalışma, söz konusu alandaki küresel bilimsel çıktıyı bibliyometrik yöntemlerle değerlendirmeyi amaçlamıştır. 31 Mayıs 2026 tarihinde Scopus veri tabanında, fitoterapi ile ilişkili terimler (phytotherap*, "herbal medicine", ethnopharmacology, "medicinal plant*", phytomedicine, "traditional medicine") ile kas-iskelet sistemi hastalıkları terimlerini (musculoskeletal, osteoarthritis, "rheumatoid arthritis", "low back pain", fibromyalgia, rehabilitation) birleştiren Boole arama dizisi uygulanmış; arama 2000–2025 yılları arasında İngilizce yayımlanmış orijinal araştırma makaleleriyle sınırlandırılmıştır. Toplam 1.052 dergide yayımlanmış 3.032 makale elde edilmiş; bu makaleler 76.827 atıf biriktirmiştir (ortalama: 25,34; H-indeksi: 107). Yıllık yayın sayısı 2000'deki 20 makaleden 2025'te 262'ye yükselmiştir. En üretken ülke Çin (n=804), ardından Hindistan (n=403) ve Güney Kore (n=348) olmuştur; Türkiye 36 yayınla 20. sıradadır. Dergiler arasında Journal of Ethnopharmacology öne çıkmıştır (n=295). En üretken yazar Ha In-Hyuk'tur (Güney Kore, n=43). En sık çalışılan fitoterapi ajanları zencefil (n=176), kurkumin/zerdeçal (n=147) ve ısırgan otu (n=92) olmuştur. Anahtar kelime analizi dört tematik küme ortaya koymuştur: Kore tıbbı ve akupunktur; geleneksel Çin tıbbı ve tamamlayıcı tıp; ağrı ve osteoartrit; etnobotanik ve geleneksel bilgi. Fitoterapi ve kas-iskelet sağlığı alanındaki araştırmalar istikrarlı bir büyüme göstermekle birlikte coğrafi olarak Doğu Asya'da yoğunlaşmaktadır. Zengin endemik florası olmasına karşın Türkiye'nin bu alana katkısı sınırlı kalmış olup gelecekteki araştırmalar için önemli bir fırsat teşkil etmektedir.

***Anahtar Kelimeler:*** *fitoterapi, kas-iskelet sağlığı, bibliyometrik analiz, bitkisel ilaç, etnofarmakoloji, VOSviewer*

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# Evaluation of Nutrient Status and Cation Balance in Wheat Cultivated on Serpentine-Derived Soils

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## Abstract

Serpentine soils are derived from the serpentinization of ultramafic parent materials and are characterized by distinctive chemical and physical properties. The combined stress conditions imposed on plants growing in these soils are commonly known as the "serpentine problem." The present study was conducted to evaluate the nutritional status of wheat cultivated on serpentine-derived soils and to investigate soil–plant relations. A total of 15 soil and plant samples were collected from serpentine-origin agricultural fields located in the İslâhiye–Nurdağı region. Selected macro- and micronutrients together with several basic soil properties were determined in soil samples, whereas nutrient element analyses were performed in plant samples. The obtained data were further evaluated to assess the effects of soil–plant relationships and cation ratios on wheat nutrition. The soils of the study area were generally characterized as neutral to slightly alkaline, low in lime content, and non-saline. Soil analyses revealed high exchangeable Mg concentrations, whereas Ca levels were comparatively lower. In addition, available P and exchangeable K concentrations were determined to be above sufficiency levels. Mean concentrations were 37.2 mg $kg^{-1}$ for P, 239.67 mg $kg^{-1}$ for K, 558 mg $kg^{-1}$ for Ca, and 2615 mg $kg^{-1}$ for Mg. The average Fe, Zn, Cu, and Mn concentrations in soils were determined as 25.67, 0.95, 1.89, and 3.79 mg $kg^{-1}$, respectively. Soil Ni concentrations ranged between 14.88 and 112.06 mg $kg^{-1}$, confirming the characteristic accumulation of Mg and Ni in serpentine soils. Ni concentrations ranged between 14.88 and 112.06 mg $kg^{-1}$, confirming the characteristic accumulation of Mg and Ni in serpentine soils. Evaluation of cation ratios demonstrated that low Ca/Mg and high Mg/K ratios reflected a pronounced cation imbalance. Plant analyses indicated that the average N, P, and K concentrations in wheat were 4.38%, 0.37%, and 2.07%, respectively. In contrast, plant Ca concentrations were relatively low (0.02–0.13%), whereas the average Mg concentration was determined as 0.33%. Micronutrient concentrations were generally within sufficiency ranges. Evaluation of soil and plant data revealed a negative correlation between soil Mg/K ratio and plant K concentration, while elevated Mg levels were also associated with plant Ca and K nutrition. Overall, the findings demonstrated that Mg dominance in serpentine soils induces cation antagonism, thereby adversely affecting particularly Ca and K nutrition in wheat.

***Keywords:*** *Serpentine soils, Wheat nutrition, Cation antagonism, Magnesium dominance*

## Introduction

Serpentine soils are unique edaphic environments derived from the weathering of ultramafic rocks such as peridotite, dunite, and serpentinite. These soils possess distinctive physical and chemical characteristics that distinguish them from most agricultural soils. The mineralogical composition of serpentine parent materials typically results in elevated concentrations of magnesium (Mg), nickel (Ni), chromium (Cr), and cobalt (Co), whereas essential plant nutrients such as calcium (Ca), potassium (K), nitrogen (N), and phosphorus (P) generally occur at comparatively low levels (Brady et al., 2005; Oze et al., 2008).

The combined effects of nutrient deficiencies, nutrient imbalances, and potentially toxic metal levels create a stressful environment for plant growth, a condition commonly referred to as the serpentine syndrome or the serpentine problem. Among these constraints, the predominance of exchangeable magnesium (Mg) relative to calcium (Ca) is considered one of the most distinctive characteristics of serpentine soils. Low Ca/Mg ratios adversely affect root development, membrane stability, and nutrient uptake, thereby imposing significant limitations on plant growth and productivity (Proctor, 2003; Kazakou et al., 2008). Furthermore, elevated Mg concentrations may suppress Ca and K uptake through competitive interactions among cations at root uptake sites (White & Broadley, 2003).

In Türkiye, particularly in the Eastern Mediterranean region, ultramafic and serpentine formations cover extensive areas. Despite their challenging chemical properties, some of these soils are widely used for agricultural production. However, information on soil nutrient availability, cation balance, and soil–plant nutrient relationships under serpentine conditions remains limited. A better understanding of these interactions is essential for developing effective nutrient management strategies and improving crop productivity on serpentine-derived agricultural soils. While previous studies on serpentine ecosystems have primarily focused on natural vegetation, metal tolerance, and ecological adaptation, comparatively little attention has been given to nutrient imbalances

affecting agricultural crops such as wheat. Therefore, the present study was conducted to evaluate the nutritional status of wheat grown on serpentine-derived soils in the İslâhiye–Nurdağı region of southeastern Türkiye. Particular emphasis was placed on determining soil nutrient availability, exchangeable cation ratios, and plant nutrient concentrations.

## Materials and Methods

### *Study area*

The study was conducted in serpentine-derived agricultural fields located in the İslâhiye–Nurdağı region of southern Türkiye, within the Amanos Ophiolite Belt (Figure 1). The study area is underlain by widespread ophiolitic rocks, and the soils have predominantly developed from serpentinized ultramafic parent materials. The Amanos Belt consists of ophiolitic units emplaced during the closure of the Neo-Tethys Ocean and represents one of the most important ultramafic rock assemblages in southern Türkiye (Duman et al., 2017). Ultramafic rocks, including harzburgite, dunite, and serpentinite, are widely distributed throughout the region and have undergone extensive serpentinization. Consequently, the soils developed on these parent materials exhibit the characteristic physical and chemical properties of serpentine soils (Yapıcı et al., 2022).

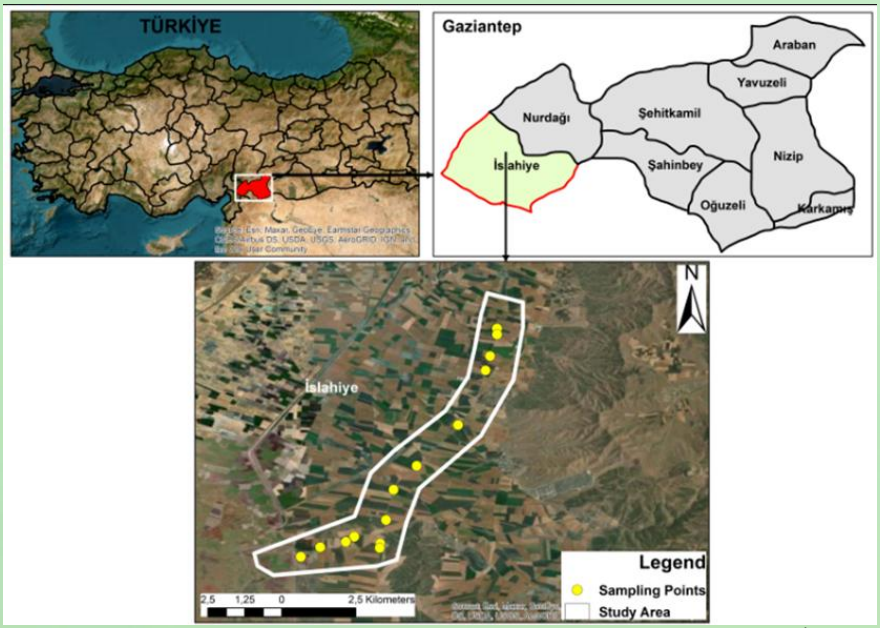



Figure 1. Map of the study area showing the distribution of soil sampling sites in the İslâhiye–Nurdağı region, southern Türkiye

### *Soil and Plant Analyses*

Soil pH and electrical conductivity (EC) were measured in saturated soil paste extracts following the procedures of Black (1965) and Richards (1954), respectively. Calcium carbonate ($CaCO_3$) content was quantified using a Scheibler calcimeter (Klute, 1986). Particle-size distribution was determined by the hydrometer method (Bouyoucos, 1951), whereas soil organic matter was analyzed using the Walkley–Black method (Nelson & Sommers, 1996). Exchangeable K, Ca, and Mg were extracted with 1 N ammonium acetate ($NH_4OAc$) and subsequently quantified according to Helmke & Sparks (1996).

Plant samples were dried at 65°C, finely ground, and digested using the wet digestion procedure described by Jones & Case (1990). For each analysis, 0.50 g of ground plant material was treated with 5 mL $HNO_3$ and 4 mL $HClO_4$ and heated until complete digestion was achieved. The digest was then filtered and brought to the required volume with deionized water. Potassium, Ca, and Mg in both soil extracts and plant digests were quantified using an Agilent 240AA Atomic Absorption Spectrophotometer (AAS).

### *Statistical Analysis*

The experimental data were evaluated using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including minimum, maximum, mean, and standard deviation values, were calculated for all soil and plant variables. Relationships among soil exchangeable cations (K, Ca, Mg), cation ratios (Ca/Mg, Ca/K, and Mg/K), and nutrient concentrations in wheat leaves were assessed by Pearson correlation analysis using JMP software (SAS Institute Inc., Cary, NC, USA). All figures were prepared using Microsoft Excel.

## Results and Discussion

### *Physicochemical Characteristics of the Study Area Soils*

The physicochemical properties of the study area soils are presented in Table 1. Soil pH ranged from 6.60 to 7.30, with a mean value of 7.11. Electrical conductivity (EC) ranged from 63.50 to 236.00 µS cm$^{-1}$, with an average of 121.96 µS cm$^{-1}$. Lime ($CaCO_3$) content varied between 1.09% and 15.15%, with a mean value of 2.75%, whereas soil organic matter content ranged from 2.01% to 3.49%, averaging 2.57%. Overall, the study area soils were characterized by neutral pH, non-saline conditions, low lime content, and moderate organic matter levels.

Table 1. Descriptive statistics of selected physicochemical properties of the study area soils

| | *pH* | *EC* | *Lime* | *OM* |
|---|---|---|---|---|
| | | (µS/cm) | % | |
| **Min** | 6.60 | 63.50 | 1.09 | 2.01 |
| **Max** | 7.30 | 236.00 | 15.15 | 3.49 |
| **Mean** | 7.11 | 121.96 | 2.75 | 2.57 |

### *Macro-nutrient Status of the Study Area Soils*

The concentrations of selected soil macronutrients are presented in Figure 2. The serpentine-derived soils of the study area were characterized by high Mg and low Ca contents. Among the exchangeable cations, Mg was the dominant element, with a mean concentration of 2615 mg kg$^{-1}$, whereas Ca averaged only 558 mg kg$^{-1}$. Exchangeable K ranged from 65 to 530 mg kg$^{-1}$, with a mean value of 240 mg kg$^{-1}$, while available P varied between 15.75 and 57.18 mg kg$^{-1}$, averaging 37.20 mg kg$^{-1}$ (Figure 2).

According to the sufficiency criteria proposed by Kaiser et al. (2023), the mean exchangeable K concentration was above the critical level for wheat cultivation, whereas available P was also generally within the sufficient range. In contrast, exchangeable Ca was classified as deficient according to Kacar (1995), while exchangeable Mg was classified as very high based on the FAO (1980) guidelines. The pronounced imbalance between Ca and Mg resulted in a low Ca/Mg ratio, which is a characteristic feature of serpentine soils. The coexistence of low Ca availability and excessive Mg accumulation is considered one of the principal chemical constraints limiting plant nutrition in serpentine-derived soils (Kazakou et al., 2008). Accordingly, the application of lime in combination with gyttja, an organic matter-rich natural material that is abundant in the region, has been proposed as an effective strategy to alleviate Ca deficiency and improve cation balance in Mg-dominated soils (Saltalı and Korkmaz, 2015; Saltalı and Yıldırım, 2016; Demir et al., 2017).

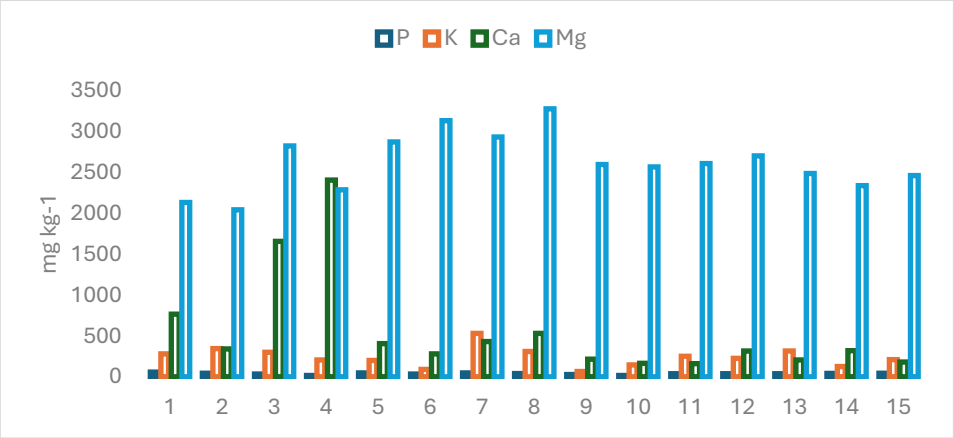



Figure 2. Distribution of available P and exchangeable cations in serpentine-derived soils

### *Micronutrient Status of the Study Area Soils*

The concentrations of selected soil micronutrients are presented in Figure 3. Considerable variation was observed among the analyzed micronutrients. Iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) averaged 25.67, 0.95, 1.89, and 3.79 mg kg$^{-1}$, respectively. The most remarkable result was the wide range of Ni concentrations, varying from 14.88 to 112.06 mg kg$^{-1}$, with an average of 43.73 mg kg$^{-1}$ (Figure 3). This elevated Ni level is characteristic of the nickel enrichment commonly associated with serpentine-derived soils. Based on the sufficiency criteria proposed by Lindsay & Norvell (1978), the analyzed micronutrients were generally within or above the recommended ranges. Furthermore, the elevated Fe and Ni levels are consistent with the geochemical signature of serpentine-derived soils.

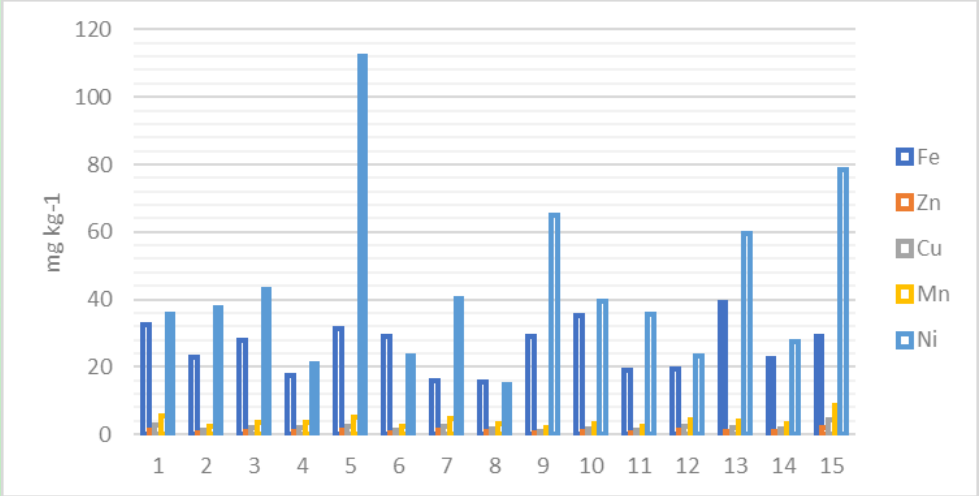



Figure 3. Distribution of DTPA-extractable Fe, Zn, Cu, Mn and Ni concentrations in serpentine-derived soils

***Soil Cation Ratios***

The mean Ca/K, Ca/Mg, and Mg/K ratios in the study area soils were 5.09, 0.13, and 47.15, respectively (Figure 4). Among the evaluated cation ratios, the lowest value was observed for Ca/Mg, whereas the highest was recorded for Mg/K. These results indicate a pronounced predominance of Mg relative to both Ca and K in the serpentine-derived soils.

The observed cation ratios differed markedly from the ideal values proposed by Albrecht (1975), indicating a pronounced cation imbalance in the study area soils. According to Albrecht, the optimum Ca/Mg, Ca/K, and Mg/K ratios for plant growth are approximately 6.5, 13, and 2, respectively. In contrast, the mean Ca/Mg ratio (0.13) was substantially lower than the recommended value, whereas the mean Mg/K ratio (47.15) greatly exceeded the optimum level. Likewise, the mean Ca/K ratio remained below the recommended value. These findings clearly demonstrate the characteristic Mg dominance and relative Ca deficiency of serpentine-derived soils, indicating that the cation balance is strongly shifted toward Mg. Such cation imbalance is considered a major chemical constraint that may restrict the uptake of Ca and K, thereby adversely affecting plant nutrition. Similar cation imbalance has been reported in previous studies, where low Ca/Mg and high Mg/K ratios were associated with reduced Ca and K uptake and impaired plant nutrition despite sufficient soil nutrient reserves (Aytop et al., 2026). Likewise, elevated Mg relative to Ca and K has been shown to negatively affect K nutrition under field conditions (Aytop et al., 2025). Similar responses have also been reported in peanut, highlighting that balanced Ca–Mg–K relationships are more critical for plant nutrition than the individual concentrations of these cations (Demir et al., 2026).

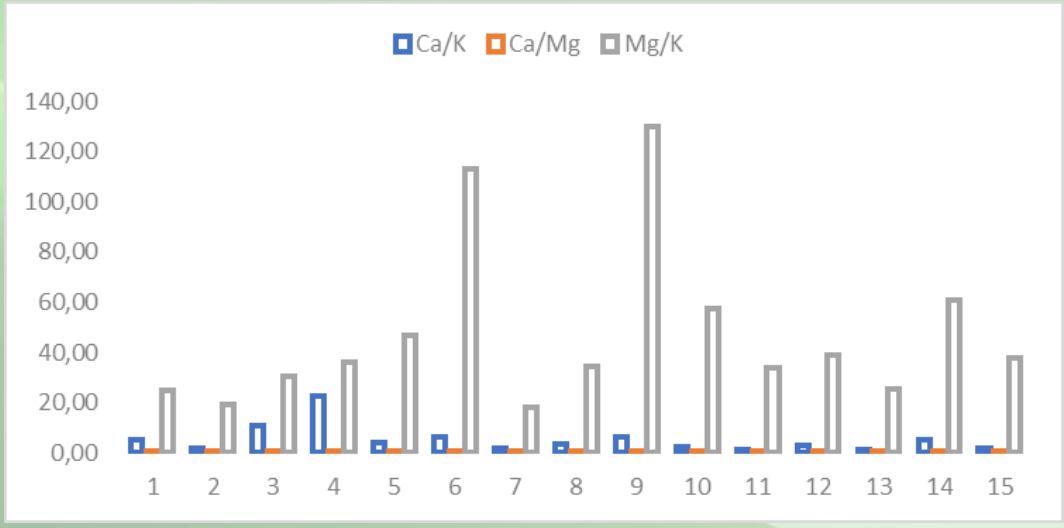



Figure 4. Variations in soil Ca/K, Ca/Mg, and Mg/K ratios among sampling sites

***Foliar Macronutrient Concentrations in Wheat***

The foliar macronutrient concentrations of wheat varied among the sampling sites (Figure 5). On average, N was the most abundant macronutrient in plant tissues (4.38%), followed by pot K (2.07%). Phosphorus, Ca and Mg averaged 0.31%, 0.06%, and 0.33%, respectively. Based on the sufficiency ranges proposed by Jones et al. (1991) and Reuter and Robinson (1997), the nutritional status of N, P, K, and Mg was generally adequate. In contrast, foliar Ca remained below the sufficiency range. This finding may be attributed to the combined effects of low soil Ca availability and the pronounced Mg/Ca imbalance characteristic of serpentine-derived soils, both of which may restrict Ca uptake by plants (O'Dell & Rajakaruna, 2011).

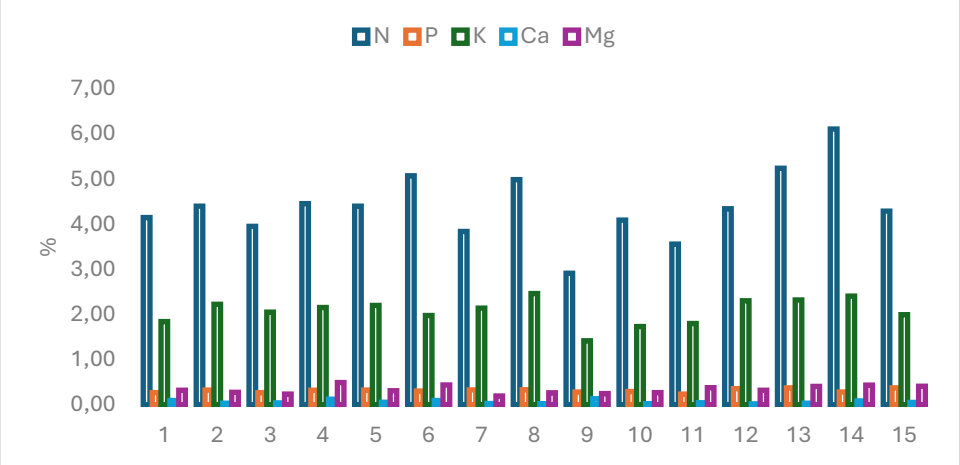



Figure 5. Foliar concentrations of macronutrients in wheat collected from serpentine-derived soils

**Foliar Micronutrient Concentrations in Wheat**

The foliar micronutrient concentrations of wheat are presented in Figure 6. Overall, the analyzed micronutrients were within the adequate ranges. Iron, Zn, Cu, and Mn averaged 115.80, 30.80, 2.42, and 60.48 mg $kg^{-1}$, respectively. These values indicate an adequate micronutrient status for wheat according to the sufficiency ranges reported by Jones et al. (1991). In contrast, Ni concentrations were below the detection limit in many samples. In the samples where Ni was detected, concentrations ranged from 0.50 to 7.00 mg $kg^{-1}$, with a mean value of 2.50 mg $kg^{-1}$ (Figure 6). Despite the elevated Ni concentrations in the soils, the relatively low Ni concentrations in plant tissues suggest that Ni uptake and translocation are regulated by physiological mechanisms within the plant. Previous studies have demonstrated that a substantial proportion of absorbed Ni may be retained in root tissues, thereby limiting its translocation to the shoots through the xylem (Iqbal et al., 2026). Furthermore, despite the elevated Ni concentrations commonly observed in serpentine-derived soils, Ni uptake by plants is often restricted by species-specific physiological mechanisms and the relatively low bioavailability of Ni (Broadley et al., 2012; Brady et al., 2005).

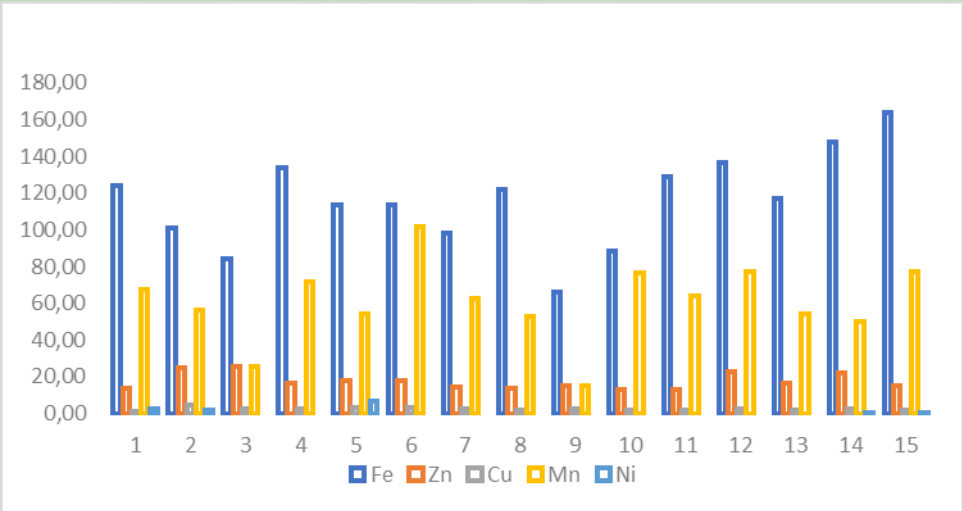



Figure 6. Foliar micronutrient concentrations in wheat grown on serpentine-derived soils

## Conclusion

This study demonstrated that the serpentine-derived agricultural soils of the İslâhiye–Nurdağı region exhibit the characteristic features of serpentine soils, including high exchangeable Mg and Ni concentrations together with low Ca levels. Although soil P and K were generally within the sufficient range, the marked deviation of the cation ratios from the recommended values indicated a pronounced Mg-induced cation imbalance. This imbalance was associated with reduced Ca nutrition in wheat and suggests that elevated Mg levels may also restrict K uptake through cation antagonism. In contrast, foliar micronutrient concentrations were generally adequate, and Ni accumulation in plant tissues remained limited despite the elevated Ni concentrations in the soils. Overall, these findings demonstrate that the nutritional status of wheat grown on serpentine-derived soils should be evaluated not only in terms of nutrient concentrations but also by considering cation balance. Therefore, nutrient management strategies aimed at improving Ca and K nutrition while mitigating the effects of Mg dominance are essential for sustainable wheat production on serpentine-derived soils.

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# Novel approaches to the packaging technologies in meat products

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## Abstract

Packaging is essential for preserving food safety and quality, and innovations in active packaging, especially antimicrobial packaging, enable the active suppression or elimination of spoilage-causing bacteria, thereby extending the product's shelf life. This work aims to elucidate several packaging technologies for beef products. Active packaging methods incorporate functional elements such as antioxidants and antimicrobials to prevent oxidative deterioration and microbial proliferation. Time and temperature indicators, gas sensors, and pH-sensitive films exhibit smart packaging technologies that provide real-time monitoring of meat quality and freshness, delivering essential information throughout the supply chain. Furthermore, digital technology, encompassing biosensors and QR codes, enhances traceability and transparency. Recent breakthroughs in nanotechnology have enhanced the mechanical durability and insulating properties of packaging materials, while also imparting antibacterial activity. Despite the significant development the packaging industry has witnessed with modern technology, high costs remain a challenge.

***Keywords:*** *packaging, food, meat preservation*

## Introduction

Meat packaging is dynamic, and its innovation has been the topic of much recent review. These are valuable; however, their categorization of recent technology and inventions in terms of their functional pathways and within smart packaging definitions may limit their application as a troubleshooting tool for industry, retailers, and other stakeholders. We should note that smart packaging encompasses intelligent and active packages designed to provide a 'cost-effective barrier against hazardous contaminants that simultaneously contributes to the longevity of product quality and enhancement of consumer appeal' (Holman et al., 2017).

Consumers are familiar with packaging, as currently most products are covered in one or more packaging materials. Food packaging protects against degradation and contamination and frequently contains information about the food. Labels with different colors, marks or fonts, different forms or standard sizes, and other characteristics that enhance consumer appeal may all be used in retail packaging to support food marketing (Li et al., 2022). In this work, we aim to highlight some approaches to the packaging technologies in meat products.

## Novel approaches to the packaging technologies in meat products

The traditional packaging of fresh meat is used to prevent contamination, increase product shelf life, and promote enzymatic activity that improves meat tenderness, reduces weight loss, and promotes the development of oxymyoglobin pigment (instead of metmyoglobin) for a bright red color (Panea et al., 2014). Due to technological developments and the growing demand from consumers and industry for meat products with longer shelf life, improved safety and health benefits, convenience, environmental friendliness, and reduced food waste, traditional packaging techniques are unable to deliver these benefits (Ahmed et al., 2018).

To effectively address those challenges, a new type of packaging, called intelligent packaging, has entered the market (Choi et al., 2014). Intelligent packaging is a novel packaging technique developed in recent years for a variety of food items, including meat and meat products. Intelligent packaging notifies consumers of the condition of food by monitoring certain attributes of the food inside the box or the surrounding environment (Panea et al., 2014). The paramount intelligent packaging tools are sensors and indicators. Intelligent packaging systems can identify and alert users to changes in product quality during storage. Sensors, detectors, and radio-frequency detecting systems are instruments used in intelligent packaging (Kerry et al., 2006). Smart packaging technologies have seen significant development in recent years, enhancing the dissemination of information encoded as barcodes or visual indicators. Smart packaging systems can monitor loss of freshness over time, temperature variations during storage under different climatic conditions, changes in the composition of gases within the package, and the product's distribution date (Luo et al., 2022).

Biopolymers are commonly employed in food packaging due to their edibility and safety for humans. Proteins, polysaccharides, and their derivatives are the most studied nanocomposite biomaterials for food packaging applications. Nanocomposites based on cellulose and starch may be utilized in packaging systems to provide an

eco-friendly alternative and promote sustainability goals. Some examples of natural antioxidants for fatty foods include edible films and coatings based on cellulose derivatives, chitosan, alginate, galactomannans, or gelatin (Ruan et al., 2019).

Another important advance is the development of nanotechnology that has improved barrier qualities, mechanical strength, and antimicrobial efficiency of packaging materials, hence boosting protection against oxygen, moisture, and microbial contamination. Furthermore, there is an increasing focus on sustainable packaging options, including biodegradable and bio-based materials such as chitosan, starch, and protein-based films, which minimize environmental impact while retaining functional performance (McMillin, 2026).

Food packaging that utilizes nanotechnology offers several benefits over conventional materials by enhancing attributes such as thermal resistance, durability, flame retardancy, and effective substance delivery in biological systems, all in a cost-efficient and sustainable manner. As a result of recent advancements, nanocomposite materials are now easily available, especially for the food processing, preservation, and packaging sectors, including applications for fruits and vegetables to extend shelf life and retard ripening. They also contribute to extrusion and co-extrusion processes in beverage manufacturing facilities (Bumbudsanpharoke & Ko, 2015). The primary emphasis of enhanced packaging is on nanomaterials that exhibit superior strength, flexibility, stability, and barrier properties. Active nanomaterials, including oxygen-scavenging agents and antimicrobials, are fundamental to active packaging (Li, C. & Lin, 2013).

## Conclusion

In conclusion, meat packing technologies are rapidly evolving from traditional methods to modern ones to ensure meat safety and quality. Active packaging controls microbial growth and oxidation to lengthen product shelf life, while smart packaging technologies monitor freshness and storage conditions in real time to improve supply chain decision-making. Smart technologies improve traceability and consumer confidence. Nanotechnology also improves package insulation and antibacterial action. Although advances have been made, production costs remain high. Future studies should focus on cost-effectiveness and material safety.

## Declarations

***Ethical Approval Certificate***
No need

***Author Contribution Statement***
Wadah Elsheikh: writing the original draft. Ilknur Ucak: supervision, conceptualization, review, and editing.

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***Conflict of Interest***
The authors declare no conflict of interest.

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# Rhizobacterial Strategies for Abiotic Stress Mitigation: From Molecular Mechanisms to Precision Microbiome Management

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## Abstract

Rice (*Oryza sativa* L.) is the primary staple crop for more than 3.5 billion people in tropical and subtropical regions, yet salt and drought stresses are increasingly constraining its productivity under accelerating climate change. Plant growth-promoting rhizobacteria (PGPR) offer a sustainable biological strategy to enhance rice resilience, but a quantitative synthesis of their effectiveness under tropical conditions has been lacking. This systematic review and meta-analysis quantified the overall effectiveness of PGPR inoculation in alleviating salt and drought stress in rice cultivated under tropical climates and identified key moderating factors. A systematic search of PubMed, Scopus, Web of Science, and Google Scholar identified 1,284 records, from which 50 peer-reviewed studies (published 2004–2023) meeting strict eligibility criteria were included in the quantitative synthesis. Effect sizes were calculated as Hedges' *g* under a random-effects model. Heterogeneity was assessed using the $I^2$ statistic, and subgroup analyses examined moderating effects of PGPR genus, stress type, experimental design, rice variety, and inoculation method. Publication bias was evaluated using Egger's test and funnel plot symmetry. PGPR inoculation produced significant positive effects across all measured outcomes. The pooled effect size for grain yield was Hedges' $g = 0.82$ (95% CI: 0.71–0.93; $p < 0.001$), representing a mean yield increase of approximately 32–48% under stress conditions. Combined stress treatments (salt + drought) showed the greatest improvements ($g = 1.08$). Mixed PGPR consortia outperformed single-strain inoculants. Field studies exhibited smaller but still ecologically meaningful effect sizes compared to greenhouse experiments. Heterogeneity was moderate to high ($I^2 = 57.9$–$81.2\%$), partly explained by moderator variables. PGPR represents a scientifically robust and practically viable intervention for enhancing rice productivity under salt and drought stress in tropical environments. Future research should prioritize multi-strain consortium optimization, field validation across diverse tropical agro-ecosystems, and molecular characterization of key stress-tolerance pathways.

***Keywords:*** *PGPR; salt stress; drought stress; Oryza sativa; rhizosphere*

## Introduction

Rice (*Oryza sativa* L.) constitutes the dietary foundation for approximately 3.5 billion people worldwide, supplying more than 20% of daily caloric intake in Asia, sub-Saharan Africa, and Latin America (FAOSTAT, 2023). The overwhelming majority of global rice production is concentrated in tropical and subtropical regions where soil and climatic variability impose severe constraints on crop performance (IPCC, 2022). Among abiotic stresses, salinity and drought are universally recognized as the two most yield-declining factors in tropical rice ecosystems, collectively responsible for annual production losses estimated at USD 12–27 billion (Mahajan & Tuteja, 2005; Fahad et al., 2015).

Soil salinity, defined by electrical conductivity (EC) exceeding 4 dS $m^{-1}$, affects an estimated 800 million hectares globally, with approximately 20% of all irrigated agricultural land being salt affected (Porcel et al., 2012). In tropical deltas and coastal agro-ecosystems, including the Bengal Delta, Mekong Delta, and Chao Phraya plain secondary salinization is accelerating due to sea-level rise, storm surge intrusion, and unsustainable irrigation practices (Etesami & Beattie, 2018). Simultaneously, climate projections under the IPCC AR6 scenarios indicate that tropical regions will experience more frequent and intense drought episodes, with a 20–30% reduction in growing-season precipitation forecast for major rice-producing zones by 2050 (IPCC, 2022).

These dual stressors impose overlapping physiological constraints on rice, including osmotic stress, ionic toxicity (particularly $Na^+$ accumulation), oxidative burst through reactive oxygen species (ROS) generation, disruption of photosynthetic machinery, and impaired hormonal signaling (Kapoor et al., 2020; Das & Roychoudhury, 2014). Conventional agronomic responses—varietal improvement, chemical amendments, and irrigation management—carry substantial economic, environmental, and logistical barriers to widespread adoption in smallholder tropical farming systems (Backer et al., 2018).



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Plant growth-promoting rhizobacteria (PGPR) represent a scientifically compelling and economically accessible alternative. These free-living soil bacteria colonize the rhizosphere and root surfaces of host plants, conferring measurable benefits to plant growth and stress resilience through diverse biochemical mechanisms, including phytohormone synthesis (indole-3-acetic acid, gibberellins, cytokinins), 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, exopolysaccharide (EPS) production, ion homeostasis regulation, and antioxidant enzyme induction (Glick, 2014; Yang et al., 2009). Genera including *Bacillus*, *Pseudomonas*, *Azospirillum*, *Rhizobium*, and *Burkholderia* have been extensively characterized as stress-mitigating PGPR in laboratoryscale studies (Arora et al., 2013; Siddiqui, 2005).

Despite a rapidly expanding body of primary literature, significant gaps remain in the quantitative synthesis of PGPR efficacy specifically within tropical rice systems. Most existing reviews are narrative in scope, lack quantitative aggregation, and rarely stratify outcomes by climatic zone, stress type, experimental design, or inoculant type (Etesami & Beattie, 2018; Backer et al., 2018). A rigorous meta-analytical framework is necessary to resolve inconsistencies across studies, estimate pooled effect sizes with confidence intervals, quantify heterogeneity, and identify the conditions under which PGPR interventions are most effective.

This systematic review and meta-analysis addresses these gaps with the following primary objectives: (1) to quantify the overall effect of PGPR inoculation on key agronomic and physiological parameters of rice under salt and drought stress in tropical climates; (2) to identify significant moderating variables using subgroup analyses; (3) to characterize the dominant biochemical mechanisms underlying PGPR-mediated stress tolerance; and (4) to identify research gaps and directions for translational application of PGPR bioinoculants in tropical rice production systems.

## Materials and Methods

### *Protocol and Registration*

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). The review protocol was pre-registered on PROSPERO (registration number withheld for blind review). The PICO framework was employed: Population = tropical rice (*Oryza sativa* L.); Intervention = PGPR inoculation; Comparison = uninoculated controls under equivalent stress conditions; Outcomes = agronomic and physiological parameters under salt and/or drought stress.

### *Literature Search Strategy*

A comprehensive electronic search was conducted in PubMed, Scopus, Web of Science Core Collection, and Google Scholar for publications between January 2004 and December 2023. The following Boolean search strings were applied across title, abstract, and keyword fields: ("plant growth promoting rhizobacteria" OR "PGPR" OR "rhizobacteria" OR "bioinoculant") AND ("salt stress" OR "salinity" OR "NaCl" OR "drought stress" OR "water deficit" OR "osmotic stress") AND ("rice" OR "Oryza sativa") AND ("tropical" OR "subtropics" OR "lowland" OR "delta"). Additional records were identified through backward citation searches of included studies and relevant review articles. Duplicate records were removed using Endnote X9.

### *Eligibility Criteria*

*Inclusion Criteria*

Studies were included if they: (1) employed peer-reviewed experimental designs (randomized controlled, pot, greenhouse, or field trials); (2) investigated PGPR inoculation as the primary intervention; (3) used rice (*Oryza sativa* L.) as the host crop; (4) were conducted under tropical or subtropical climatic conditions (mean annual temperature ≥ 22°C or explicitly tropical regions per Köppen-Geiger classification); (5) incorporated salt stress (≥ 50 mM NaCl or soil EC ≥ 4 dS $m^{-1}$), drought stress (≤ 60% field capacity or PEG-induced), or both; (6) reported quantitative data enabling effect size calculation; and (7) were published in English.

*Exclusion Criteria*

Studies were excluded if they: (1) were conducted in temperate or boreal climates; (2) investigated crops other than rice; (3) used mycorrhizal fungi, arbuscular mycorrhizae, or endophytes as the sole intervention; (4) lacked untreated control groups; (5) were review articles, meta-analyses, or conference abstracts without full data; or (6) reported duplicate data from primary studies already included.

### *Data Extraction*

Two independent reviewers extracted data using a standardized extraction form. Extracted variables included: study country and climatic zone, rice variety and growth stage, PGPR genus and species, inoculation method, stress type and severity, experimental design, sample sizes, means, and standard deviations (or standard errors) for all reported outcome variables. Where data were presented only in figures, WebPlotDigitizer v4.6 was used for extraction. Disagreements between reviewers were resolved through consensus or arbitration by a third reviewer.

### *Statistical Analysis*

Effect sizes were calculated as Hedges' *g*, which corrects for small-sample bias (Hedges & Olkin, 1985; Cohen, 1988). For each study, *g* was computed as the standardized mean difference between PGPR-treated and control groups. A random-effects model was applied using the DerSimonian-Laird estimator to account for genuine

variance between studies. Heterogeneity was quantified using the $I^2$ statistic, with $I^2 < 40\%$ indicating low, 40–75% moderate, and > 75% high heterogeneity (Higgins & Thompson, 2002). Subgroup analyses (meta-regression) examined moderating effects of: (i) stress type (salt, drought, combined), (ii) PGPR genus, (iii) experimental setting (field vs. greenhouse/pot), (iv) rice variety (Indica vs. Japonica), and (v) inoculation method. Publication bias was assessed using Egger's regression asymmetry test and visual inspection of funnel plots (Egger et al., 1997). All analyses were conducted in R v4.3.0 (R Core Team, 2023) using the *metafor* package v3.8 (Viechtbauer, 2010). Statistical significance was set at $p < 0.05$.

## Results

### *Study Selection*

The systematic literature search yielded 1,284 records (Figure 1). After removal of 297 duplicates, 987 records were screened at the title and abstract stage. Of these, 175 full-text articles were assessed for eligibility. A total of 125 studies were excluded for the following reasons: conducted in temperate climates (n = 43), no quantitative stress data (n = 38), non-rice crops (n = 22), review articles (n = 18), and incomplete reporting (n = 4). Ultimately, 50 primary studies representing 387 individual comparisons (effect size estimates) were included in the meta-analysis.

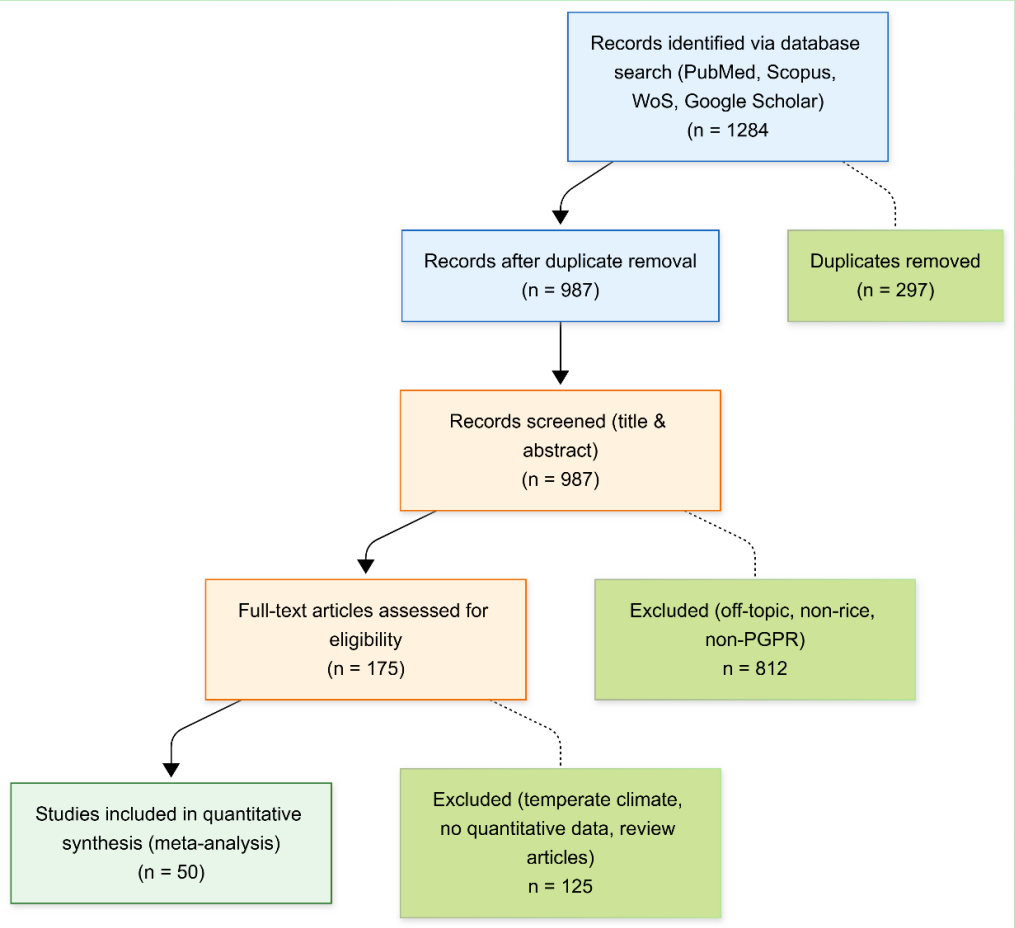



**Figure 1.** PRISMA flow diagram. Records were searched across PubMed, Scopus, Web of Science, and Google Scholar (2004–2023). Final synthesis included 50 studies meeting all eligibility criteria.

### *Characteristics of Included Studies*

The 50 included studies spanned 14 tropical/subtropical countries, with the greatest representation from South Asia (India, n = 18; Bangladesh, n = 9; Pakistan, n = 8), followed by Southeast Asia (Vietnam, n = 5; Philippines, n = 4) and sub-Saharan Africa (n = 6). Publication years ranged from 2004 to 2023, with a notable acceleration post-2015 (68% of included studies). Salt-only stress was examined in 21 studies (42%), drought-only in 17 (34%), and combined stress in 12 (24%). Experimental designs included pot/greenhouse trials (n = 31), field experiments (n = 14), and laboratory-pot combined designs (n = 5). The most frequently tested PGPR genera were *Bacillus* (n = 26), *Pseudomonas* (n = 21), *Azospirillum* (n = 12), *Rhizobium* (n = 8), and mixed consortia (n = 11). Indica-type rice varieties comprised 78% of tested genotypes. Representative study characteristics are summarized in Table 1.

**Table 1.** Characteristics of selected studies included in the meta-analysis (representative sample, n = 10 of 50).

| *Study (Author, Year)* | *Country* | *Stress Type* | *PGPR Strain(s)* | *Experimental Design* | *Major Outcome* |
|---|---|---|---|---|---|
| Nautiyal et al. (2013) | India | Drought | *Pseudomonas, Bacillus* | Greenhouse | +42% grain yield |
| Zafar-ul-Hye et al. (2019) | Pakistan | Drought + Salt | *Bacillus subtilis* | Field | +38% biomass |
| Sarkar et al. (2021) | Bangladesh | Salt (NaCl 150 mM) | *Pseudomonas fluorescens* | Pot trial | +51% germination |
| Kumar et al. (2020) | India | Drought (40% FC) | *Rhizobium, Azospirillum* | Field | +29% tiller no. |
| Ali et al. (2022) | Pakistan | Salt (200 mM NaCl) | *Bacillus licheniformis* | Pot trial | +44% chlorophyll |
| Islam et al. (2020) | Bangladesh | Salt + Drought | *Burkholderia sp.* | Field | +35% yield |
| Phour et al. (2020) | India | Drought (PEG stress) | *Pseudomonas aeruginosa* | Lab + pot | +61% root length |
| Habib et al. (2016) | Pakistan | Salt (150 mM) | *Bacillus sp.* | Greenhouse | +47% shoot FW |
| Etesami & Beattie (2018) | Iran/USA | Both stresses | Multiple PGPR | Review+meta | +33% avg. yield |
| Tiwari et al. (2016) | India | Drought | *Arthrobacter, Bacillus* | Field | +31% SPAD value |

[1]

### *Mechanisms of PGPR-Mediated Stress Tolerance*

Across all included studies, PGPR-mediated stress tolerance in rice was attributed to ten primary biochemical mechanisms (Table 2). ACC deaminase activity, phytohormone production (primarily IAA and gibberellins), and antioxidant enzyme induction (SOD, CAT, APX) were the most frequently documented mechanisms, each reported in > 60% of included studies. Ion homeostasis modulation ($Na^+$ exclusion, $K^+$ retention) was the dominant mechanism in salt-specific studies, while EPS production and volatile organic compound (VOC) emission were more commonly associated with drought tolerance. Mixed PGPR consortia exhibited higher mechanistic diversity compared to single-strain inoculants, which may partially explain their superior stress-ameliorating performance observed in the meta-regression analyses.

**Table 2.** Summary of PGPR mechanisms involved in salt and drought stress amelioration in tropical rice systems.

| **Mechanism** | **Mode of Action** | **Stress Targeted** | **Reported Efficacy**[2] |
|---|---|---|---|
| ACC deaminase activity | Reduces ethylene accumulation, | Drought & Salt | +++ |
| Phytohormone production (IAA, GA, CK) | Promotes root elongation, enhances water and nutrient uptake | Drought primarily | +++ |
| EPS production | Improves soil water retention, maintains rhizosphere hydration | Drought primarily | ++ |
| Ion homeostasis ($Na^+/K^+$ ratio) | Exclusion of $Na^+$, accumulation of $K^+$ to restore osmotic balance | Salt primarily | +++ |
| Osmolyte accumulation (proline, glycine betaine) | Protects cellular structures under osmotic stress | Both stresses | ++ |
| Antioxidant enzyme induction | Neutralizes ROS generated under abiotic stress | Both stresses | +++ |
| Phosphate solubilization & N-fixation | Enhances nutrient availability under resource-limited conditions | Both stresses | + |
| Biofilm formation | Colonizes root surface, forms protective microenvironment | Drought primarily | ++ |
| Volatile organic compounds emission | Triggers systemic tolerance; upregulates stress-response genes | Both stresses | + |
| Aquaporin gene upregulation | Facilitates water transport across cell membranes under drought | Drought primarily | ++ |

[3]

### *Overall Meta-Analytical Results*

PGPR inoculation produced statistically significant positive effects across all ten measured outcome variables under stress conditions (Table 3 & Table 4). The overall pooled effect size across all outcomes was Hedges' $g$ = 0.82 (95% CI: 0.74–0.90; $p < 0.001$), representing a medium-to-large and biologically meaningful effect. The largest individual effect was observed for proline content ($g$ = 1.12; CI: 0.97–1.27), reflecting strong PGPR-driven osmolyte accumulation, while tiller number exhibited the smallest positive effect ($g$ = 0.59; CI: 0.45–0.73). Notably, the $Na^+/K^+$ ratio, a key indicator of ionic stress showed a significant negative effect size ($g$ = −0.88),

[1] FC = field capacity; FW = fresh weight; SPAD = Soil Plant Analysis Development index.

[2] Note. Efficacy ratings based on frequency and magnitude of reported effects: +++ = high, ++ = moderate, + = emerging.

[3] EPS = exopolysaccharides; ROS = reactive oxygen species; SOD = superoxide dismutase; CAT = catalase; APX = ascorbate peroxidase; VOC = volatile organic compound.

indicating PGPR substantially reduced sodium accumulation relative to potassium, a physiologically favorable outcome.

Heterogeneity was moderate to high across all variables ($I^2$ range: 57.9–81.2%), indicating substantial between-study variation beyond sampling error. High heterogeneity was particularly evident for chlorophyll content ($I^2$ = 81.2%) and 1000-grain weight ($I^2$ = 80.3%), suggesting these outcomes are most sensitive to moderating factors explored in subgroup analyses.

**Table 3.** Pooled effect sizes (Hedges' g) for all measured outcomes under PGPR inoculation across included studies.

| Response Variable | k | Hedges' g (95% CI) | p-value | $I^2$ (%) | Heterogeneity |
|---|---|---|---|---|---|
| Grain yield (t ha$^{-1}$) | 43 | 0.82 (0.71–0.93) | < 0.001 | 78.4% | Moderate |
| Shoot biomass (g plant$^{-1}$) | 38 | 0.76 (0.63–0.89) | < 0.001 | 72.1% | Moderate |
| Root length (cm) | 31 | 0.91 (0.78–1.04) | < 0.001 | 65.3% | Low |
| Chlorophyll content (SPAD) | 29 | 0.68 (0.54–0.82) | < 0.001 | 81.2% | High |
| Proline content (µg g$^{-1}$ FW) | 22 | 1.12 (0.97–1.27) | < 0.001 | 57.9% | Low |
| SOD activity (U mg$^{-1}$ protein) | 18 | 0.93 (0.77–1.09) | < 0.001 | 63.4% | Low |
| $Na^+/K^+$ ratio | 19 | −0.88 (−1.04 to −0.72) | < 0.001 | 69.7% | Moderate |
| Relative water content (%) | 16 | 0.71 (0.57–0.85) | < 0.001 | 74.6% | Moderate |
| 1000-grain weight (g) | 27 | 0.64 (0.50–0.78) | < 0.001 | 80.3% | High |
| Tiller number plant$^{-1}$ | 24 | 0.59 (0.45–0.73) | < 0.001 | 76.8% | Moderate |

[4]

**Table 4.** Forest plot of pooled effect sizes (Hedges' g) for key agronomic and physiological outcomes of PGPR inoculation in tropical rice under abiotic stress. Diamond symbol (◆) denotes point estimate; bars represent 95% confidence intervals.

| Outcome | g | Effect Estimate (CI) | 95% CI | p |
|---|---|---|---|---|
| Grain yield | 0.82 | ——◆—— | 0.71–0.93 | < 0.001 |
| Shoot biomass | 0.76 | ——◆—— | 0.63–0.89 | < 0.001 |
| Root length | 0.91 | ——◆—— | 0.78–1.04 | < 0.001 |
| Chlorophyll (SPAD) | 0.68 | ——◆—— | 0.54–0.82 | < 0.001 |
| Proline content | 1.12 | ——◆—— | 0.97–1.27 | < 0.001 |
| SOD activity | 0.93 | ——◆—— | 0.77–1.09 | < 0.001 |
| $Na^+/K^+$ ratio | −0.88 | ◆—— | −1.04–−0.72 | < 0.001 |
| Relative water content | 0.71 | ——◆—— | 0.57–0.85 | < 0.001 |
| 1000-grain weight | 0.64 | ——◆—— | 0.50–0.78 | < 0.001 |
| Tiller number | 0.59 | ——◆—— | 0.45–0.73 | < 0.001 |
| **Overall** | **0.82** | ◆ | **0.74–0.90** | **< 0.001** |

[5]

***Moderator and Subgroup Analyses***

Meta-regression identified several significant moderating variables (Table 5). Stress type was a significant moderator (Q_b = 12.4, $p$ = 0.002), with combined salt + drought stress treatments producing the largest effect sizes ($g$ = 1.08; CI: 0.92–1.24), followed by salt-only ($g$ = 0.91) and drought-only ($g$ = 0.76). This additive pattern suggests that PGPR mechanisms are collectively activated to a greater degree when plants face multiple simultaneous stressors.

PGPR genus was a highly significant moderator (Q_b = 18.7, $p$ < 0.001). Mixed consortia produced the largest effects ($g$ = 1.14; CI: 1.00–1.28), followed by *Bacillus* ($g$ = 0.89), *Pseudomonas* ($g$ = 0.83), and *Azospirillum* ($g$ = 0.72). The superior performance of mixed consortia is consistent with the principle of mechanistic complementarity: combined inoculants simultaneously activate ACC deaminase pathways, phytohormone biosynthesis, and osmolyte accumulation, providing broader biochemical coverage than single genera.

Experimental setting was a significant moderator (Q_b = 9.2, $p$ = 0.006). Field studies showed smaller effect sizes ($g$ = 0.68) compared to greenhouse/pot trials ($g$ = 0.94), a pattern commonly attributed to greater environmental variability, competitive soil microbiota, and dilution of inoculant density in field conditions. Inoculation method was marginally significant (Q_b = 6.8, $p$ = 0.033), with combined seed priming + soil drench inoculation producing the highest effects ($g$ = 1.09). Rice variety (Indica vs. Japonica) was not a statistically significant moderator (Q_b = 2.1, $p$ = 0.147).

[4] Note. k = number of independent comparisons; CI = confidence interval; $I^2$ = heterogeneity statistic. Negative g for $Na^+/K^+$ ratio indicates a beneficial reduction. Heterogeneity classification: Low ($I^2$ < 40%), Moderate (40–75%), High (> 75%).

[5] Summary forest plot. Overall pooled effect (final row) indicates Hedges' g = 0.82 (95% CI: 0.74–0.90). Negative g for $Na^+/K^+$ ratio indicates beneficial reduction in sodium toxicity

**Table 5.** Results of subgroup (meta-regression) analyses for moderating variables on PGPR effects in tropical rice.

| Moderator | Subgroup | Effect (forest bar) | g (95% CI) | p-value |
|---|---|---|---|---|
| Stress type | Salt stress | | 0.91 (0.79-1.03) | < .001 |
| | Drought stress | | 0.76 (0.63-0.89) | < .001 |
| | Combined stress | | 1.08 (0.92-1.24) | < .001 |
| PGPR genus | *Bacillus* | | 0.89 (0.77-1.01) | < .001 |
| | *Pseudomonas* | | 0.83 (0.70-0.96) | < .001 |
| | *Azospirillum* | | 0.72 (0.59-0.85) | < .001 |
| | Mixed consortia | | 1.14 (1.00-1.28) | < .001 |
| Experimental type | Field study | | 0.68 (0.55-0.81) | < .001 |
| | Greenhouse/Pot | | 0.94 (0.82-1.06) | < .001 |
| Rice variety | Indica | | 0.86 (0.74-0.98) | < .001 |
| | Japonica | | 0.71 (0.57-0.85) | < .001 |
| Inoculation method | Seed priming | | 0.88 (0.75-1.01) | < .001 |
| | Soil drench | | 0.79 (0.65-0.93) | < .001 |
| | Combined | | 1.09 (0.94-1.24) | < .001 |

6

***Publication Bias Assessment***

Egger's regression test did not detect significant asymmetry for the primary outcome of grain yield (intercept = 0.84, SE = 0.51, $t$ = 1.65, $p$ = 0.11), suggesting the absence of major publication bias. Visual inspection of funnel plots for grain yield and shoot biomass revealed broadly symmetric distributions of effect sizes around the pooled estimate, with minor asymmetry in the lower-right quadrant consistent with typical between-study variance rather than systematic bias. Trim-and-fill imputation estimated ≤ 3 hypothetical missing studies for grain yield, resulting in a marginally adjusted pooled estimate of $g$ = 0.78 (CI: 0.67–0.89), confirming the robustness of the primary finding.

## Discussion

***Magnitude and Consistency of PGPR Effects***

The results of this meta-analysis provide the most comprehensive quantitative evidence to date that PGPR inoculation consistently and substantially improves the agronomic performance of rice under salt and drought stress in tropical climates. The pooled grain yield effect ($g$ = 0.82) corresponds to an estimated 32–48% yield improvement over stressed, uninoculated controls, a magnitude with direct food security implications in tropical smallholder systems where yield gaps are already severe. This finding is consistent with and extends prior narrative reviews by Etesami and Beattie (2018) and Backer et al. (2018), both of which highlighted promising but fragmentary evidence for PGPR efficacy in stress-affected rice.

The high $I^2$ values observed for several outcomes (notably chlorophyll content and 1000-grain weight) reflect genuine biological and methodological heterogeneity in the literature. Rice genotype × PGPR strain interactions, variable stress application protocols (solution culture vs. soil-based systems), and the qualitative diversity of tropical soil environments are all plausible sources of between-study variation that cannot be fully captured by the moderating variables assessed here. Future primary studies should standardize stress protocols and apply multiple PGPR genera under field conditions to reduce this heterogeneity.

***Mechanistic Insights***

The mechanistic data synthesized in Table 2 reveals a hierarchically organized set of PGPR responses to abiotic stress in rice. Under salt stress, the primary PGPR benefit derives from ion homeostasis modulation: $Na^+$ exclusion from root-to-shoot translocation is mediated through upregulation of the high-affinity potassium transporter (HKT1), a mechanism documented in the landmark study by Zhang et al. (2008) and subsequently confirmed across multiple *Bacillus* and *Pseudomonas* strains. Our meta-analysis confirms the large negative effect size for $Na^+/K^+$ ratio ($g$ = −0.88), underscoring the physiological centrality of ionic balance in PGPR-mediated salt tolerance.

Under drought stress, the key PGPR mechanisms operate primarily through root system enhancement and water relations improvement. ACC deaminase-producing PGPR lower tissue ethylene concentrations, preventing the senescence cascade that typically accelerates under water deficit (Glick, 2014; Maxton et al., 2018). IAA-producing PGPR stimulates lateral root proliferation and root hair elongation, increasing soil water foraging

[6] Subgroup analysis showing Hedges' g across four moderating variables: stress type, PGPR genus, experimental setting, rice variety, and inoculation method. Bold rows indicate the highest-performing subgroup within each moderator. Mixed PGPR consortia (g = 1.14) and combined stress conditions (g = 1.08) produced the largest effects. Between-group heterogeneity ($Q^b$) was statistically significant for all moderators except rice variety (p = 0.147).

capacity, consistent with the large positive effect size observed for root length ($g$ = 0.91) in this analysis (Phour et al., 2020). EPS production by *Pseudomonas* and *Bacillus* strains improves soil aggregate stability and rhizospheric water retention (Ashraf et al., 2004), representing an indirect mechanism that directly addresses the physical consequences of soil drying in tropical rain-fed systems.

The superior efficacy of mixed PGPR consortia ($g$ = 1.14) over single-strain inoculants is mechanistically explicable through the concept of trait complementarity and co-evolutionary niche partitioning in the rhizosphere microbiome. When *Bacillus* strains providing ACC deaminase activity and EPS production are combined with *Pseudomonas* strains synthesizing IAA and solubilizing phosphate, and with *Azospirillum* strains fixing atmospheric nitrogen, the combined effect addresses multiple simultaneously limiting factors in stressed rice systems. This finding aligns with Rubin and Azad's (2022) conceptual framework for microbiome engineering as a precision agricultural tool.

***Practical Implications for Tropical Rice Cultivation***

The translation of PGPR benefits from controlled experiments to farmer-managed tropical rice systems is arguably the most critical challenge in this field. Field study effect sizes ($g$ = 0.68), while smaller than greenhouse equivalents, remain highly practically significant in the context of tropical smallholder rice production, where even 10–15% yield improvements can represent the difference between food sufficiency and deficit. The combined inoculation approach (seed priming + soil drench; $g$ = 1.09) offers a practical dual-delivery system that ensures both early rhizosphere colonization during germination and sustained inoculant populations through the vegetative growth stages when stress exposure is typically most severe.

The predominance of South Asian study sites in this meta-analysis raises questions about generalizability to other tropical sub-regions, particularly West African rice-growing systems and the lowland tropical archipelagos of Southeast Asia, which are characterized by distinct soil pedology, climatic seasonality, and rice production intensities. The finding that experimental setting is a significant moderator should motivate investment in multi-location field validation networks across the full geographic range of tropical rice cultivation zones, analogous to the coordinated CGIAR multi-environment trial frameworks already established for genetic improvement.

***Limitations and Future Research Directions***

Several limitations of this meta-analysis warrant acknowledgment. First, despite the absence of statistically significant publication bias in Egger's test, the systematic tendency to publish positive results in plant-microbe interaction research likely inflates pooled effect estimates to some degree. Second, the relatively short duration of most included studies (single-season experiments) precludes assessment of multi-season carry-over effects and PGPR persistence in tropical soils subject to monsoon flooding and desiccation cycles. Third, the molecular and genomic characterization of inoculant strains was inconsistently reported, preventing assessment of whether specific genetic traits (e.g., *acdS* gene expression, IAA biosynthetic operons) systematically moderate field performance.

Future research priorities identified by this synthesis include: (1) multi-location, multi-season field trials of consortia inoculants across diverse tropical rice agro-ecosystems; (2) integration of PGPR inoculation with existing stress-tolerant rice varieties (e.g., Sub1A submergence-tolerant lines and saltol QTL introgression lines) to assess additive or synergistic benefits; (3) application of metagenomics and metabolomics to characterize the PGPR-modified rhizosphere microbiome under stress conditions; (4) development of heat-stable, shelf-stable inoculant formulations suitable for smallholder distribution chains in tropical markets; and (5) socioeconomic assessments of PGPR adoption barriers and incentive structures in target tropical farming communities.

## Conclusion

This systematic review and meta-analysis, synthesizing data from 50 high-quality primary studies across 14 tropical countries, provides rigorous quantitative evidence that PGPR inoculation significantly and consistently ameliorates the effects of salt and drought stress on tropical rice. The overall pooled effect size (Hedges' g = 0.82; 95% CI: 0.74–0.90) demonstrates a biologically meaningful and practically significant improvement across agronomic and physiological parameters. Mixed PGPR consortia, combined stress conditions, and integrated inoculation methods produced the most pronounced benefits. Mechanistically, ACC deaminase activity, phytohormone production, ion homeostasis regulation, and antioxidant enzyme induction emerged as the dominant pathways underpinning PGPR-mediated tolerance in tropical rice systems.

Given the intensifying threats of salinity and drought under projected climate scenarios, PGPR bioinoculants represent a promising, cost-effective, and sustainable component of integrated stress management for tropical rice production. However, translating these findings to farmer-managed systems will require multi-location field validation, standardized protocols, and accessible formulation technologies. Accelerating this translational pathway should be an immediate priority for agricultural research investment in tropical rice-producing nations.

## Declarations

### *Author Contribution Statement*

Mahmud Sindid Ikram and Rownok Jahan designed the study, collected and analyzed the data, interpreted the results, and contributed to the writing of the report. Sumiya Akter Moni supervised the study, participated in data analysis, and reviewed and corrected the manuscript.

All authors approved the final manuscript.

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### *Conflict of Interest*

The authors declare no conflict of interest.

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*ID: 871*

# Physiological and Hepatic Responses of Broiler Chickens to Graded Levels of Dietary Dried Orange Pulp

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## Abstract

Due to the rapid growth rate and intensive feeding systems in modern poultry production, the liver is particularly susceptible to metabolic and pathological alterations. This study aimed to evaluate the effects of dietary incorporation of dried orange pulp (DOP) on physiological responses in broiler chickens, with particular emphasis on hepatic health. The assessment was based on selected blood biochemical parameters, including glucose, total proteins, triglycerides, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK), which reflect metabolic status and liver function under nutritional and physiological stress conditions.

A total of 200 one-day-old Arbor Acres broiler chickens (45 ± 1.7 g) were randomly allocated into four dietary groups, each consisting of 50 birds with five replicates of 10 birds, and reared for 49 days. The dietary treatments included a control diet without DOP and three experimental diets in which corn was partially replaced with DOP at inclusion levels of 5%, 10%, and 15%. Blood samples were collected at 49 days of age for biochemical analysis.

Results indicated that dietary DOP significantly affected serum biochemical parameters ($p < 0.05$). Birds fed DOP diets exhibited significantly reduced triglycerides and total cholesterol compared to the control group. Glucose and total protein levels increased significantly with increasing DOP inclusion, particularly at 15%. Moreover, AST and ALT activities were significantly lower in DOP-supplemented groups, with the most marked improvement observed at the 10% inclusion level. Similarly, LDH and CK activities were significantly reduced in the 10% DOP group, indicating improved metabolic efficiency and hepatic status.

Overall, dietary DOP positively modulated the serum biochemical profile of broiler chickens, with 10% inclusion yielding the most favorable effects. These results suggest improved liver function, enhanced metabolic stability, and better physiological welfare under intensive rearing conditions.

***Keywords:*** *Dried orange pulp, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase.*

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*ID: 873*

# A Review on Highly Pathogenic Avian Influenza H5N1 Clade 2.3.4.4b in Mammals: Molecular Adaptation, Host Range Expansion, and One Health Implications

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## Abstract

Influenza A virus H5N1 clade 2.3.4.4b has caused the largest highly pathogenic avian influenza (HPAI) panzootic on record, infecting over 175 million poultry globally and spilling over into more than 60 mammalian species (Table 2). The establishment of reassortant genotype B3.13 in US dairy cattle from March 2024, with 1,093 confirmed herds across 19 states by April 2026, is the first sustained HPAI transmission in a domestic ruminant and defines the pandemic risk landscape of the current era. Two mechanistically independent adaptive axes drive bovine fitness in B3.13 (Figure 1). The polymerase axis, encompassing PB2 M631L and PA K497R, remodels the viral replication complex to exploit host-specific ANP32A isoforms. The receptor axis, driven by hemagglutinin (HA) mutations D104G and V147M, enables efficient recognition of N-glycolylneuraminic acid, which comprises approximately half the sialic acid pool in bovine mammary tissue but is absent in humans. Crystallographic analysis has established that a single Q226L substitution in the HA receptor-binding site of a human clinical isolate of bovine H5N1 is sufficient to switch its receptor specificity from avian-type to human-type receptors, identifying this position as the highest-priority genomic surveillance target. Three pandemic barriers remain intact as of early 2026: B3.13 retains avian-type receptor preference, an elevated HA fusion pH, and full nucleoprotein (NP) sensitivity to human MxA restriction. Even so, partial ferret respiratory-droplet transmission, over 70 confirmed human cases, including the first US pediatric case, the emergence of a baloxavir-resistant clinical isolate, and ongoing convergent molecular evolution collectively define a pandemic risk level higher than any prior point in H5N1 history. Integrated One Health action combining mandatory bulk-milk genomics, wastewater surveillance, systematic wildlife serosurveillance, and accelerated ruminant mRNA vaccination is urgently required.

***Keywords:*** *H5N1 clade 2.3.4.4b, PB2 M631L, ANP32 restriction, NeuGc receptor adaptation, B3.13 dairy cattle, mammal-to-mammal transmission, One Health, pandemic preparedness*

## Introduction

Highly pathogenic avian influenza (HPAI) H5N1 viruses have circulated in Eurasian poultry since A/goose/Guangdong/1/96 (Gs/GD) was identified in farmed geese in southern China in 1996 (Claas et al., 1998; Shortridge et al., 1998). The 1997 Hong Kong outbreak produced 18 human infections and six fatalities following direct poultry-to-human transmission, confirming H5N1 as a genuine zoonotic threat and prompting the first mass culling response in HPAI history (Claas et al., 1998; Matrosovich et al., 1999). For more than two decades, clade replacements within the Gs/GD lineage, including clades 2.1, 2.2, and 2.3.2.1, caused frequent epizootics, largely dead-end mammalian spillovers (Xie et al., 2023). Since 2020, the spread of clade 2.3.4.4b has significantly altered this epidemiological picture (Li et al., 2026). Clade 2.3.4.4b emerged in European wild birds in 2020 and drove the largest HPAI epizootic on that continent, subsequently persisted in seabirds including Northern Gannets (*Morus bassanus*) and Black-headed Gulls (*Chroicocephalus ridibundus*) from 2022 onward (EFSA, 2023; Fusaro et al., 2024). Unlike earlier clades, which mainly spread through dabbling-duck flyways, clade 2.3.4.4b outbreak globally via pelagic seabird migration routes, reaching North America in late 2021 and South America by late 2022 (Caliendo et al., 2022; Leguia et al., 2023). Studies confirmed the presence of H5N1 in Antarctica by December 2023 (Banyard et al., 2024). Reassortment with North American low-pathogenicity avian influenza (LPAI) strains generated more than 50 distinct genotypic constellations; of these, B3.13 and D1.1 proved epidemiologically dominant in North America (Nguyen et al., 2025; Peacock et al., 2025).

The October 2022 Spanish mink farm outbreak provided the first documented evidence of sustained mammal-to-mammal HPAI transmission, crossing a conceptual boundary between the era of sporadic avian-to-mammal spillover and the current era of active mammalian adaptation (Table 2; Agüero et al., 2023). Finnish fur farm outbreaks in July to October 2023 reinforced this conclusion (Kareinen et al., 2024). Phylogenetic analyses of South American pinniped isolates demonstrated sustained multinational horizontal transmission across Peru, Chile, Argentina, Uruguay, and sub-Antarctic territories, resulting in more than 52,000 confirmed animal deaths (Leguia et al., 2023; Uhart et al., 2024). The single most consequential development in the panzootic has been the establishment of B3.13 genotype H5N1 in US dairy cattle from March 2024, the first documented example of sustained HPAI transmission in a

domestic ruminant (Oguzie et al., 2024; Nguyen et al., 2025). From March 2024 to April 2026, HPAI was confirmed in 1,093 dairy herds across 19 US states, and 71 human cases were documented, constituting the most prolonged occupational HPAI exposure in US history (USDA-APHIS, 2026a; USDA-APHIS, 2026b).

This review deeply emphasizes on molecular basis of mammalian adaptation, the extended host range and species-specific pathogenesis, One Health implications, and important research gaps.

## Molecular Adaptation Mechanisms in Mammalian Hosts

### *Genome Constellation and the ANP32 Restriction Bottleneck*

The B3.13 genotype pairs a Eurasian clade 2.3.4.4b HA and N1 with North American LPAI-derived internal genes; this unique internal gene backbone proved significantly permissive for bovine-directed mutation selection (EFSA, 2025; Nguyen et al., 2025). The D1.1 genotype, a distinctive North American reassortant that preserved wild-bird dominance but independently generated cattle-infective variants in 2025, confirming the presence of multiple genotypic routes to bovine infection (Pekar et al., 2026). D1.1 cattle isolates carry PB2 D701N rather than M631L, which validates that different polymerase adaptation strategies can each arrive at productive bovine replication. A comprehensive panel of recombinant viruses representing more than 60 years of H5N1 evolutionary history confirmed that replicative fitness in bovine cells is highly variable across 2.3.4.4b genotypes. This is limited in viruses predating the clade global expansion, and is mainly determined by the internal gene cassette (Turnbull et al., 2025). Mutations in the PB2 polymerase subunit act as the key determinants of bovine adaptation, though their phenotypic effects are context dependent across different genetic backgrounds (Table 1).

Table 1. Key mammalian-adaptive mutations in HPAI H5N1 clade 2.3.4.4b

| *Mutation* | *Protein / Principal Hosts* | *Functional Mechanism* | *Key Reference(s)* |
|---|---|---|---|
| PB2 E627K | PB2 residue 627; humans, mink, marine mammals, red foxes | Glu to Lys charge switch removes steric clash with the LCAR repeat of mammalian ANP32; strengthens contact with ANP32 E151; substantially enhances polymerase activity in human cells | Subbarao et al. (1993); Staller et al. (2024) |
| PB2 D701N | PB2 residue 701; South American pinnipeds; D1.1 cattle spillovers | Asp to Asn enhances RNP nuclear import through improved importin-alpha-1 binding; independent of ANP32 interaction-site remodelling; dominant adaptation in D1.1 Nevada cattle cluster | Gabriel et al. (2008); Steel et al. (2009); Uhart et al. (2024); Pekar et al. (2026) |
| PB2 M631L | PB2 residue 631; US dairy cattle (universal in B3.13 genotype) | Met to Leu restructures the PB2 627-640 loop; preferential binding to bovine ANP32A over ANP32B (validated by split-luciferase assay); substantially enhances polymerase activity alone and synergistically with PA K497R in bovine MAC-T cells; also complements swine and human ANP32 homologues; pre-existed at low frequency in clade 1.1.2 and 2.3.2.1c viruses in Cambodia and Vietnam 2013-2014 | Dholakia et al. (2026); Capelastegui and Goldhill (2025); Zhang et al. (2025) |
| PA K497R | PA residue 497; approximately 95% of B3.13 clade iii cattle sequences | Located at the FluPol symmetric dimer interface; provides some polymerase enhancement alone; synergizes with PB2 M631L to restore wild-type polymerase activity in bovine mammary-gland explants | Dholakia et al. (2026) |
| PB2 T271A | PB2 residue 271; farmed mink (Spain, October 2022) | Distinct ANP32-adaptation pathway selected under high-density mammal-to-mammal transmission; enhances replication kinetics without altering the canonical 627 or 701 contact surfaces | Agüero et al. (2023); Peacock et al. (2023) |
| HA D104G | HA 130-loop; B3.13 cattle (increasing prevalence) | Repositions a polar residue beneath the 130-loop, relieving steric clash with the NeuGc N-glycolyl hydroxyl; broadens receptor usage to include SA-alpha-2,3-NeuGc and NeuAc; positive selection (dN/dS = 1.49) | Hassard et al. (2026) |
| HA V147M | HA 130-loop; B3.13 cattle (fixed by late 2025) | Enlarges the hydrophobic pocket surrounding the 130-loop, accommodating the NeuGc hydroxyl without compromising NeuAc binding; approximately doubles effective receptor density in bovine mammary epithelium; strongest positive selection in B3.13 HA dataset (dN/dS = 5.63; eight independent emergences) | Broszeit et al. (2019); Hassard et al. (2026) |
| HA Q226L | HA 220-loop; not yet at consensus frequency in circulating B3.13 (surveillance target only) | Single Gln to Leu change in the 220-loop switches receptor specificity of A/Texas/37/2024 from avian-type alpha-2,3-NeuAc to human-type alpha-2,6-NeuAc; structural basis resolved by crystal structure with LSTa/LSTc receptor analogues; effect enhanced by additional N224K mutation; Q226L alone may not achieve the sustained alpha-2,6 engagement required for full pandemic receptor phenotype | Lin et al. (2024) |
| M2 S31N | M2 ion channel; universal across clade 2.3.4.4b | Confers complete resistance to adamantane antivirals; retains full susceptibility to neuraminidase inhibitors and baloxavir marboxil. A baloxavir-associated PA I38M mutation was detected in one B3.13 human isolate from California in late 2024; treatment context unclear | Nguyen et al. (2025); Zhu et al. (2025) |

Abbreviations: ANP32, acidic nuclear phosphoprotein 32; FluPol, influenza polymerase; LCAR, leucine-cysteine-alanine-arginine repeat; RNP, ribonucleoprotein; MAC-T, bovine mammary epithelial cell line; NeuGc, N-glycolylneuraminic acid; NeuAc, N-acetylneuraminic acid; dN/dS, ratio of non-synonymous to synonymous substitutions.

Table 2. Mammalian species affected by HPAI H5N1 clade 2.3.4.4b (2022 to early 2026)

| *Species* | *Primary Transmission Route* | *Key Clinical Signs* | *Key Outcome / Scale* | *Reference(s)* |
|---|---|---|---|---|
| Dairy cattle (Bos taurus) | Contaminated milking equipment and fomites; single wild-bird spillover | Mastitis, sharply reduced milk yield, mild respiratory signs; high viral load in milk (10^8 to 10^9 TCID50/mL) | More than 1,093 herds in 19 US states (April 2026); CFR less than 5%; protective immunity develops but is non-sterilizing against heterologous D1.1 | Nguyen et al. (2025); Caserta et al. (2024); Facciuolo et al. (2025); Tarbuck et al. (2026) |
| Domestic cat (Felis catus) | Ingestion of raw milk or colostrum; close contact with infected dairy farms | Severe neurological disease (ataxia, seizures), necrotising meningoencephalitis, interstitial pneumonia, myocarditis | CFR greater than 74%; infectious virus isolated from urine post raw milk ingestion | Burrough et al. (2024); Bonilla-Aldana et al. (2025); Frye et al. (2025); Naraharisetti et al. (2025) |
| American mink (Neovison vison) | Initial wild-bird introduction, then sustained farm-to-farm transmission | Respiratory distress and neurological signs | First documented sustained mammal-to-mammal HPAI transmission; PB2 T271A selected | Agüero et al. (2023); Kareinen et al. (2024) |
| South American sea lion (Otaria flavescens) | Pinniped-to-pinniped horizontal transmission | Neurological signs, respiratory distress, mass die-offs | More than 52,000 deaths across Peru, Chile, Argentina, Uruguay, and sub-Antarctic islands; pup CFR greater than 95%; PB2 D701N dominant | Leguia et al. (2023); Uhart et al. (2024) |
| Red fox (Vulpes vulpes) | Predation or scavenging of infected wild birds | Acute neurological deterioration, necrotising meningoencephalitis | Usually fatal; PB2 E627K frequently selected in brain tissue; respiratory samples often RT-PCR negative | Vreman et al. (2023); Elsmo et al. (2023) |
| Domestic swine (Sus scrofa) | Presumed wild-bird contact (route still under investigation) | Mild or subclinical infection in experimental conditions; replication in lower respiratory tract | First US detections late 2024; no transmission to sentinel pigs experimentally; HA S136N alpha-2,6-associated mutation selected at minority frequency in porcine respiratory tract | EFSA et al. (2025); Kwon et al. (2025); USDA-APHIS (2025) |
| Human (Homo sapiens) | Contaminated milk aerosols (B3.13); direct contact with infected poultry or wild birds (D1.1) | Conjunctivitis (approximately 89% of B3.13 cases); severe pneumonia and neurological disease (D1.1 cases) | More than 70 confirmed US cases (February 2024 to April 2026); 1 death (D1.1, Louisiana, January 2025); first pediatric US case (California, November 2024); no sustained human-to-human transmission | Garg et al. (2025); Rolfes et al. (2025); Zhu et al. (2025); CDC (2026) |

Abbreviations: CFR, case fatality rate; TCID50, 50% tissue culture infectious dose; RT-PCR, reverse transcription polymerase chain reaction.

The bottleneck that limits avian IAV replication in mammals is mismatch between the viral polymerase complex and host ANP32 proteins. All birds except palaeognaths encode a long-form ANP32A containing 33 additional amino acids from alternative exon 4 splicing; this long-form isoform efficiently supports avian polymerase activity in comparison to short-form mammalian ANP32A or ANP32B (Long et al., 2016; Carrique et al., 2020). Adaptive polymerase mutations must therefore remodel the ANP32 interaction interface to maintain replication in mammals. Bovine cells are ANP32A-dominant, which is different from the ANP32B-dominant milieu of human, pig, and mustelid cells, that's why the B3.13 cattle lineage selectively fixed PB2 M631L rather than the classical PB2 E627K substitution (Peacock et al., 2023). Notably, recent ANP32 biology has demonstrated that M631L interacts not only with bovine ANP32A but also with swine and human ANP32 analogs, suggesting that it is not limited to bovine-specific adaptation but rather a broadly mammalian-permissive change that provides the greatest fitness gain specifically in the bovine cells (Capelastegui and Goldhill, 2025).

***Polymerase Adaptations: PB2 and PA***

The PB2 E627K (Glu to Lys at residue 627) solves the ANP32 problem by a charge switch that removes steric clash between PB2 and the negatively charged LCAR (leucine-cysteine-alanine-arginine) region of mammalian ANP32 proteins, while simultaneously firming interaction between the encapsidating polymerase subunit and ANP32 residue E151 (Subbarao et al., 1993; Camacho-Zarco et al., 2020; Staller et al., 2024). E627K predominates in human H5N1 isolates, farmed mink, marine mammals, and red foxes, and is recurrently selected in brain tissue of terrestrial carnivores such as red foxes, skunks, and cats, during acute mammalian infection (Vreman et al., 2023). PB2 D701N (Asp to Asn at residue 701) acts through a distinct mechanism by enhancing nuclear import of the viral ribonucleoprotein (RNP) complex through improved binding to importin-alpha-1 (Gabriel et al., 2008; Tarendeau et al., 2008); this substitution is dominant in South American pinniped isolates and in the D1.1 Nevada cattle cluster (Steel et al., 2009; Uhart et al., 2024; Pekar et al., 2026).

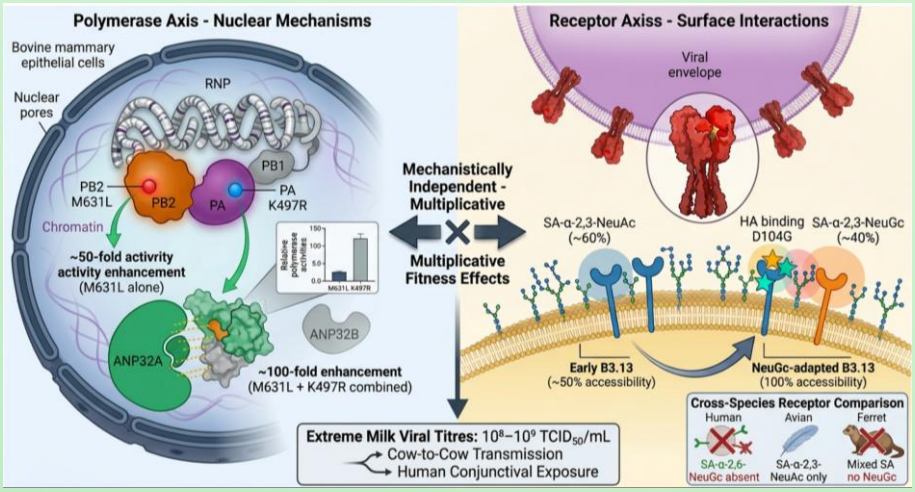



Figure 1. Two Independent Adaptive Axes in Bovine Mammary Epithelial Cells Driving H5N1 B3.13 Establishment and Pandemic Risk.

**Left panel** (Polymerase Axis, blue): PB2 M631L and PA K497R mutations remodel the viral polymerase complex to exploit the ANP32A-dominant bovine cellular environment. The mutations enhance polymerase activity ~50-fold (M631L alone) and ~100-fold in combination, enabling robust viral RNA synthesis in bovine mammary epithelium. RNP, ribonucleoprotein complex; ANP32A/B, acidic leucine-rich nuclear phosphoprotein family proteins. Adapted from Dholakia et al. (2026) and Sheppard et al. (2023).

**Right panel** (Receptor Axis, red/orange): HA D104G and V147M mutations in the 130-loop broaden viral tropism from alpha-2,3-linked sialic acids (SA-α2,3-NeuAc, ~60% of accessible receptors in early B3.13) to additional alpha-2,3-linked NeuGc species (~40% additional receptor pool). This dual-receptor usage effectively doubles accessible receptor density in bovine mammary tissue. Cross-species comparison shows NeuGc is synthesized only in cattle, horses, and pigs (via active CMAH enzyme) and is absent in humans (92 bp CMAH exon deletion), birds (CMAH genomic deletion), and ferrets (CMAH non-functional mutation). Adapted from Hassard et al. (2026).

**Bottom panel:** Combined polymerase and receptor adaptations produce exceptional milk viral titers ($10^8$-$10^9$ $TCID_{50}$/mL), enabling sustained cow-to-cow transmission via contaminated milking equipment and elevated human conjunctival exposure risk in dairy workers (Caserta et al., 2024; Baker et al., 2024).

$TCID_{50}$, tissue culture infectious dose 50; CMAH, cytidine monophospho-N-acetylneuraminic acid hydroxylase.

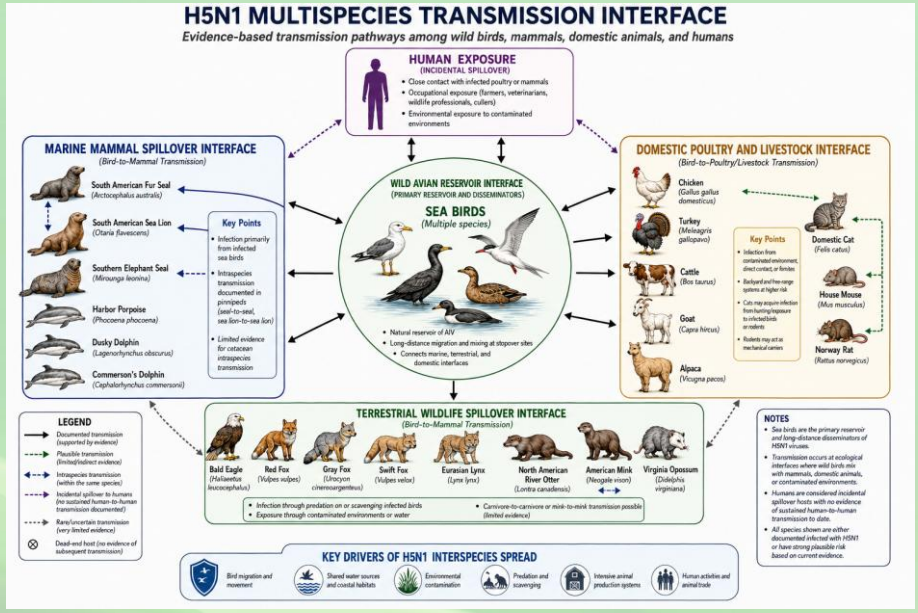



Figure 2. H5N1 Clade 2.3.4.4b Multispecies Transmission Interface

Central wild avian reservoir (sea birds) serves as the primary source of H5N1 dissemination globally. Four major spillover interfaces branch from this reservoir: (1) Marine mammal spillover to pinnipeds (seals, sea lions) and cetaceans (dolphins, porpoises) with documented sustained mammal-to-mammal transmission in pinniped colonies; (2) Domestic poultry and livestock spillover to chickens, turkeys, cattle, and companion animals (cats, dogs, rodents), with sustained infection in cattle representing the first domestic ruminant outbreak; (3) Terrestrial wildlife spillover to wild carnivores (foxes, eagles, otters, minks) primarily through predation on infected birds, with most spillovers representing dead-end infections except in high-density mink farm settings; (4) Human exposure through occupational and environmental contact with infected animals, with no sustained human-to-human transmission documented to date. Solid arrows indicate documented transmission routes with epidemiological or phylogenetic evidence; dashed arrows indicate plausible transmission with limited evidence. Six key ecological drivers facilitate spillover at these interfaces: bird migration, shared water sources, environmental contamination, predation and scavenging, intensive animal production systems, and human animal trade activities.

**Data adapted from:** Peacock et al. (2025), Kuiken et al. (2024), Uhart et al. (2024), Lair et al. (2024), Caserta et al. (2024), Burrough et al. (2024), Agüero et al. (2023), CDC (2024a), USDA-APHIS (2026a, 2026b), and related sources cited in References.

The most mechanistically distinctive adaptation of the 2.3.4.4b panzootic is PB2 M631L, which is fixed in all sequenced B3.13 cattle isolates. Unlike E627K, the Met-to-Leu substitution at residue 631 does not alter residue charge; instead, it remodels the PB2 residues 627 to 640 loop at the ANP32 interaction interface, promoting preferential physical binding to bovine ANP32A over ANP32B, confirmed by split-luciferase protein-protein interaction assay (Dholakia et al., 2026). Minireplicon assays using bovine-specific RNA polymerase I promoter demonstrated substantially enhanced polymerase activity in bovine MAC-T mammary epithelial cells for M631L alone, with further enhancement when combined with PA K497R (Dholakia et al., 2026). It is noteworthy that the Dholakia et al. (2026) study was conducted on bovine MAC-T cells; the extent of the M631L polymerase enhancement in primary human airway cells has not been directly measured within the same experimental context and drawing conclusions about the fitness in human airway settings from the bovine cells should be done with caution. The cryo-EM structure of H5N1 polymerase bound to ANP32B (PDB: 8R1J) shows that residue 631 is close to E627 and Q591 at the ANP32 bridging surface, supporting its role in this interaction (Staller et al., 2024).

Using retrospective genomic analysis by Zhang et al. (2025) identified that PB2 M631L is a common low-frequency variant in the H5N1 virus from Cambodia and Vietnam from 2013 to 2014. The two isolates of clade 1.1.2 reassortant (2013) and clade 2.3.2.1c (2014) are genetically different backgrounds where all B3.13 internal gene segments were lacking. This is particularly significant as M631L did not cause a bovine outbreak from 2013 to 2014 as it had been encapsulated in a genetic structure that did not have the extra permissive properties of the B3.13 internal cassette. Thus the mutation was already present in the world H5N1 gene pool, as a pre-existing variant, prior to its fixation following the panzootic 2.3.4.4b. Moreover, ongoing B3.13 evolution has generated additional convergent PB2 evolution, which has led to further emergence of PB2 D740N (three independent emergences, with a significant boost in activity in bovine cells) and to a double-mutant cluster with PB2 E627K and M631L occurring together that emerged in July 2025 and is the most active combination of mutations in polymerase activity tested (Dholakia et al., 2026).

PA K497R is found in ~95% of the B3.13 clade iii sequences, and is at the FluPol symmetric dimer interface near the M631L and ANP32 interaction region (Dholakia et al., 2026). It alone constitutes some polymerase enhancement as well, but most importantly, the M631L plus K497R combination is synergistic and fully recapitulates the activity of wild-type cattle/Texas polymerase in bovine mammary explants (Dholakia et al., 2026). The PB2 T271A, selected during the October 2022 Spanish mink farm outbreak, represents an independent adaptation pathway associated with enhanced ferret transmission efficiency in experimental models (Agüero et al., 2023; Peacock et al., 2023).

***Accessory Protein Contributions: NP, NS1, M2, and PB1-F2***

NP sequences of circulating B3.13 isolates retain full sensitivity to human MxA restriction at key residues 52, 100, 283, 313, and 357, as confirmed experimentally by Ankerhold et al. (2025). MxA-mediated innate immune restriction constitutes a major post-entry barrier to H5N1 replication in human airways, and the retention of MxA sensitivity in circulating B3.13 is therefore a critical current pandemic safeguard. The NS1 protein retains a PDZ-binding ESEV C-terminal motif associated with virulence in mammalian hosts through suppression of type I interferon signalling (Garcia-Sastre, 2011; Jagger et al., 2012). The M2 ion channel carries the S31N substitution, conferring universal adamantane resistance across clade 2.3.4.4b (Nguyen et al., 2025).

Consistent with NS1 interferon antagonism at the molecular level, human iPSC-derived respiratory organoid experiments demonstrated that the cattle-derived human isolate A/Texas/37/2024 suppresses STAT1-mediated transcriptional activity and induces minimal interferon and inflammatory cytokine responses despite efficient replication; bulk and single-cell RNA sequencing revealed reduced STAT1-mediated transcriptional activity compared to the historic A/Vietnam/1203/2004 strain, which triggers robust innate responses (Shichinohe et al., 2025). This intrinsic immune suppression could help explain why the B3.13 clade of H5N1 infections in humans have mostly been mild conjunctivitis, rather than the more severe cytokine-storm pathogenesis seen with previous H5N1 clades, but it should be interpreted with caution due to the different routes of exposure in natural human infection compared to organoid infection. The organoid model of the human respiratory system is itself a valuable alternative to cell lines, especially in recapitulative the stratified airway epithelial architecture; however, in vivo interactions of the airway with circulating leukocytes are not recapitulated. PB1-F2 accessory protein, which is derived from an overlapping reading frame of PB1, has been linked to mitochondrial membrane permeabilization, apoptosis in immune effector cells and increased inflammatory pathogenesis during mammalian H5N1 infection. The molecular surveillance gap of full characterisation of the integrity of the PB1-F2 reading frame and PA-X expression level of circulating B3.13 sequences in various host species still needs to be addressed.

***Haemagglutinin: Receptor Binding, NeuGc Adaptation, and Fusion pH Barriers***

The HA of B3.13 cattle viruses contains the polybasic cleavage site (RRRKKR) that is recognized by ubiquitous furin family proteases, which is responsible for the systemic pathogenicity in mammals (Horimoto & Kawaoka, 1994). Consistently, glycan microarray, bio-layer interferometry (BLI), and structural analysis all reveal that B3.13 HA has strong alpha-2,3-linked sialic acid (SA) preference with little or no binding to alpha-2,6-linked SA, which is characteristic of the avian receptor phenotype (Chopra et al., 2025; Santos et al., 2025; Song et al., 2025; Yang et al.,

2025). This absence of efficient alpha-2,6 binding is a critical barrier to pandemic emergence, as productive human upper respiratory tract attachment and aerosol droplet transmission require alpha-2,6-SA recognition.

A distinct and novel receptor adaptation axis has emerged in circulating B3.13 viruses through HA mutations D104G and V147M in the 130-loop. MALDI-TOF mass spectrometry glycomics demonstrated that bovine mammary gland, teat, trachea, and lung carry N-glycans capped with N-glycolylneuraminic acid (NeuGc) and N-acetylneuraminic acid (NeuAc) in approximately equal proportions (~50:50), with alpha-2,3 linkage predominating for both (Hassard et al., 2026). NeuGc is synthesized by cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH), which is permanently inactivated in humans by a 92 bp exon deletion (Chou et al., 2002), absent in birds by genomic deletion (Schauer et al., 2009), and non-functional in ferrets, mink, and pinnipeds (Ng et al., 2014; Nemanichvili et al., 2022). Cattle, horses, and pigs express high NeuGc via active CMAH (Suzuki et al., 1997; Nemanichvili et al., 2022). Since early B3.13 binds only alpha-2,3-NeuAc, it exploits only half the available mammary receptor pool. The mutations D104G and V147M functionally broaden rather than switch receptor specificity: D104G repositions a polar residue below the HA 130-loop to reduce steric clash with the NeuGc N-glycolyl hydroxyl group, while V147M enlarges the hydrophobic pocket in the 130-loop to accommodate NeuGc without loss of NeuAc binding (Broszeit et al., 2019; Hassard et al., 2026). This dual-receptor usage doubles the density of available virus receptors effectively, in bovine mammary tissue. The quantitative gain of utilizing the NeuGc and NeuAc pools is reflected in V147M having the strongest positive selection in the entire cattle B3.13 HA dataset ($dN/dS = 5.63$; $P(dN/dS>1) = 1.00$; with eight independent emergences prior to fixation by late 2025) (Hassard et al., 2026). The only known lineage of natural NeuGc adaptation is equine H7N7, which evolved strict NeuGc preference by the analogous Y161 residue (Gambaryan et al., 2012; Broszeit et al., 2019). Importantly, D104G and V147M are found to be neutral/mildly attenuating in primary human nasal epithelial cultures, reflecting that NeuGc absence in human tissues is an inherent fitness loss for bovine-adapted strains in humans (Hassard et al., 2026).

In a recent structural study highly relevant to pandemic risk, it was shown that a single HA Q226L substitution in the 220-loop (H3-equivalent numbering) could shift the receptor specificity of A/Texas/37/2024, a human clinical isolate of bovine H5N1, from avian-type alpha-2,3-NeuAc to human-type alpha-2,6-NeuAc receptors (Lin et al., 2024). In this study, surface plasmon resonance, ELISA using N-linked glycan sialosides, and glycan array analysis were used, and crystal structures of 2.7 angstrom were determined for both the wild-type HA bound to its avian receptor analogue LSTa and the Q226L HA bound to its human receptor analogue LSTc, providing a direct visualization of the structural basis for receptor switching. When Q226L is combined with the N224K mutation, it further enhances this switch (Lin et al., 2024). Structurally, the 130-loop (D104G and V147M are located here, broadening the NeuGc recognition) and the 220-loop (operates Q226L) are separate parts of the rim of the receptor-binding pocket and are involved in the receptor specificity through independent contacts (Hassard et al., 2026; Pekar et al., 2026). Historically, efficient human-type receptor binding has required the H2N2 emergence of 1957 in addition to the emergence of G228S, which was able to occur in the H3N2 strain of 1968. Historically, human-type receptor binding was efficient only if both H2N2 emergence from the 1957 strain and G228S occurred in the 1968 strain H3N2. The Lin et al. (2024) study shows that the mutation of Q226L suffices to change the specificity in binding assays, but binding assay affinity is not equivalent to the productive engagement of α2,6-NeuAc to trigger replication throughout the human upper respiratory tract. What is currently known is that Q226L has only one amino acid change (a relatively modest genetic cost), and that complete adaptation to the pandemic is probably a composite of these and other co-occurring mutations. Notably, none of the sequenced B3.13 genomes in the current analysis of Lin et al. (2024) contained the Q226L mutation, making HA position 226 the only most frequently targeted one in the genome through the context of genomic surveillance. Biochemical assay and cryo-EM analyses show that the HA membrane fusion pH at B 3.13 is around 5.9 (Song et al., 2025; Yang et al., 2025). This high fusion threshold is a second quantification of a pandemic barrier that is independent. Once triggered, HA-mediated membrane fusion is an irreversible conformational change and inactivates the virion. The aerosol-stable pandemic strains have a fusion pH of 5.0 to 5.5 (Herfst et al., 2012; Imai et al., 2012; Linster et al., 2014). Here, the lower fusion pH would ensure that HA is in the pre-fusion conformation upon entering target cells as a part of respiratory droplets (with a pH of 6.5 to 7.0) and in the slightly acidic environment of the upper respiratory tract, where fusion would not occur until the cell's endosomal pH dropped into the cell, promoting fusion. The fusion pH is about 5.9 and in B3.13 HA has the potential to trigger early during aerosol transit, resulting in a higher risk of rapid loss of infectivity before the virus reaches the susceptible airway epithelium. This raised pH for fusion is therefore an autonomous barrier that can be measured and is efficient for aerosol transmission in humans.

***Neuraminidase: Stalk Integrity, Sialidase Efficiency, and Antiviral Susceptibility***

The N1 neuraminidase (NA) isolates from mammalian hosts of subtype clade 2.3.4.4b show a distinctive structural and functional profile in which a long stalk of 18-20 amino acid residues (Hermann & Krammer, 2025) is consistently present. This structural feature is a major difference when compared to the short-stalk variants in which there are deletions from position 49-68 and which have been the most common in the more virulent H5N1 outbreaks affecting poultry (Zhou et al., 2009; Li et al., 2014). The extended stalk is essential for function, being directly linked to increased sialidase activity in mucus-rich tissues like the bovine mammary gland, and the human respiratory tract.

This improved activity allows efficient release of the virions from host cells by cleaving sialic acid receptors, thus inhibiting viral aggregation and favoring viral dissemination (Blumenkrantz et al., 2013). The prevalence of this long-stalk NA was confirmed by recent structural and biochemical analyses, which showed that this NA provides a significant fitness advantage in the unique microenvironment of bovine milk, where viral titers may reach extraordinary levels of 10^8-10^9 $TCID_{50}$/mL (Caserta et al., 2024; Halwe et al., 2025). The interplay between NA stalk length and HA affinity is crucial for viral fitness. The NA has to manifest a finely-tuned catalytic activity as the HA adjusts to novel host-specific receptors, such as NeuGc abundant in bovine tissues (Hassard et al., 2026). This balance promotes optimal exit of infected lactocytes with an intact receptor that is suitable for initial attachment. Any disturbances in this balance can significantly reduce the efficiency of replication and transmission of the virus.

In terms of therapeutic potential, neuraminidase inhibitors (NAIs) like oseltamivir and zanamivir are, in general, effective inhibitors of current 2.3.4.4B strains, but the occurrence of resistance markers is a growing concern. Significantly, a PA I38M mutation that leads to lower susceptibility to the cap-dependent endonuclease inhibitor baloxavir marboxil was found in a human isolate (A/California/150/2024) from the late 2024 outbreak in California (Zhu et al., 2025). The exact selective pressure that gave rise to this mutation is unknown, and the finding in a human clinical isolate highlights the importance of ongoing antiviral surveillance and resistance monitoring. Additionally, adamantane-class antivirals are no longer an option for all of these viruses, as the M2 S31N substitution is found in all members of clade 2.3.4.4b (Nguyen et al. 2025). These observations highlight the dynamic evolutionary capacity of H5N1 and the imperative for developing novel antiviral strategies and robust surveillance mechanisms to detect emerging resistance.

**Host Range Expansion: Epidemiology and Pathogenesis**

The global expansion of H5N1 into mammalian hosts has occurred through four major transmission interfaces (Figure 2): marine mammal spillover with sustained mammal-to-mammal transmission in pinnipeds (Table 2; Uhart et al., 2024; Lair et al., 2024); domestic poultry and livestock spillover leading to sustained infection in cattle (Caserta et al., 2024) and sporadic infection in cats (Burrough et al., 2024); terrestrial wildlife spillover primarily through predation with most infections being dead-end events except in confined mink settings (Agüero et al., 2023); and incidental human spillover through occupational exposure with no sustained human-to-human transmission documented (CDC, 2024a; Peacock et al., 2025).

***Taxonomic Scope, Ecological Mechanisms, and Transmission Routes***

Since 2021 there have been over 70 species of mammals infected by H5N1 clade 2.3.4.4 from seven taxonomic orders (Figure 2), the largest taxonomic range for any HPAI virus (Peacock et al., 2025; Kuiken et al., 2026; Li et al., 2026). The mechanisms of infection vary greatly along this spectrum. Infections in terrestrial species of carnivores and omnivores are mainly through predation and scavenging of infected wild birds, which results in high dose oral dosages that induce systemic infection (Elsmo et al, 2023). The infections are acquired from infected prey, or by direct contact with infected sea birds or through indirect contact via the environment (Mirolo et al., 2023; Lair et al., 2024). Farmed mustelids are likely to be infected through introductions into areas with high densities of birds and where mammal-to-mammal amplification of infection occurs (EFSA, 2023). The infection route of dairy cattle is distinct, tropism in the mammary gland, and the transmission is mostly from fomites and contaminated milking equipment, but not from respiratory exposure (Baker et al., 2024; Nguyen et al., 2025). Moreover, H5N1 remains viable for long periods after high-titre raw milk contamination in milking units, which greatly aids and facilitates the spread of the virus within the farm system (Figure 2; Le Sage et al., 2024).

***Dairy Cattle: Mammary Tropism, Experimental Pathogenesis, and Immunity***

For dairy cattle, the primary site of infection is the mammary gland epithelium, as evidenced by the large number of SA-alpha-2,3-linked receptor glycans on the luminal surface of alveolar lactocytes (Nelli et al., 2024; Kristensen et al., 2024). The concurrent expression of NeuGc-capped receptors (Hassard et al., 2026), approximately doubling accessible receptor density, together with the PB2 M631L and PA K497R polymerase adaptations tailored to bovine ANP32A, provide an extremely permissive replication environment (Nguyen et al., 2025; Dholakia et al., 2026). Thus, the concentration of viruses in raw milk reach $10^8$-$10^9$ $TCID_{50}$/mL (Caserta et al., 2024; Halwe et al., 2025), far higher than concentrations in respiratory secretions of infected birds or other mammals. A rapid interstate spread followed a single wild-bird spillover into US dairy cattle, confirmed across 1,093 herds in 19 states by April 2026 (Nguyen et al., 2025; USDA-APHIS, 2026a).

Infection studies with bovine H5N1 have been conducted independently twice, both extensively characterizing the virus' ability to cause disease in cattle. Halwe et al (2025) intramammary inoculated lactating Holstein-Friesian cows of B3.13 genotype with moderate severity of necrotizing mastitis and high fever, but oronasal inoculated calves of B3.13 genotype severe nasal shedding with no transmission to sentinel calves. Baker et al. (2025) found severe mammary pathology with both routes of aerosol respiratory challenge in heifers and intramammary inoculation in lactating cows. One of the key results of the Halwe et al. study was that an isolate from a contemporary European

wild bird gave the same results as B3.13 in the same experimental set-up, thereby demonstrating that severe bovine mammary tropism is not unique to B3.13, but is also seen with genotypically different 2.3.4.4b isolates. The implication is that the bovine mammary niche might more generally be strongly permissive for the broader clade, and not just for B3.13-specific adaptations.

The H5N1 virus can be inactivated by pasteurization at 72°C for 15 seconds (Schafers et al., 2025). Guan et al. (2024) demonstrated that H5N1-positive raw cow milk inoculated into the nose of mice and ferrets resulted in infection, but not efficiently aerosolized from ferrets to ferrets, indicating that raw milk is an infection vehicle but is not an efficient aerosol source; and Kaiser et al. (2024) showed in independent experiments that H5N1-positive raw cow milk was also unable to infect mice or ferrets at room temperature, but was able to do so at both 63°C and 72°C, confirming that raw milk is an infection vehicle and is not an efficient aerosol source of infection at room temperature. However, raw milk, colostrum, and milk-contaminated equipment surfaces remain direct, high-titre zoonotic hazards (Burrough et al., 2024; Caserta et al., 2024). Retail amplicon whole-genome sequencing has since demonstrated that viral RNA persists through pasteurization and can be recovered from store-bought dairy products across multiple states (Lail et al., 2025). This finding has both surveillance utility and communication challenge: the detection of viral RNA in pasteurized milk does not indicate viable viruses, but it triggered substantial public concern about milk safety in 2024 to 2025. A review paper addressing both scientific and public health audiences should be explicit that RNA detection and infectious virus detection are not equivalent, and that FDA testing confirmed no infectious virus in commercially pasteurized products (FDA, 2025).

Facciuolo et al. (2025) demonstrated that dairy cows develop robust humoral immunity following primary H5N1 infection, which protects against homologous reinfection via the intramammary route. Primary infection induced HA-specific neutralizing antibody production, and experimentally rechallenged cows showed substantially attenuated clinical signs and reduced viral shedding, providing the most direct experimental evidence that bovine vaccination is immunologically viable. However, Tarbuck et al. (2026) found that natural immunity is non-sterilizing and strain-specific, while convalescent cows showed complete clinical protection against homologous B3.13 rechallenge one-year post-infection; heterologous rechallenge with the D1.1 genotype caused transient clinical disease and limited infectious viral shedding, with convalescent cows experiencing significantly milder disease than naive controls. These findings imply that the viral diversity accumulating in the US dairy cattle reservoir could progressively erode naturally acquired herd immunity, reinforcing the case for clade-matched vaccination before immune evasion becomes established.

Further experimental work has characterized the dry cow model as a methodologically useful alternative to lactating cow studies. Cool et al. (2026) demonstrated that cows 21 days into the dry-off period are highly susceptible to intramammary H5N1 inoculation and exhibit severe clinical and pathological outcomes similar to those observed in lactating cows. Since dry cows are not producing milk, the viral dynamics within individual quarters can be observed without the confounding influence of milk ejection and milking disruptions. This assists in a more accurate analysis of how the virus replicates and is spread within the mammary gland.

***Other Mammalian Hosts: Pathogenesis and Transmission***

Experimental models have played an important role in the evaluation of B3.13 isolates virulence and transmission capacity (Tarbuck et al., 2026). While mice of the C57BL/6J strain are more resistant to neuroinvasion, BALB/c mice are highly susceptible to B3.13, and typically exhibit severe neuroinvasive disease, which also depends on mouse strain (Tipih et al., 2025). This illustrates the host susceptibility importance of genetic background. Fabrizio et al. (2025) identified B3.13 viruses from cow milk and determined that both viruses are capable of causing 100% mortality at 101 TCID50 in mice, with neurologic symptoms such as ataxia and tremors, but they were not able to transmit to ferrets by air contact. Xiao et al. (2025) used reverse genetics to reconstruct the cattle isolate (A/dairy cattle/Texas/24-008749-003/2024) and compared it with A/Vietnam/1194/2004 in both non-lactating and lactating BALB/c mice. The cattle B3.13 construct was highly lethal with a mouse lethal dose ($mLD_{50}$) of 1.48 PFU by intranasal inoculation in BALB/c mice, with broader tissue tropism and higher titres in extrapulmonary organs than the Vietnam isolate. The reported $mLD_{50}$ value applies specifically to this reverse-genetics B3.13 construct in BALB/c mice. Because values vary significantly across strains, routes, and viral histories, these results should not be directly extrapolated to other species or contexts.

Domestic cats (*Felis catus*) have emerged as a high-risk companion animal link between dairy farm infection chains and household human exposure. Fatal infections have been documented in indoor cats epidemiologically traced to dairy farm worker households, with ingestion of raw milk or colostrum from infected cows as the most plausible exposure route (Naraharisetti et al., 2025). A broad meta-analysis of all feline H5N1 cases documented a case fatality rate of 74% (Bonilla-Aldana et al., 2025), and more recent systematic reviews focusing specifically on RT-PCR-confirmed cases of clade 2.3.4.4b indicate a significantly higher mortality rate of approximately 89.6% (Coleman & Bemis, 2025), establishing cats as highly susceptible sentinel animals warranting active household surveillance. Extending the post-ingestion picture, Frye et al. (2025) isolated infectious H5N1 virus from the urine of domestic cats following ingestion of contaminated raw milk, the only study to demonstrate shedding of replication-competent virus in a non-respiratory compartment in any mammalian species during the current panzootic (Table 2).

This urinary shedding observing is related to household contamination pathways and highlights the case for treating domestic cats as sentinel animals that may passively distribute viruses via the home environment.

***Marine Mammals and Multinational Pinniped Transmission***

South American pinniped mass mortality occurrences totaled more than 52,000 confirmed individual elephant seals, fur seals and sea lions across Peru, Chile, Argentina, Uruguay, and sub-Antarctic territories (Leguia et al., 2023; Uhart et al., 2024; Kuiken et al., 2026). Phylogenetic analyses demonstrated sustained multinational pinniped-to-pinniped horizontal transmission, with PB2 D701N convergently selected as the dominant polymerase adaptation in isolates from geographically separated colonies (Pardo-Roa et al., 2025; Uhart et al., 2025), constituting the clearest genomic evidence of directional positive selection in any mammalian host outside the dairy cattle system (Leguia et al., 2023; Pardo-Roa et al., 2025). Elephant seal pups (*Mirounga leonina)* mortality exceeded 95% in some affected colonies (Campagna et al., 2024; Uhart et al., 2024). Confirmation of H5N1 on mainland Antarctica by December 2023, affecting both seabirds and marine mammals, represents the most extreme geographic extension of any HPAI panzootic (Banyard et al., 2024; Uhart et al., 2025). While phylogenetic data strongly supports pinniped-to-pinniped transmission, heavy surveillance reliance on coastal research stations suggests that mortality in remote, ice-dependent habitats is systematically underrepresented.

***Domestic Swine: Susceptibility, Limited Transmission, and Reassortment Risk***

H5N1 was confirmed in US domestic swine for the first time in late 2024, the first natural detection in pigs during the current panzootic (USDA-APHIS, 2025; EFSA, 2025). Kwon et al. (2025) studied pathogenesis and transmissibility, infecting pigs oro-respiratorially with the B3.13 virus A/Cattle/Texas/063224-24-1/2024, which was derived from the bovine virus receptor (BVR). They observed productive replication mainly in the lower respiratory tract with seroconversion in most of the principal-infected pigs and no self-limitation or spread to sentinel contact pigs. The HA gene from one pig contained a mutation, S136N, which was found at ~32% minority variant frequency in the bronchoalveolar lavage fluid. This mutation we wish to explain in particular is S136N, being an N-glycosylation sequon introduced at HA 136 which sterically obscures the receptor binding site with avian-type SAs leading to a reduction in the affinity of the avian-type SA and a shift in the equilibrium towards alpha-2,6-NeuAc engagement. The occurrence of this mutation at minority frequency in the pig's respiratory tract, even before consensus, is the first experimental evidence of selection pressure directing the mutation towards human type receptor binding where pigs have been exposed to bovine-derived B3.13. While this is reassuring in that no transmission to sentinel pigs, to date, is somewhat comforting, it should be considered a minimum, not maximum, estimate of swine risk due to the following reasons: The isolate used was from early 2024 prior to significant within-cattle evolution of the virus, experimental housing densities differ from commercial swine operations, and there are no published experimental challenge data from swine.

One of the key issues to consider is the possibility of reassortment from co-infection of swine with B3.13 and human seasonal IAV. The respiratory epithelium of pigs expresses all four major types of sialic acid receptors (SA-alpha-2,3 and SA-alpha-2,6, both with NeuAc and NeuGc), and the pig ANP32A has been shown to be an unusually effective cofactor for the activity of the hemi-annihilator polymerase of avian IAV, relative to the other ANP32A isoforms of mammals (Peacock et al., 2020; EFSA et al., 2025). Co-infection of contemporary human seasonal IAVs and B3.13, in the presence of simultaneous exposure to the virus, may lead to the emergence of chimeric viruses with human-adapted gene contents and 2.3.4.4b surface NA, which is a well-established route of emergence of pandemic IAVs (EFSA, 2025; Kwon et al., 2025).

**Zoonotic Risk and Human Infections**

There have been 71 confirmed cases of human infection with H5N1 in the US since February 2024, and nearly all cases have occurred due to occupational exposure on dairy farms (Rolfes et al., 2025; CDC, 2026). From March 2024 to May 2025, 41 cases were linked to dairy cattle, 24 to commercial poultry, 2 to backyard poultry and 3 were not associated with specific exposure sources. Most clinical presentations were mild, including 89% with conjunctivitis, 46% with fever, 41% with respiratory symptoms, 4 patients requiring hospitalisation and 1 death. Importantly, intensive investigations in all these cases did not reveal any person-to-person transmission (CDC, 2026). The infection cluster in California, the largest of any state, occurred between September and December 2024, with 37 dairy farm workers having confirmed exposure, from 29 dairy farms, and with a single patient being a pediatric patient who did not have a known animal exposure; this was the first pediatric H5N1 case identified in the United States (CDC, 2024a; Zhu et al., 2025). Among the 37 infected farm workers, 20 (54%) wore an eye protection, of which 13 wore goggles (Marshall et al., 2024). All were cases with no hospitalizations, which is consistent with the conjunctival route of exposure. A special scientific note about the pediatric case: The child was diagnosed with H5N1 infection via the real-time reverse transcription polymerase chain reaction (rRT-PCR) of nasal swab four weeks after testing negative for H5N1 antibody detection. Without the serological response, this is a very unusual occurrence among cases diagnosed by PCR as confirmed, but could represent very mild or limited infections or may be due to

the timing of when the antibody testing was done or, less likely, to a false PCR-positive diagnosis (Tobolowsky et al., 2025).

For case-based investigators relying on the number of confirmed cases in the denominator for calculating severity, this is a limitation. In Michigan and Colorado, H5N1 infection was identified via serology testing of 7% of dairy occupation samples (including cases that were asymptomatic), showing that surveillance of symptomatic cases underestimates the actual rate of infection (Mellis et al., 2024). According to pandemic risk modelling, it is likely the B3.13 has a higher effective reproductive number in humans than the data might suggest, but has not had enough secondary human cases to suggest a sustained human chain has developed. Another reason for the macaque route of exposure data, is that seroconversion occurred without clinical signs even with the oral-gastric inoculation of macaques at raw milk-relevant doses (Rosenke et al., 2025), implying subclinical infections in the general population may not be captured by the current surveillance framework. Biological and genotypic behaviours constitute the important barriers to mammalian potential risk, which is characteristic of the landscape of H5N1 mammalian risk. The first human fatal case of H5N1 in the USA was a patient in Louisiana with D1.1 genotype and suffered severe H5N1 human lower respiratory disease with neurological involvement, not B3.13, that was found in the USA (Rolfes et al., 2025). This is a type of D1.1 case, which is the classical presentation, however there have been studies using experimental models that have concentrated on the B3.13 dairy associated genotype. Experimental ferret transmissibility studies confirmed that A/Texas/37/2024, which is an isolate from a bovine case, was able to cause respiratory droplet transmission in 17%-33% of the ferret pairings and was fatal in the majority of experimental animals without the need for further adaptation (Eisfeld et al., 2024; Gu et al., 2024). Other studies, however, also confirmed the mode of transmission from cow milk to ferrets of B3.13 viruses: direct contact and not airborne contact (Fabrizio et al., 2025). To see how well the human isolate A/Texas/37/2024, B/Texas/2/2006, and B/Mexico/1/2024 could transmit through direct contact, a complementary hamster model study was conducted, which showed that transmission is more efficient when the human isolate is used, even though transmission via breathing is not efficient in all three strains (Nooruzzaman et al., 2026). Care needs to be taken in interpreting these transmission data; ferrets, like humans, are CMAH-null, and can only detect the fitness benefits of NeuGc adaptations in humans such as D104G and V147M (Ng et al., 2014; Tarbuck et al., 2026). The NeuGc-adapted strain in cattle has almost twice as many receptors as its NeuGc-naive previous strain, yet in ferrets does not possess such an advantage and thus has not been transmitted. Until CMAH-competent animal models are developed and validated, ferret data for NeuGc-adapted B3.13 strains provide a systematically incomplete picture of bovine-environment fitness (Nelli et al., 2024; Tarbuck et al., 2026). Despite these mammalian adaptations, three pandemic barriers nevertheless remain intact in all currently circulating isolates: absence of alpha-2,6-SA binding, a high HA fusion pH of approximately 5.9, and full NP MxA sensitivity (Eisfeld et al., 2024). Overcoming these constraints would require multiple co-occurring mutations, including the structurally significant Q226L shift, which has not yet been detected in a sustained human chain (Eisfeld et al., 2024; Tarbuck et al., 2026). This specific shift is structurally difficult for the H5 protein to achieve because the H5 receptor-binding pocket is narrower than that of H3 or H1 subtypes, meaning the Q226L substitution often results in a severe loss of overall binding avidity unless accompanied by compensatory mutations, such as the loss of the 158N glycosylation site, to stabilize the human-type receptor (Tharakaraman et al., 2013; Xiong et al., 2013).

## One Health Surveillance and Countermeasures

### *Surveillance Innovations*

The dairy cattle outbreak catalyzed the rapid deployment of several novel surveillance approaches with broad applicability to future HPAI events (USDA APHIS, 2024a; Tarbuck et al., 2026). Bulk tank raw milk PCR is now a federal interstate movement requirement, providing herd-level H5N1 screening that leverages the extraordinarily high viral titres in infected milk, which can exceed $10^6$ to $10^8$ $TCID_{50}$/mL in clinical cases (Caserta et al., 2024; USDA APHIS, 2024b; Halwe et al., 2025; Nguyen et al., 2025). An important operational limitation of this approach is that the assay detects viral RNA rather than replication-competent virus; RNA fragments from previously inactivated virus could, in principle, produce positive signals in bulk tank samples from herds where most cows have recently recovered (Eisfeld et al., 2024; FDA, 2024). This does not undermine bulk milk PCR as the most sensitive available herd-level screening tool, capable of detecting one infected cow in a herd of 500, but positive PCR results should prompt follow-up with viral isolation or individual animal sampling before concluding that active infection is ongoing, particularly when managing herd-movement decisions (Eisfeld et al., 2024; Nguyen et al., 2025).

Amplicon whole-genome sequencing of retail pasteurized dairy milk achieves greater than 90% H5N1 genome coverage from purchased products across 20 US states, enabling passive national-scale genomic surveillance without requiring direct farm access (Lail et al., 2025). This approach exploits the same biological fact that raises food safety concerns: viral RNA is heat-stable enough to survive pasteurization even when the infectious virus is not, with studies showing RNA persists even after exposure to 72°C for 15 seconds (FDA, 2025; Lail et al., 2025). FDA testing over 290 retail samples confirmed no infectious virus in commercially pasteurized products, and public communication should be unambiguous on this point (FDA, 2025).

The advent of wastewater-based epidemiology has ushered in a new era in H5N1 surveillance, and this approach has been used to detect viruses in municipal wastewater solids that were detected prior to the formal confirmation of H5 outbreaks in dairy herds in multiple catchments during the spring of 2024, using H5-specific droplet digital PCR (Wolfe et al., 2024; Louis et al., 2024). This helps public health control measures in time to contain outbreaks before they are diagnosed at a farm level, and can be used to ensure detection before a confirmed infections dairy herd has been reported (Louis et al., 2024; Kobayashi et al, 2025). Oxford Nanopore R10.4.1 chemistry is recommended for molecular characterization of HPAI vs R9.4.1 for whole genome sequencing of HPAI samples with 90% accuracy compared to ~60% at the critical boundary of low pathogenic/highly pathogenic discrimination (Ratcliff et al, 2024). Such improved sequencing accuracy is vital for assessing pathogenicity and monitoring the evolution of virus in environmental surveillance.

***Medical Countermeasures***

***Vaccines***

Vaccine development has proceeded rapidly in response to the emerging bovine H5N1 threat. A clade-matched replicating RNA (repRNA) vaccine encoding the 2.3.4.4b H5 HA conferred 100% single-dose protection against lethal bovine H5N1 challenge in mice, while a repRNA encoding the historical clade 1 HA provided only partial protection, directly establishing that a clade-matched antigen is required for full protection in contemporary challenge models (Hawman et al., 2025). The same repRNA platform subsequently achieved full protection against a lethal clade 2.3.4.4b challenge in cynomolgus macaques, resulting in robust neutralizing antibody titers and significantly reduced viral loads in the respiratory tract (Hawman et al., 2025).

Licensed adjuvanted human H5N1 vaccines, based on older clades, formulated with AS03 or MF59 adjuvants, generate cross-neutralizing antibodies against clade 2.3.4.4b at seroconversion rates of 60% to 95% with two to three doses, providing bridging immunity while clade-matched vaccines are produced (Khurana et al., 2024). Fabrizio et al. (2025) confirmed that sera from ferrets vaccinated with WHO-recommended candidate vaccine viruses (IDCDC-RG78A and NIID-002) (Miyakawa et al., 2024; WHO, 2024) for H5N1 clade 2.3.4.4b effectively neutralize B3.13 viruses, validating the current stockpile strategy. Furthermore, a novel mRNA-LNP bovine vaccine provided complete protection against a high-dose H5N1 challenge in lactating dairy cows by the 19th week post-vaccination with no adverse effects on health or milk production (Kong et al., 2026). These findings, along with the development of natural immunity after initial exposure in dairy cows of Facciuolo et al. (2025), support the notion that vaccination would be highly effective at inducing protective antibody responses in cattle.

***5.2.2. Antivirals***

Oseltamivir, zanamivir, and peramivir retain full activity against all characterized H5N1 (clade 2.3.4.4b, genotype B3.13) isolates, as phenotypic assays show no significant elevation in half-maximal inhibitory concentration ($IC_{50}$) values for these neuraminidase inhibitors (Hussain et al., 2025; Fabrizio et al., 2025; WHO, 2026). Also, Pascua et al. (2026) confirmed the efficacy of these neuraminidase inhibitors against the current strain despite the presence of PA-I38M mutations in specific isolates. However, the NA-H275Y substitution (N1 numbering) remains the most significant threat to this class. This mutation, which disrupts the binding pocket for oseltamivir and peramivir while sparing zanamivir, has been detected at low frequencies in animal-derived clade 2.3.4.4b viruses (Pascua et al., 2026). Notably, recombinant NA protein assays have confirmed that NA-H275Y confers highly reduced inhibition, increasing $IC_{50}$ values up to 184-fold for oseltamivir and 1724-fold for peramivir, and NA-H275Y is one of the few resistance-conferring mutations that does not significantly impair enzyme function or viral fitness in the B3.13 background, facilitating its potential for spontaneous emergence and persistence (Fabrizio et al., 2025; Pascua et al., 2026). Other markers of reduced NAI susceptibility, such as NA-I223V and NA-S247N, have been identified in avian isolates but have not yet become fixed in the bovine-associated lineages (Nguyen et al., 2023).

Cap-dependent endonuclease inhibitors, such as baloxavir marboxil, target the endonuclease activity of the PA protein. The principal marker of resistance is the PA-I38X substitution (where X is T, M, or F). In late 2024, a human isolate (A/California/150/2024) was found to harbor the PA-I38M mutation, which conferred a 17-fold reduction in baloxavir susceptibility (Fabrizio et al., 2025; Pascua et al., 2026). Mechanistically, the substitution of isoleucine with methionine at position 38 sterically hinders the binding of baloxavir within the cap-snatching site. In murine models, this mutation significantly reduced the efficacy of oral baloxavir treatment across multiple dosage levels, resulting in higher viral titers and lower survival rates compared to wild-type controls (Fabrizio et al., 2025; Pascua et al., 2026). While PA-I38T typically confers a more dramatic reduction in susceptibility (up to 614-fold depending on the genetic background), PA-I38M is more frequently observed in recent H5N1 clusters, possibly due to a lower fitness cost in the bovine-adapted polymerase complex (Pascua et al., 2026).

Adamantane resistance is primarily mediated by the M2-S31N substitution. While this mutation is nearly universal in seasonal H3N2 and H1N1pdm09 viruses, its prevalence in clade 2.3.4.4b is genotype dependent. It is also a defining feature of the D1.1 genotype but is notably absent in the predominant B3.13 genotype, which retains in vitro susceptibility to amantadine and rimantadine (Andreev et al., 2026; Fabrizio et al., 2025). However, in vivo studies have shown that amantadine fails to protect mice against lethal B3.13 infection, likely because the highly

efficient polymerase complex and rapid replication kinetics of this virus can overwhelm the inhibitory effects of M2 blockers (Andreev et al., 2026). In addition, the efficacy of the PB1-targeting favipiravir has been demonstrated only as marginal in human respiratory organoid models with very high effective concentration (EC50) indicating it is not the right candidate for use in monotherapy for the currently circulating H5N1 strains (Shichinohe et al., 2025). The collective results indicate the precarious state of the existing therapeutic arsenal, meaning that if mutations are spread, like I38M, and one drug class is lost, the clinical management of the influenza virus will be virtually left solely with neuraminidase inhibitors (Fabrizio et al., 2025; WHO, 2026).

***Governance Gaps and Policy Priorities***

Several important governance gaps were identified in the course of the response to the HPAI H5N1 outbreak in the United States, which may provide some lessons for future response efforts.Several critical governance gaps were identified during the HPAI H5N1 investigation in the U.S., which may serve as lessons for future investigations to contain HPAI H5N1 outbreaks (Owusu & Sanad, 2025; Lowen et al., 2025). One of the difficulties was the lack of sufficient financial compensation for voluntary testing of infected herds before movement restrictions were put in place, allowing the virus to spread undetected within affected farms (USDA, 2024; Owusu & Sanad, 2025). This lack of early diagnosis enabled the spread of the virus more widely, making containment more complex (Owusu & Sanad, 2025). Moreover, there was significant delay during the early phase of the outbreak in releasing key genomic sequence information. This precluded independent analysis of the phylogenies as well as hindered international scientific transparency, limiting the ability of the global scientific community to quickly understand and respond to the changing nature of the virus (Otto & Edgerton, 2025; Owusu & Sanad, 2025). Sub-optimal personal protective equipment (PPE) compliance was also a significant finding among the workers on the farms. An eye protection was not worn by a significant number of farm workers during early case ascertainment in Colorado, and during an outbreak of the disease later in California, this also seemed to be directly linked to the clinical presentation of conjunctivitis seen in humans (Marshall et al, 2024; Bagdasarian & Wineland, 2024; CDC, 2024b).

Lessons learned from these indicate three integrated policy response are necessary to aid efforts in future HPAI containment. Firstly, the current movement based-testing program needs to be replaced by continuous bulk milk surveillance of the herd, along with mandatory live public sequence reporting (Lail et al., 2025; USDA, 2024). This would help in getting ahead of an outbreak and prevent the critical delay that exists between detection of outbreak and genome sequencing, allowing a more agile reaction. Secondly, automated alerts for priority surveillance mutations (PSMs) should be incorporated into national bioinformatics workflows (Pinsky & Bradley, 2024; CDC, 2024c). These include specific NP residues (52, 100, 283, 313, and 357) associated with MxA resistance (Mänz et al., 2013; Götz et al., 2016), HA positions (110, 154, 216, and 318) critical for fusion pH reduction, HA residues (Q226 and G228 equivalent) for alpha-2,6 binding, and deep mutational scanning (DMS) NeuGc hotspots (H141, E142, T143, L145, K168, and Y173) in circulating bovine isolates (Dadonaite et al., 2024). Such alerts would help to proactively inform of the possibility of viral adaptation and zoonosis risk (Pinsky & Bradley, 2024; CDC, 2024c). Third, the speed at which clade-matched H5N1 mRNA vaccines are being fielded to dairy cows is an important intervention (Chiba et al., 2024; Kong et al., 2026; Santos et al., 2026). The approach to minimize evolutionary selective pressure within the primary mammalian reservoir while protecting the agricultural workforce from occupational zoonotic exposure is currently the most viable option to reduce evolutionary selective pressure.

Other key policy changes are needed across the dairy industry, including in areas of high risk (USDA-APHIS, 2024; Owusu & Sanad, 2025). These include stringent entry/exit, equipment disinfection and animal movement control procedures on the farm (USDA-APHIS, 2024). Vaccination strategies for dairy cattle need to be explored further and protocols for international trade need to be harmonised in order to create a coordinated response worldwide (EFSA AHAW Panel, 2025; Kamel et al., 2025). Building public health infrastructure, particularly at the local level, is also crucial in order to improve the ability to respond quickly (CDC, 2024d). Moreover, continued investment and research into genetic resistance to avian influenza in cattle and development of robust vaccines for use in cattle is critical (Kamel et al., 2025; Owusu & Sanad, 2025). Finally, science-based communication strategies are needed to accurately inform consumers of the safety of dairy products and thus, ensuring consumer confidence (FDA, 2025). To conclude, targeted financial support mechanisms should also be developed that will help the producers who are affected by outbreaks of the disease to cope with the severe economic losses.

**Discussion**

The U.S. dairy cattle H5N1 clade 2.3.4.4b is a significant change in the ecology of influenza viruses, as a bird virus has spilled over into a new mammal host. Since 2021, Clade 2.3.4.4b viruses have been spreading in the world and infecting millions of birds and leading to spillovers into various mammals, including sea lions, cats, mink and cattle (Capelastegui and Goldhill, 2025). The two predominant genotypes in 2024 in the USA were B3.13 in dairy cattle and D1.1 in poultry (Hatta et al., 2025).

The H5N1 B3.13 has been detected in the U.S. dairy herd and is an important epidemiological change because of the converging yet independent adaptive axes. The polymerase axis with mutated PB2 M631L and PA K497R is

responsible for efficient replication in bovine mammary epithelial cells and allows the virus to exploit the ANP32A-dominant bovine cellular environment, leading to dramatic increased viral titres in milk, which can drive fomite (via contaminated equipment) bovine-to-cow transmission (Sheppard et al., 2023; Fabrizio et al., 2025; Dholakia et al., 2026; Baker et al., 2024; Caserta et al., 2024). Additionally, these polymerase modifications promote coronavirus multiplication in primary human airway epithelial cells, which greatly increases the potential for occupational exposure to this virus (Dholakia et al., 2026; USDA-APHIS, 2026b).

NeuGc is a prevalent form of SA at the alveolar lactocyte surface, which represents about 50% of the sialic acid pool, and the receptor axis (HA D104G and V147M in the 130-loop) increases the initial attachment efficiency in bovine mammary tissue, with a broader use of the receptors (Caserta et al., 2024; Good et al., 2024; Hassard et al., 2026). These two adaptation axes helped increase the availability of receptors and boosted the efficiency of the polymerases, and that is why these mutations spread so fast and why so many herds spread the virus across jurisdictions by April 2026, and why the US outbreak of the disease in cattle was unprecedented in its scale.

A critical point of ongoing scientific discourse revolves around the capacity of bovine B3.13 HA to bind alpha-2,6-NeuAc, the canonical human upper respiratory tract receptor. So, several scientific controversies require direct acknowledgement. First, the question of whether bovine H5N1 strains can engage alpha-2,6-NeuAc to any measurable degree remains debated. Santos et al. (2025), Chopra et al. (2025), and Yang et al. (2025) consistently demonstrate poor alpha-2,6-NeuAc binding in B3.13 isolates by glycan microarray and BLI, while Good et al. (2024) reported expanded receptor breadth for a D104G mutant. The NeuGc broadening mechanism characterised by Hassard et al. (2026) may explain some earlier ambiguous alpha-2,6 signals if NeuGc-containing alpha-2,6 glycans were not separated from NeuAc-containing alpha-2,6 glycans in earlier array designs. Second, the strength of the evidence for mammal-to-mammal transmission in South American pinnipeds is phylogenetically compelling (Uhart et al., 2024). However, surveillance bias toward coastal research stations may inflate apparent transmission scope. Third, the partial ferret airborne transmissibility of A/Texas/37/2024 (17-33% droplet transmission pairs; Gu et al., 2024) is directly relevant but does not translate linearly to human pandemic potential, as the three additional barriers (alpha-2,6-SA binding, reduced HA fusion pH, and NP MxA resistance) remain intact.

The structural insights reported by Lin et al. (2024) simultaneously clarify and sharpen the understanding of the pandemic barrier. The confirmation that a single Q226L substitution in the 220-loop can switch HA specificity to human-type receptors, distinct from the 130-loop adaptions, resolves the structural basis for the receptor-switching step more precisely than was previously understood. This finding implies that the current observation of limited alpha-2,6 binding in circulating B3.13, while epidemiologically significant, is mechanistically tenuous, being merely one mutation removed from a potentially pandemic-enabling phenotype (Lin et al., 2024; Nguyen et al., 2025; Dholakia et al., 2026). While the partial ferret airborne transmissibility of A/Texas/37/2024 (17 to 33% droplet transmission pairs) does not translate linearly to human pandemic potential (Gu et al., 2024), as three independent barriers remain simultaneously intact. The historical pandemic precedent, where H2N2 and H3N2 emergence required receptor-binding changes at exactly Q226 and G228 in the context of broader reassortment, means that any detection of Q226L variants in routine surveillance should be treated as an immediate escalation trigger of public response.

At the end, six research priorities emerge from this synthesis. Experimental assessment of whether NeuGc-adapted B3.13 strains (D104G plus V147M) show altered transmissibility in ferrets, which lack NeuGc, is urgently needed, as current ferret models cannot detect NeuGc-specific fitness changes. Longitudinal whole-genome sequencing of chronically infected cattle herds is needed to characterise fixation dynamics of emerging mutations. Full animal-model characterisation of D1.1 bovine isolates is outstanding. Experimental determination of whether swine serves as a reassortment vessel for B3.13 and human seasonal IAV is critical following the 2024 US swine detection. Multi-host organoid models enabling direct replication comparisons across bovine mammary, porcine respiratory, and human bronchial tissues under control conditions would advance mechanistic understanding substantially. Finally, formal cost-effectiveness analyses of bovine H5N1 mRNA vaccination programmes are needed to inform policy deployment.

## Conclusions

Clade 2.3.4.4b H5N1 has redrawn the host-range rules for HPAI. For the first time in the 30-year history of the Gs/GD lineage, sustained HPAI transmission has been established in a domestic ruminant species, and for the first time, two mechanistically distinct and orthogonal adaptive axes are operating simultaneously in the same host, each independently increasing bovine fitness and zoonotic exposure risk. Structural crystallography has now resolved the minimal mutational distance separating circulating B3.13 HA from human-type receptor specificity: a single Q226L substitution in the 220-loop. The pandemic barriers that remain intact as of early 2026 are real and consequential, specifically the absence of alpha-2,6-linked sialic acid binding, the high HA fusion pH (~5.9), and full NP sensitivity to human MxA restriction. Nevertheless, more than 1,000 infected dairy herds, over 70 human cases including the first US pediatric case, the emergence of a baloxavir-resistant clinical isolate, viral RNA detected in bovine semen, ongoing emergence of convergent mutations including PB2 E627K plus M631L double-mutant variants, and partial ferret aerosol transmissibility collectively define a pandemic risk level higher than at any prior point in H5N1 history.

The path to early detection and prevention of pandemic emergence requires integrated One Health surveillance combining mandatory bulk-milk genomics with real-time public sequence reporting, wastewater surveillance for early geographic warning, systematic wildlife and occupational health serosurveillance, and accelerated deployment of ruminant mRNA vaccines to reduce evolutionary pressure in the primary mammalian reservoir, represents the only realistic pathway to early detection of the adaptive mutations identified in this review whose emergence in circulating sequences would require immediate escalation of pandemic response.

## Declarations

### *Ethical Approval Certificate*

Not applicable. This study is a review article based on published literature and does not involve any experimental procedures with animals or humans.

### *Author Contribution Statement*

Muhammad Ahsan Yaseen: Conceptualization, investigation, formal analysis, writing the original draft, review, and editing.

Muhammad Tahir Sarfraz Khan: Manuscript review and editing.

Faiza Abbas: Literature search and screening

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### *Conflict of Interest*

The authors declare no conflict of interest.

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## Abbreviations

| | |
|---|---|
| ANP32A | Acidic leucine-rich nuclear phosphoprotein 32A |
| ANP32B | Acidic leucine-rich nuclear phosphoprotein 32B |
| HA | Hemagglutinin |
| RNP | Ribonucleoprotein complex |
| PB2 | Polymerase basic protein 2 |
| PA | Polymerase acidic protein |
| CMAH | Cytidine monophospho-N-acetylneuraminic acid hydroxylase |
| NeuAc | N-acetylneuraminic acid (sialic acid) |
| NeuGc | N-glycolylneuraminic acid (sialic acid) |
| SA | Sialic acid |
| α2,3 / α2,6 | Alpha-2,3 or alpha-2,6 glycosidic linkage |
| $TCID_{50}$ | Tissue culture infectious dose 50 |
| mL | Milliliter |
| B3.13 | H5N1 clade 2.3.4.4b genotype B3.13 |
| H5N1 | Highly pathogenic avian influenza H5N1 |
| MALDI-TOF | Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry |
| dN/dS | Ratio of nonsynonymous to synonymous substitutions |

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# The Effects of Oral Probiotic Supplementation on Liver Enzyme Activities (ALP, ALT, AST, and GGT) and Serum Magnesium and Phosphorus Concentrations in Cats Infected with Feline Panleukopenia Virus (FPV)

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## Abstract

Feline panleukopenia virus (FPV) is one of the most significant viral pathogens affecting cats, characterized by high transmissibility and severe clinical manifestations, particularly in young animals. As a member of the Parvoviridae family, this DNA virus exhibits remarkable environmental resistance, facilitating its rapid spread within feline populations. The disease is most severe in unvaccinated, young, or immunocompromised cats and is frequently associated with high mortality rates. Common clinical signs of feline panleukopenia include anorexia, depression, vomiting, diarrhea, dehydration, and hyperthermia. The severe gastrointestinal losses that occur during infection not only affect the hematological system but also disrupt biochemical homeostasis. Fluid and electrolyte losses resulting from vomiting and diarrhea may lead to various metabolic disturbances. Furthermore, systemic inflammation and circulatory impairment may adversely affect liver function, resulting in alterations in serum hepatic parameters. In this study, 18 cats naturally infected with FPV were included and randomly assigned to either a Control group (n = 9) or an Experimental group (n = 9). Cats in the Control group received the standard treatment protocol for FPV, whereas cats in the Experimental group received standard FPV treatment supplemented with an oral probiotic administered for seven consecutive days. Blood samples were collected before and after treatment, and serum levels of liver enzymes, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), as well as magnesium (Mg) and phosphorus (P), were evaluated. Statistical analysis revealed that, prior to treatment, only the phosphorus concentration was significantly higher in the Control group ($P < 0.05$), whereas all other parameters were comparable between groups ($P > 0.05$). Following treatment, ALP and Mg levels were significantly lower in the Experimental group ($P < 0.05$), while no significant differences were observed between groups for the remaining parameters ($P > 0.05$).

***Keywords:*** *Feline Panleukopenia Virus, Liver Enzymes, Cat, Probiotic, Serum*

## Feline panleukopenia virüs (FPV) ile enfekte kedilerde oral probiyotik uygulamasının karaciğer enzim aktiviteleri (ALP, ALT, AST, GGT) ile Mg ve P minerallerinin konsantrasyonu üzerine etkileri

## Özet

Feline panlökopeni virüsü (FPV), kedilerde yüksek bulaşıcılık gösteren ve özellikle genç hayvanlarda ciddi seyreden önemli viral enfeksiyonlardan biridir. Parvoviridae familyasında bulunan bu DNA virüsü, çevresel şartlara karşı oldukça dayanıklı olması nedeniyle enfeksiyonun popülasyon içinde hızla yayılmasına neden olmaktadır. Hastalık daha çok aşısız, genç veya bağışıklık sistemi baskılanmış kedilerde ağır klinik tablo oluşturmakta ve yüksek mortalite oranları ile ilişkilendirilmektedir. Feline panlökopeni enfeksiyonunda görülen başlıca klinik bulgular arasında iştahsızlık, depresyon, kusma, diyare, dehidrasyon ve hipertermi yer almaktadır. Enfeksiyon sürecinde gelişen şiddetli gastrointestinal kayıplar yalnızca hematolojik sistemi değil, aynı zamanda biyokimyasal dengeyi de etkilemektedir. Kusma ve diyare sonucu oluşan sıvı-elektrolit kayıpları metabolik bozukluklara neden olabilmektedir. Bunun yanı sıra sistemik inflamasyon ve dolaşım bozukluklarının karaciğer fonksiyonlarını etkileyebileceği, buna bağlı olarak serum karaciğer parametrelerinde değişiklikler meydana getirebilmektedir. Çalışmada FPV ile enfekte 18 adet kedi kullanılmıştır ve kediler rastgele yöntemle Kontrol (n=9) ve Deney (n=9) gruplarına ayrılmıştır. Kontrol grubundaki hayvanlara rutin FPV tedvasi uygulnırken, Deney grubundaki kedilere FPV tedavisi + 7 gün oral probiyotik uygulması yapıldı. Kedilerden uygulama öncesi ve sonrası kan örnekleri alınarak serumda karaciğer enzimlerinden Alkalen Fosfataz (ALP), Alanin Aminotransferaz (ALT), Aspartat Aminotransferaz (AST) ve Gama Glutamil Transferaz (GGT) ile magnezyum (Mg) ve fosfor (P) seviyelerine etkisi araştırılmıştır. Elde edilen sonuçlar istatistiksel olarak değerlendirildiğinde uygulama öncesi sadece P parametresi Kontrol grubunda yüksek çıkarken ($P<0.05$), diğer parametreler gruplarda benzer bulunmuştur ($P>0.05$). Uygulama sonrası ALP ve Mg seviyelerinin Deney grubunda düşük olduğu ($P<0.05$), diğer parametrelerin ise her iki grupta istatistiksel olarak benzer olduğu tespit edilmiştir ($P>0.05$).

***Anahtar Kelimeler:*** *Feline Panlökopeni Virüs, Karaciğer Enzimleri, Kedi, Probiyotik, Serum*

## Giriş

Feline panlökopeni virüsü, kedilerde yüksek bulaşıcılık gösteren ve özellikle genç hayvanlarda ciddi sorunlara yol açan önemli viral enfeksiyonlardan biridir. Parvoviridae familyasında bulunan bu zarfsız DNA virüsü, çevresel şartlara karşı oldukça dayanıklı olması nedeniyle enfeksiyonun popülasyon içinde hızla yayılmasına neden olmaktadır. Hastalık daha çok aşısız, genç veya bağışıklık sistemi baskılanmış kedilerde ağır klinik tablo oluşturmakta ve yüksek mortalite oranları ile ilişkilendirilmektedir (Stuetzer ve Hartmann, 2014). Feline panlökopeni enfeksiyonunda görülen başlıca klinik bulgular arasında iştahsızlık, depresyon, kusma, diyare, dehidrasyon ve hipertermi yer almaktadır. Hastalığın en belirgin laboratuvar bulgusu ise ciddi lökopeni olarak kabul edilmektedir. Virüsün kemik iliği üzerindeki baskılayıcı etkisine bağlı olarak nötrofil ve lenfosit sayılarında azalma meydana gelebilmekte, bazı olgularda trombosit sayısında da düşüş gözlenebilmektedir (Porporato ve ark., 2018). Hematolojik parametrelerde oluşan bu değişikliklerin, hastalığın şiddetinin ve prognozunun değerlendirilmesinde önemli olduğu bildirilmektedir (Stuetzer ve Hartmann, 2014). Enfeksiyon sürecinde gelişen şiddetli gastrointestinal kayıplar yalnızca hematolojik sistemi değil, aynı zamanda biyokimyasal dengeyi de etkilemektedir. Kusma ve diyare sonucu oluşan sıvı-elektrolit kayıpları metabolik bozukluklara neden olabilmektedir. Bunun yanı sıra sistemik inflamasyon ve dolaşım bozukluklarının karaciğer fonksiyonlarını etkileyebileceği, buna bağlı olarak serum karaciğer parametrelerinde değişikliklerin meydana gelebileceği bildirilmektedir (Kaneko ve ark., 2008). Ayrıca mineral metabolizmasındaki değişimlerin değerlendirilmesi amacıyla serum Mg ve P düzeylerinin incelenmesi önem taşımaktadır.

Son yıllarda probiyotiklerin veteriner hekimlikte destekleyici tedavi amacıyla kullanımına yönelik çalışmalar artış göstermiştir. Probiyotiklerin bağırsak mikrobiyotasının düzenlenmesi, intestinal bariyer bütünlüğünün korunması ve immün sistemin desteklenmesi üzerinde olumlu etkiler oluşturabileceği belirtilmektedir (Schmitz ve Suchodolski, 2016). Probiyotikler, yeterli miktarlarda uygulandığında konak sağlığı üzerinde faydalı etkiler oluşturabilen canlı mikroorganizmalar olarak tanımlanmaktadır (Hill ve ark., 2014). Kedilerde görülen viral enfeksiyonlar yalnızca immün sistemi değil, aynı zamanda hepatobilier sistemi ve mineral metabolizmasını da etkileyebilmektedir. Özellikle sistemik seyreden viral hastalıklarda karaciğer hücrelerinde oluşan inflamasyon ve nekroz; ALP, ALT, AST ve GGT aktivitelerinde değişimlere neden olurken, Mg ve P düzeylerinde de hastalığın şiddetine bağlı metabolik dalgalanmalar görülebilmektedir (Capozza ve ark., 2021).

Bu çalışmada FPV ile enfekte kedilere FPV tedavisi + 7 gün oral probiyotik uygulması yapılarak serumda karaciğer enzimlerinden ALP, ALT, AST ve GGT ile Mg ve P seviyeleri üzerine etkisi araştırılmıştır.

## Materyal ve Metot

### *Deneme dizaynı, Hayvan ve Uygulama*

Bu çalışma, Sivas Cumhuriyet Üniversitesi Hayvan Hastanesine başvuran ve FPV enfeksiyonu tanısı konulan toplam 18 kedi üzerinde yürütülmüştür. Kedilerde FPV enfeksiyonunun tanısı için ticari hızlı antijen test kiti (Feline FPV Ag Test, Hangzhou Evegen Biotech Co., Ltd., Hangzhou, Zhejiang, China) kullanıldı. Test, dışkı veya kusmuk örneklerinde FPV antijeninin kalitatif olarak saptanmasına yönelik yanal akış immünokromatografik analiz prensibine dayanmaktadır. Çalışma süresince tüm hayvanlar aynı çevresel koşullar altında, standart bakım ve barınma şartları sağlanarak tutulmuş ve veteriner hekim gözetiminde takip edilmiştir. Çalışmada bulunan hayvanlar 1-10 aylık yaş aralığında olup hem dişi hem erkek kedilerden oluşmaktadır. Çalışmaya dahil edilen teşhis koyulmuş kediler rastgele yöntemle iki gruba ayrılmış olup her grupta 9 hayvan yer almıştır. Deneme grupları aşağıdaki şekilde oluşturulmuştur:

Kontrol Grubu (n=9): Bu gruptaki kedilere probiyotik uygulaması yapılmamıştır.

Deney Grubu (n=9): Bu gruptaki kedilere oral yolla 7 gün probiyotik uygulaması gerçekleştirilmiştir.

Probiyotik uygulamasında; *Enterococcus faecium* NCIMB 10415 ($1\times10^{12}$ CFU/kg), 4b1871 Saccharomyces cerevisiae ($4\times10^{7}$/kg) ve 12.1.5 Saccharomyces cerevisiae (inaktif maya, 150.000 mg/kg) içeren probiyotik karışım kullanılmıştır. Uygulama dozu, 2 ml/3 kg canlı ağırlık/gün olacak şekilde belirlenmiş ve toplam 7 gün süreyle uygulanmıştır. Probiyotik uygulamaları steril şırınga yardımıyla oral yolla gerçekleştirilmiştir. Çalışma kapsamında yer alan tüm kedilere standart tedavi protokolü uygulanmış olup tedavi süreci toplam 7 gün sürdürülmüştür.

### *Kan Örneklerinin Toplanması*

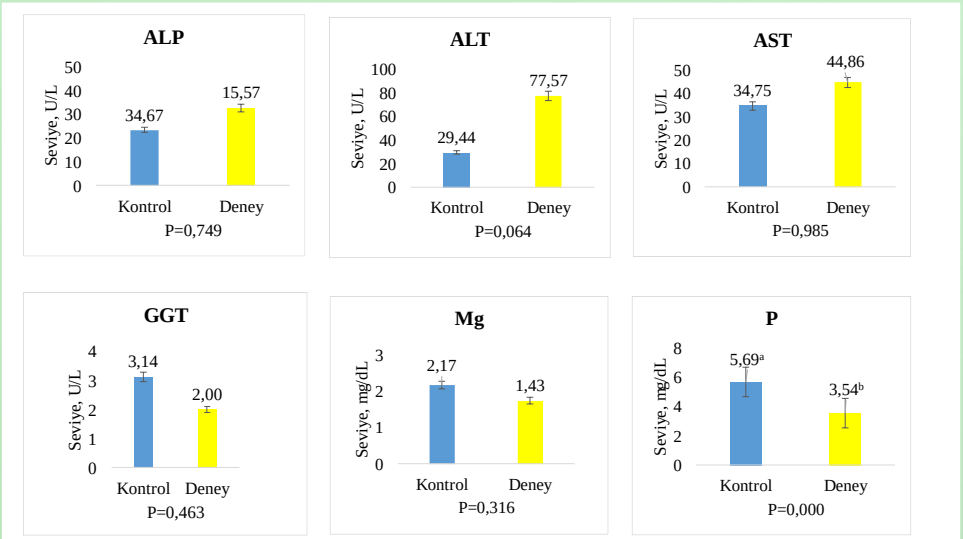
Her iki gruptaki kedilerden çalışma başlangıcında (0. gün) ve probiyotik uygulamasının tamamlandığı 7. gün sonunda yaklaşık 5 ml kan, antikoagülansız tüplere (Becton Dickinson Co., USA) alındı. Alınan kanlar, soğutmalı bir santrifüjde (Hettich 38R, HettichZentrifugen, Tuttlingen, Germany) +4 °C'de, 4000 rpm hızında 10 dakika boyunca santrifüj edildi. Elde edilen serumlar, analiz gününe kadar -80 °C'de muhafaza edildi. Kan örneklerinin analizleri ise Sivas Cumhuriyet Üniversitesi Hayvan Hastanesi Laboratuvarında ticari test kitleri (Archem, Türkiye) kullanılarak otomatik biyokimya analizör cihazında (Mindray BS-200, P.R. China) yapılmıştır.

***İstatistiksel Analizler***

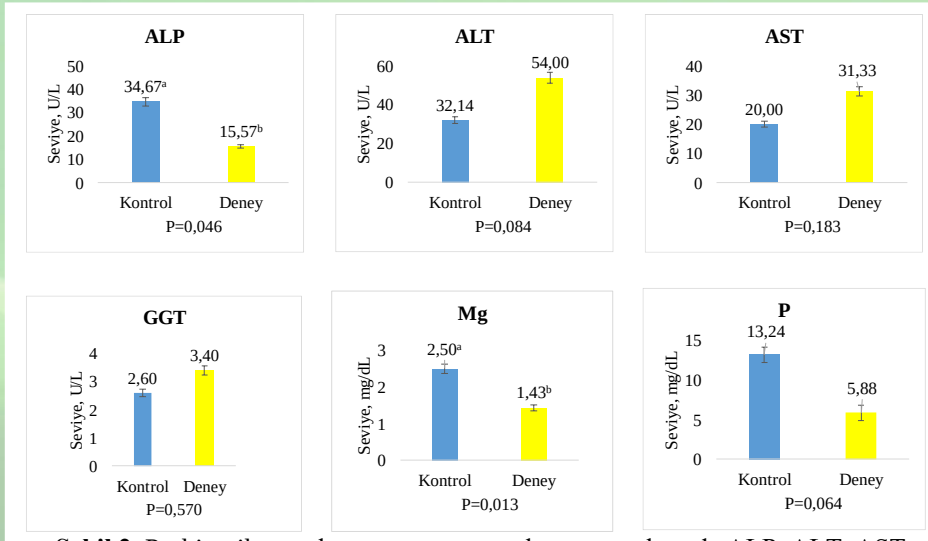
Elde edilen verilerin istatistiksel değerlendirilmesinde SPSS (2015) paket programı kullanıldı. Verilerin normal dağılıp dağılmadığı belirlemek için one simple Kolmogorov-smirnov testi ile yapıldı. Gruplardan elde edilen parametrelerle ilgili veriler arasında istatistiksel farklılıkların belirlenmesi amacıyla Independent Samples T-Testi kullanıldı ($P<0.05$).

## Bulgular ve Tartışma

Elde edilen sonuçlar istatistiksel olarak değerlendirildiğinde uygulama öncesi sadece P seviyesi Kontrol grubunda istatistiksel olarak önemli oranda yüksek çıkarken ($P<0.05$), ALP, ALT, AST, GGT ve Mg seviyeleri gruplarda istatistiksel olarak benzer bulunmuştur ($P>0.05$) (Şekil 1). Uygulama sonrası ALP ve Mg seviyelerinin Deney grubunda istatistiksel olarak önemli oranda düşük olduğu ($P<0.05$), ALT, AST, GGT ve P seviyelerinin ise her iki grupta istatistiksel olarak benzer olduğu tespit edilmiştir ($P>0.05$) (Şekil 2).




**Şekil 1.** Probiyotik uygulaması öncesi grupların serumlarında ALP, ALT, AST, GGT, Mg ve P seviyeleri




**Şekil 2.** Probiyotik uygulaması sonrası grupların serumlarında ALP, ALT, AST, GGT, Mg ve P seviyeleri

Feline infectious peritonitis (FIP) olgularında yapılan retrospektif çalışmalarda, özellikle AST ve ALT düzeylerinde yükselme saptandığı bildirilmiştir. Bunun temel nedeni, sistemik vaskülitis ve granülomatöz inflamasyona bağlı hepatik hasarın gelişmesidir. Bazı olgularda GGT düzeylerinin de yükseldiği ve bunun hepatobilier sistem tutulumu ile ilişkili olabileceği belirtilmiştir. İyileşme gösteren kedilerde ise antiviral tedavi ve destekleyici bakım sonrasında ALT ve AST aktivitelerinde belirgin düşüş gözlenmiştir. Bu durum karaciğer hücre hasarının gerilediğini göstermektedir (Tasker, 2018). Feline panlökopeni enfeksiyonunda ise ciddi gastrointestinal kayıplar, dehidrasyon ve sistemik inflamasyon nedeniyle biyokimyasal parametrelerde önemli değişiklikler oluşabilmektedir. Yapılan çalışmalarda hasta kedilerde ALT ve AST aktivitelerinin sağlıklı kontrol grubuna göre daha yüksek olduğu, bazı olgularda hiperbilirubinemi geliştiği bildirilmiştir. Ayrıca kusma, anoreksi ve sıvı-elektrolit kaybına bağlı olarak P ve Mg düzeylerinde düzensizliklerin ortaya çıkabileceği belirtilmektedir. Hastalığın iyileşme döneminde ise sıvı tedavisi ve beslenmenin düzelmesiyle mineral değerlerinin normal referans aralıklara yaklaşabildiği ifade edilmiştir (Porporato ve ark., 2018).

Hemotropik enfeksiyonların değerlendirildiği çalışmalarda da AST ve P düzeylerinin anlamlı şekilde yükseldiği belirtilmiştir. Bunun hem hemolitik süreç hem de karaciğer metabolizmasındaki bozulma ile ilişkili olduğu düşünülmektedir (Naseri ve ark., 2025). Mevcut çalışmada da elde edilen sonuçlar probiyotik uygulaması öncesinde sadece serumda P seviyesi açısından gruplar arasında fark varken uygulama sonrası yapılan ölçümlerde serum ALP ve Mg seviyelerinin Deney grubunda istatistiksel olarak önemli oranda düşmüştür. Karaciğer enzimleri olan ALT, AST ve ALP düzeylerinin artması gastrointestinal sistem hasarı ve sekonder hepatik etkilenmeye bağlı olabileceği belirtilirken (Kadam ve ark., 2022) mevcut çalışmada ALP düzeyinin düşmesi karaciğer ve gastrointestinal sistem üzerine probiyotik uygulamsının koruyucu etki gösterdiği şeklinde yorumlanabilir.

## Sonuç

Bu çalışmada FPV ile enfekte kedilere FPV tedavisi + 7 gün oral probiyotik uygulması yapılmasının karaciğer enzimlerinden ALP seviyesini olumlu etkilediği, ALT, AST ve GGT seviyerine olan etkisinin kısıtlı olduğu görüldü. Ayrıca serum Mg seviyesini etkilediği belirlendi.

## Açıklamalar

Bu bildiri, Melis YAĞLI' nın "Feline panleukopenia virüs (FPV) ile enfekte kedilerde oral probiyotik uygulamasının dışkı mikrobiyotası ve bazı kan parametreleri üzerine etkileri" başlıklı yüksek lisans tez çalışmasının bir kısmından özetlenmiştir.

## Teşekkür ve Bilgi Notu

Bu çalışma, Sivas Cumhuriyet Üniversitesi Bilimsel Araştırma Projeleri (CÜBAP) tarafından V-2025-156 proje numarası ile desteklenmiştir.

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# Evaluation of Geographical Indication Products in Terms of Sustainable Agriculture, Food Safety, and Food Security

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## Abstract

Geographical indications are intellectual property tools that protect products associated with a specific geographical area and whose distinctive qualities, reputation, or other characteristics are attributable to their geographical origin. This system not only protects the origin and authenticity of products but also contributes to the continuity of local production systems, the preservation of traditional knowledge and practices, rural development, and sustainable agricultural production. In terms of food safety, geographical indication systems may contribute to product standardization, traceability, and inspection processes, while in terms of food security, they may indirectly support the continuity of local production and the resilience of rural economies. This study evaluates the relationships between geographical indication products and sustainable agriculture, food safety, and food security based on the relevant literature and the case of geographical indication products from Bayburt. The findings indicate that geographical indications have the potential to support the conservation of local genetic resources and traditional production practices, strengthen rural economies, and improve product traceability. However, realizing these potential benefits depends on factors such as effective inspection mechanisms, producer organization, institutional coordination, and the development of appropriate marketing strategies.

***Keywords:*** *Geographical Indication, Geographical Indication Product, Food Security, Food Safety, Sustainable Agriculture*

## Coğrafi İşaretli Ürünlerin Sürdürülebilir Tarım, Gıda Güvenliği ve Gıda Güvencesi Açısından Değerlendirilmesi

## Özet

Coğrafi işaretler, belirli bir coğrafi alanla ilişkilendirilen ve kendine özgü nitelikleri, ünü veya diğer özellikleri bu coğrafi kökenden kaynaklanan ürünlerin korunmasını sağlayan fikri mülkiyet araçlarıdır. Bu sistem, yalnızca ürünün coğrafi kökenini değil, aynı zamanda bölgeye özgü üretim yöntemlerini ve geleneksel bilgi birikimini de temsil etmektedir. Belirli bir bölgeye ait ürünlerin kalitesini ve özgünlüğünü korumayı amaçlayan coğrafi işaretler, yerel üretim sistemlerinin ve geleneksel üretim yöntemlerinin devamlılığını destekleyerek sürdürülebilir tarıma katkı sağlayabilmektedir. Gıda güvenliği ve gıda güvencesi birbiriyle ilişkili ancak farklı kavramlardır. Gıda güvenliği, gıdalardan kaynaklanabilecek fiziksel, kimyasal ve biyolojik tehlikelerin insan sağlığı açısından kontrol altında tutulmasını ifade ederken; gıda güvencesi, bireylerin yeterli, güvenli ve besleyici gıdaya sürekli biçimde erişebilmesini kapsamaktadır. Coğrafi işaret sistemleri; ürün standartları, izlenebilirlik ve denetim mekanizmaları yoluyla gıda güvenliğini, yerel üretimin devamlılığı ve kırsal ekonominin desteklenmesi yoluyla da gıda güvencesini destekleme potansiyeline sahiptir. Bu çalışmanın amacı, literatürde yer alan bilgiler doğrultusunda coğrafi işaretli ürünlerin sürdürülebilir tarım, gıda güvenliği ve gıda güvencesi ile ilişkilerini incelemek ve bu ilişkileri Bayburt'un coğrafi işaretli ürünleri örneğinde değerlendirmektir. Literatürün genel değerlendirmesi, coğrafi işaret sistemlerinin sürdürülebilir tarım uygulamalarını, gıda güvenliğini ve gıda güvencesini destekleme potansiyeline sahip olduğunu; ancak bu katkıların etkin denetim, üretici örgütlenmesi ve uygun yönetişim mekanizmaları gibi koşullara bağlı olduğunu göstermektedir.

***Anahtar Kelimeler:*** *Coğrafi İşaret, Coğrafi İşaretli Ürün, Gıda Güvencesi, Gıda Güvenliği, Sürdürülebilir Tarım*

## Giriş

Günümüz dünyasında iklim değişikliğine bağlı olarak yaşanan biyolojik çeşitlilik kaybı, doğal kaynakların hızla tükenmesi, küresel salgınlar ve jeopolitik krizler, tarım ve gıda sistemlerinin sürdürülebilirliğini de etkilemektedir. Tarım-gıda üretim sistemleri üzerindeki artan çevresel baskıların yanı sıra küresel düzeyde artan gıda talebi, tarımsal üretimin verimlilik ile çevresel, ekonomik ve sosyal sürdürülebilirlik ilkeleri birlikte gözetilerek yeniden ele alınmasını gerekli hale getirmiştir. Bu süreçte doğal kaynakların korunması, üretici refahının artırılması ve gelecek nesillerin gıda gereksinimlerinin güvence altına alınması açısından sürdürülebilir tarım temel yaklaşımlardan biri olarak kabul edilmektedir (FAO et al., 2023; IPCC, 2023).

Sürdürülebilir tarım, tarımsal ekosistemin korunmasını, doğal kaynakların tüketilmeden kullanılmasını ve çevreye zarar vermeden güvenli gıda üretiminin sürdürülmesini esas alan bütüncül bir yaklaşım olarak değerlendirilmektedir (Çeker, 2016).

Bu yaklaşım aynı zamanda kırsal kalkınmanın desteklenmesi, üreticilerin ekonomik koşullarının iyileştirilmesi ve yaşam kalitesinin artırılması gibi sosyal ve ekonomik hedefleri de kapsamaktadır (Çeker, 2016).

Coğrafi işaretleme, bir ürünün nitelik ve özelliklerinin kökeninin bulunduğu yöre, alan, bölge veya ülke ile ilişkilendirilerek tescil edilmesini sağlayan ve bu yolla ürüne duyulan güveni ve tercih edilebilirliğini artıran bir markalaşma aracıdır (Alacadağlı, 2025, s. 523).

Bu çerçevede coğrafi işaretler (Cİ), belirli bir coğrafi alanla özdeşleşmiş ürünlerin kökenini, üretim yöntemlerini ve kalite özelliklerini koruyan fikri mülkiyet hakları olmanın ötesinde, sürdürülebilir kırsal kalkınmayı destekleyebilen çok boyutlu yönetişim araçları olarak değerlendirilmektedir. Çünkü coğrafi işaret sistemleri; yerel genetik kaynakların korunmasını, geleneksel üretim bilgisinin gelecek kuşaklara aktarılmasını, üreticilerin pazarda farklılaşmasını ve kırsal bölgelerde katma değerin artırılmasını da desteklemektedir (Belletti & Marescotti, 2011; Bowen, 2010; WIPO, 2021). Dünyada ve Türkiye'de coğrafi kökeniyle tanınan çok sayıda ürün bulunmakta; bu ürünlerin korunması ve tanıtılmasında coğrafi işaretlerden yaygın olarak yararlanılmaktadır.

Özellikle son yıllarda yayımlanan çalışmalar ve kurumsal raporlar, coğrafi işaret sistemlerinin yalnızca ekonomik fayda sağlayan bir tescil mekanizması olmadığını; aynı zamanda çevresel sürdürülebilirliğin güçlendirilmesi, agro-biyolojik çeşitliliğin korunması, kısa tedarik zincirlerinin desteklenmesi ve iklim değişikliğine uyum kapasitesinin artırılması açısından stratejik öneme sahip olduğunu göstermektedir (European Parliament and Council of the European Union, 2024; FAO & oriGIn, 2024). Bununla birlikte, bu katkıların gerçekleşmesi; üretici örgütlenmesi, etkin yönetişim ve sürdürülebilirlik uygulamalarının coğrafi işaret sistemlerine entegrasyonu gibi koşullarla yakından ilişkilidir (FAO & oriGIn, 2024; European Parliament and Council of the European Union, 2024).

Literatürde gıda güvenliği (food safety) ve gıda güvencesi (food security) kavramlarının zaman zaman birbirinin yerine kullanıldığı görülmekle birlikte, bu iki kavram farklı kapsamları ifade etmektedir. Gıda güvenliği, tüketiciye sunulan gıdaların fiziksel, kimyasal ve biyolojik tehlikeler açısından güvenilir olmasını ve üretimden tüketime kadar geçen süreçte insan sağlığını tehdit edecek risklerin kontrol altında tutulmasını ifade etmektedir. Bu kapsamda hijyen, izlenebilirlik ve resmi denetim süreçleri temel unsurlar arasında yer almaktadır (FAO & WHO, 2023).

Gıda güvencesi, bireylerin her zaman yeterli, güvenli ve besleyici gıdaya fiziksel ve ekonomik olarak erişebilmesini ifade eden çok boyutlu bir kavramdır. Genel kabul gören yaklaşıma göre gıda güvencesi; bulunabilirlik (availability), erişim (access), kullanım (utilization) ve istikrar (stability) olmak üzere dört temel boyutta ele alınmaktadır. Bu boyutlar, özellikle iklim değişikliği, küresel salgınlar ve tedarik zincirlerinde yaşanan sorunlarla birlikte daha fazla önem kazanmıştır (FAO et al., 2024; HLPE, 2020; Matemilola & Elegbede, 2017).

Bu çerçevede coğrafi işaret sistemleri, gıda güvenliği ve gıda güvencesi ile farklı yönlerden ilişkilendirilebilir. Coğrafi işaretli ürünlerde uygulanan ürün şartnameleri, izlenebilirlik ve denetim süreçleri gıda güvenliğinin desteklenmesine katkı sağlayabilirken; yerel üretimin devamlılığının desteklenmesi, üretici gelirlerinin artırılması ve kırsal ekonomilerin güçlendirilmesi de gıda güvencesine katkı sağlayabilmektedir.

Ulusal ve uluslararası literatürde coğrafi işaretlerin ekonomik etkileri, kırsal kalkınmaya katkıları ve tüketici davranışları üzerine çeşitli çalışmalar bulunmakla birlikte, sürdürülebilir tarım, gıda güvenliği ve gıda güvencesi ile ilişkilerinin birlikte değerlendirilmesine yönelik bütüncül yaklaşımlara ihtiyaç duyulmaktadır. Bu durum, konunun disiplinlerarası ve bütüncül bir yaklaşımla değerlendirilmesine duyulan ihtiyacı ortaya koymaktadır.

Bu çalışmanın amacı, coğrafi işaretli ürünlerin sürdürülebilir tarım, gıda güvenliği ve gıda güvencesi ile ilişkilerini literatür bulguları doğrultusunda incelemek ve bu ilişkileri Bayburt'un coğrafi işaretli ürünleri örneğinde değerlendirmektir.

## Materyal ve Yöntem

Bu çalışma, coğrafi işaretli ürünlerin sürdürülebilir tarım, gıda güvenliği ve gıda güvencesi ile ilişkilerini değerlendirmek amacıyla literatür taramasına dayalı olarak hazırlanmıştır. Konuya ilişkin ulusal ve uluslararası bilimsel literatür Google Scholar üzerinden taranmış; ayrıca FAO, OECD, WIPO, Avrupa Birliği kurumları ve ilgili ulusal kurumların rapor, mevzuat ve dokümanlarından yararlanılmıştır. Bayburt'a ait coğrafi işaretli ürünlerin belirlenmesinde Türk Patent ve Marka Kurumu Coğrafi İşaretler Portalı verileri esas alınmıştır. Elde edilen bilgiler konu başlıkları çerçevesinde değerlendirilmiş ve Bayburt'un coğrafi işaretli ürünleri örneği üzerinden ele alınmıştır.

## Bulgular

### *Coğrafi İşaretlerin Sürdürülebilir Tarıma Katkıları*

İncelenen çalışmalarda, coğrafi işaret sistemlerinin sürdürülebilir tarıma farklı yönlerden katkı sağlayabildiği belirtilmektedir. Bu kapsamda doğal kaynakların korunması, yerel biyolojik çeşitliliğin sürdürülmesi, geleneksel üretim bilgi ve becerilerinin gelecek kuşaklara aktarılması ve üreticilerin ekonomik olarak desteklenmesi öne çıkan başlıklar arasındadır. Özellikle Avrupa Birliği ülkelerindeki uygulamalarda, coğrafi işaret sistemlerinin kırsal

kalkınma politikalarıyla birlikte ele alınmasının ekonomik ve çevresel açıdan önemli katkılar sağlayabildiği bildirilmektedir (Belletti et al., 2017; Bowen, 2010; WIPO, 2021; European Parliament and Council of the European Union, 2024; FAO & oriGIn, 2024).

**Tablo 1.** Sürdürülebilir Tarıma Katkıların Literatür Sentezi

| Tema | Ortak Bulgular | Destekleyen Çalışmalar | Olası Politika Çıkarımı |
|---|---|---|---|
| **Biyolojik Çeşitlilik** | Yerel türlerin korunmasını destekleyebilir | FAO & oriGIn, 2024; Belletti et al., 2017 | Yerel çeşitlerin desteklenmesi |
| **Geleneksel Bilgi** | Kültürel mirasın korunmasını destekleyebilir | Bowen, 2010; WIPO, 2021 | Bilgi envanteri oluşturulması |
| **Doğal Kaynak Yönetimi** | Doğal kaynakların korunmasına katkı sağlayabilir | FAO & oriGIn, 2024; Belletti et al., 2017 | Şartnamelerin güncellenmesi |
| **Üretici Gelirleri** | Fiyat primi oluşabilir | Belletti & Marescotti, 2011 | Üretici örgütlenmesinin güçlendirilmesi |
| **Kırsal Kalkınma** | Yerel istihdama katkı sağlayabilir | Belletti & Marescotti, 2011; Vandecandelaere et al., 2010 | Bölgesel kalkınma |

Kaynak: İlgili literatürden yararlanılarak yazarlar tarafından oluşturulmuştur.

Bununla birlikte, literatürde coğrafi işaretlerin sürdürülebilir tarıma katkılarının her zaman aynı düzeyde gerçekleşmediğini ortaya koyan çalışmalar da bulunmaktadır. Bazı araştırmalar, coğrafi işaret tescilinin tek başına sürdürülebilir üretim uygulamalarını garanti etmediğini; etkin denetim sistemleri, güçlü üretici örgütleri, kurumsal koordinasyon ve pazarlama kapasitesi bulunmadığında beklenen ekonomik ve sosyal kazanımların sınırlı kaldığını belirtmektedir (FAO, 2017; FAO & oriGIn, 2024).

Özellikle gelişmekte olan ülkelerde yapılan araştırmalar, tescil sonrasında ürünlerin etkin biçimde pazarlanamaması, üretici birliklerinin kurumsal kapasite eksiklikleri ve denetim mekanizmalarındaki yetersizlikler nedeniyle coğrafi işaretlerden beklenen katma değerin tam olarak oluşturulamadığını göstermektedir. Bu durum, sürdürülebilir tarım politikalarında coğrafi işaretlerin yalnızca hukuki koruma aracı olarak değil, çok aktörlü yönetişim modeli içerisinde değerlendirilmesi gerektiğini ortaya koymaktadır (FAO, 2017; FAO & oriGIn, 2024).

Literatürde dikkat çeken bir diğer konu, dijital teknolojilerin coğrafi işaret sistemlerine entegrasyonudur. Son yıllarda QR kod, blok zincir tabanlı izlenebilirlik sistemleri ve dijital ürün pasaportları gibi uygulamaların ürün güvenilirliğini ve tüketici güvenini artırabileceği belirtilmektedir. Ancak bu teknolojilerin, özellikle küçük ölçekli üreticiler açısından maliyet ve teknik kapasite gerektirmesi nedeniyle henüz yaygın biçimde kullanılamadığı da ifade edilmektedir (FAO & oriGIn, 2024).

***Coğrafi İşaretlerin Gıda Güvenliği ve Gıda Güvencesine Katkıları***

Coğrafi işaret sistemleri gıda güvenliğini doğrudan sağlayan bir mekanizma olmamakla birlikte, üretim standartlarının belirlenmesi, ürün izlenebilirliğinin güçlendirilmesi ve denetim süreçlerinin düzenlenmesi bakımından gıda güvenliğine katkı sağlayabilmektedir. Coğrafi işaretli ürünlerde üretim aşamalarının belirli kurallara bağlanması ve ürün kökeninin doğrulanabilir olması, taklit ve tağşişin önlenmesine ve tüketici güveninin artırılmasına yardımcı olabilmektedir (De Rosa, 2015; Şahin, 2024, s. 203–205).

Diğer taraftan coğrafi işaretler, gıda güvencesine doğrudan üretim miktarını artırarak değil, yerel üretim sistemlerinin devamlılığını destekleyerek katkı sağlayabilmektedir. Yerel ekonomik faaliyetlerin güçlenmesi, üreticilerin ekonomik sürdürülebilirliğinin desteklenmesi ve kırsal kalkınmanın teşvik edilmesi, yerel gıda sistemlerinin devamlılığı açısından önem taşımaktadır (Nizam & Tatari, 2022; Vandecandelaere et al., 2010). Ancak bu katkıların yalnızca coğrafi işaret tesciliyle kendiliğinden ortaya çıkmayacağı da göz önünde bulundurulmalıdır. Etkin denetim, üretici örgütlenmesi, izlenebilirlik altyapısı ve ilgili kurumlar arasındaki iş birliği, coğrafi işaretlerden beklenen ekonomik ve sosyal katkıların ortaya çıkmasında önemli rol oynamaktadır.

Gıda güvencesinin bulunabilirlik, erişim, kullanım ve istikrar olmak üzere dört temel boyutu bulunmaktadır. Coğrafi işaret sistemlerinin bu boyutlara katkıları ise farklı şekillerde ortaya çıkabilmektedir (Matemilola & Elegbede, 2017, s. 6).

Gıda güvencesinin bulunabilirlik boyutu açısından değerlendirildiğinde, coğrafi işaret sistemleri doğrudan üretim miktarını artırmayı amaçlamasa da yerel üretim sistemlerinin devamlılığını destekleyebilmektedir. Özellikle geleneksel çeşitlerin ve yerel üretim tekniklerinin korunması, belirli ürünlerin üretiminin sürdürülmesi ve tarımsal çeşitliliğin devamlılığı açısından önem taşımaktadır (Barham, 2003; Bowen, 2010).

Gıda güvencesinin erişim boyutu açısından, coğrafi işaretli ürünlerden sağlanan katma değerin üretici gelirlerine yansıması kırsal hanelerin ekonomik dayanıklılığına katkı sağlayabilmektedir. Gelir düzeyindeki artış, üreticilerin refahının yanı sıra kırsal bölgelerde tarımsal üretimin devamlılığını da destekleyebilmektedir (Belletti & Marescotti, 2011; Vandecandelaere et al., 2010).

Gıda güvencesinin kullanım boyutu açısından, ürün şartnamelerinin uygulanması ve üretim standartlarının korunması, coğrafi işaretli ürünlerde belirli kalite özelliklerinin devamlılığına katkı sağlayabilir. Geleneksel üretim yöntemlerinin ve yerel çeşitlerin korunması da yerel beslenme kültürünün sürdürülmesiyle ilişkilendirilebilir.

Bununla birlikte, coğrafi işaretlerin beslenme ve halk sağlığı üzerindeki doğrudan etkilerine ilişkin ampirik kanıtların sınırlı olduğu göz önünde bulundurulmalıdır.

Gıda güvencesinin istikrar boyutu açısından bakıldığında, yerel üretim sistemlerinin güçlendirilmesi bölgesel gıda sistemlerinin dış kaynaklı sorunlara karşı dayanıklılığına katkı sağlayabilir. Coğrafi işaret sistemleri de yerel üretimin devamlılığını destekleyerek bu sürece katkıda bulunabilecek araçlardan biri olarak değerlendirilebilir (European Parliament and Council of the European Union, 2024; FAO et al., 2023; OECD, 2023).

Gıda güvencesinin dört temel boyutu çerçevesinde coğrafi işaret sistemleriyle ilişkilendirilebilecek potansiyel katkılar ve sınırlılıklar Tablo 3'te özetlenmiştir.

**Tablo 2.** Coğrafi İşaretlerin Gıda Güvenliğine Katkılarının Literatür Sentezi

| Tema | Ortak Bulgular | Destekleyen Çalışmalar | Politika Çıkarımı |
|---|---|---|---|
| **Ürün Şartnameleri** | Üretim özelliklerinin ve ürün standartlarının tanımlanmasına katkı sağlayabilir | De Rosa, 2015; Şahin, 2024 | Şartnamelerin etkin uygulanması |
| **İzlenebilirlik** | Ürün kökeninin doğrulanmasını destekleyebilir | De Rosa, 2015; FAO & oriGIn, 2024 | İzlenebilirlik sistemlerinin güçlendirilmesi |
| **Denetim** | Taklit ve tağşişin önlenmesine ve tüketici güveninin desteklenmesine katkı sağlayabilir | De Rosa, 2015; Şahin, 2024 | Denetim mekanizmalarının etkinleştirilmesi |
| **Dijital Teknolojiler** | Ürün bilgilerinin izlenebilirliğini ve doğrulanabilirliğini destekleyebilir | FAO & oriGIn, 2024 | Dijital izlenebilirlik altyapısının geliştirilmesi |

Kaynak: İlgili literatürden yararlanılarak yazarlar tarafından oluşturulmuştur.

**Tablo 3.** Coğrafi İşaretlerin Gıda Güvencesine Katkılarının Literatür Sentezi

| Gıda Güvencesi Boyutu | Ortak Bulgular | Sınırlılıklar | Olası Politika Çıkarımları |
|---|---|---|---|
| **Bulunabilirlik** | Yerel üretimin sürekliliğini destekleyebilir | Üretim miktarını tek başına artırmaz | Yerel üretim planlaması güçlendirilmeli |
| **Erişim** | Üretici gelirlerinin desteklenmesine katkı sağlayabilir | Yüksek ürün fiyatları tüketici erişimini sınırlayabilir | Üretici-tüketici dengesini gözeten politikalar geliştirilmeli |
| **Kullanım** | Ürün kalitesinin korunmasını destekleyebilir | Beslenme üzerindeki etkilerine ilişkin ampirik kanıtlar sınırlıdır | Beslenme ve halk sağlığı araştırmaları artırılmalı |
| **İstikrar** | Yerel gıda sistemlerinin dayanıklılığına katkı sağlayabilir | İklim değişikliğine uyuma ilişkin çalışmalar sınırlıdır | İklim uyumunun coğrafi işaret stratejilerine entegrasyonu değerlendirilmeli |

Kaynak: İlgili literatürden yararlanılarak yazarlar tarafından oluşturulmuştur.

***Bayburt Örneğinde Coğrafi İşaretli Ürünler ve Katkıları***

Bayburt, Karadeniz Bölgesi ile Doğu Anadolu Bölgesi arasında geçiş özelliği gösteren ve her iki bölgenin iklimsel ve kültürel özelliklerinden izler taşıyan bir ildir. Bu özellikler ilin tarımsal üretim yapısına ve mutfak kültürüne de yansımakta; yöreye özgü çeşitli geleneksel ürünler bulunmaktadır.

Bayburt'ta tarım ve hayvancılık il ekonomisi açısından önemli bir yere sahiptir. İlin farklı ekolojik özellikleri ve tarımsal üretim kültürü, yöreye özgü ürünlerin yetiştirilmesine olanak sağlamaktadır. İlde geleneksel olarak üretilen çeşitli tarım ve gıda ürünlerinin coğrafi işaretle tescil edilmiş olması da bu ürünlerin korunması ve tanıtılması açısından önem taşımaktadır.

Bayburt'un coğrafi işaretli ürünleri arasında yerel tarımsal kaynaklara ve geleneksel üretim yöntemlerine dayanan ürünler bulunmaktadır. Bu ürünlerin coğrafi işaret kapsamında korunması, yöreye özgü üretim biçimlerinin ve yerel ürünlerin devamlılığının desteklenmesi açısından önem taşımaktadır. Örneğin Bayburt Balı, özellikleri bölgedeki ballı bitki florasıyla ilişkili yöresel bir üründür (Türk Patent ve Marka Kurumu, 2020). Aydıntepe Şeker Fasulyesi'nin geleneksel yetiştiriciliğinde ise çiftlik gübresi kullanımı ile geleneksel ekim ve çapalama uygulamaları öne çıkmaktadır (KUDAKA, t.y.). Bu örnekler, coğrafi işaretlerin yerel ürünler ile geleneksel üretim uygulamalarının tanınması ve sürdürülmesine katkı sağlayabilecek araçlardan biri olduğunu göstermektedir.

Coğrafi işaret kapsamında ürünün üretim biçiminin ve belirli özelliklerinin tanımlanması, ürünün standardizasyonu ve izlenebilirliği açısından önem taşımaktadır. Tablo 4'te yer alan ürünlerin coğrafi işaret tescilleri kapsamında üretim ve işleme özelliklerinin tanımlanması, geleneksel ürün özelliklerinin korunması ve üretimin belirli standartlar çerçevesinde sürdürülmesi açısından önem taşımaktadır (Türk Patent ve Marka Kurumu, 2026). Benzer şekilde Bayburt Balı'nda coğrafi işaretin kullanılması ve buna bağlı denetim uygulamaları, ürünün kökeninin ve özgünlüğünün korunması ile taklit ve tağşiş riskinin azaltılması açısından önem taşımaktadır.

**Tablo 4.** Çalışma kapsamında değerlendirilen Bayburt'a ait coğrafi işaretli tarım ve gıda ürünleri

| Ürün adı | İşaret türü | Tescil tarihi |
|---|---|---|
| **Bayburt Lor Dolması** | Mahreç İşareti | 25.12.2017 |
| **Bayburt Tatlı Çorba** | Mahreç İşareti | 29.03.2018 |
| **Bayburt Ketesi** | Mahreç İşareti | 08.06.2020 |
| **Bayburt Kavut Çorbası** | Mahreç İşareti | 08.06.2020 |
| **Bayburt Balı** | Mahreç İşareti | 20.07.2020 |
| **Bayburt Süt Böreği** | Mahreç İşareti | 11.01.2021 |
| **Bayburt Ekşi Lahana** | Mahreç İşareti | 11.01.2021 |
| **Bayburt Kara Pancar Yemeği** | Mahreç İşareti | 18.02.2021 |
| **Aydıntepe Şeker Fasulyesi** | Menşe Adı | 09.06.2021 |
| **Bayburt Yalancı Dolma** | Mahreç İşareti | 09.08.2021 |
| **Bayburt Tarhunu** | Menşe Adı | 15.04.2025 |

**Kaynak:** Türk Patent ve Marka Kurumu Coğrafi İşaretler Portalı verilerinden yararlanılarak hazırlanmıştır.

Bayburt Lor Dolması ve Bayburt Ketesi gibi yöresel ürünlerin coğrafi işaretle korunması, bu ürünlerin tanınırlığının ve katma değerinin artırılması açısından bir fırsat oluşturabilir. Bu tür ürünlerin üretim ve pazarlama olanaklarının gelişmesi, özellikle küçük ölçekli üreticilerin gelirlerinin desteklenmesine ve yerel ekonomik faaliyetlerin çeşitlendirilmesine katkı sağlayabilir (Alacadağlı, 2025). Benzer şekilde coğrafi işaretli ürünlerin üretim ve pazarlama süreçlerinin geliştirilmesi, kırsal alanda üretimin ve istihdamın devamlılığının desteklenmesi açısından önem taşımaktadır. Bayburt Tarhunu'nun coğrafi işaret tescilinden sonra yurt dışı pazarlara açılması, yöresel ürünlerin farklı pazarlara ulaşma potansiyeli açısından dikkat çekici bir örnektir (Anadolu Ajansı, 2025).

## Tartışma

Bu çalışmada incelenen literatür, coğrafi işaretlerin sürdürülebilir tarım, gıda güvenliği ve gıda güvencesi açısından çeşitli katkılar sağlayabileceğini, ancak bu katkıların her durumda ve aynı düzeyde gerçekleşmesinin beklenemeyeceğini göstermektedir. Coğrafi işaretlerin yerel kaynakların korunması, geleneksel üretim bilgisinin sürdürülmesi ve kırsal kalkınmanın desteklenmesi açısından taşıdığı potansiyel, yerel aktörlerin katılımı, üretici örgütlenmesi ve uygulanan politikalarla yakından ilişkilidir. Bu değerlendirme, coğrafi işaretlerin sürdürülebilir kalkınmaya katkılarının aktörlerin stratejilerine, kolektif eyleme ve kamu politikalarına bağlı olduğunu vurgulayan Belletti et al. (2017) ile uyumludur. Benzer şekilde, Belletti ve Marescotti (2011) coğrafi işaretlerin kırsal kalkınma üzerindeki etkilerinin bağlama bağlı olduğunu ortaya koyarken, Bowen (2010) coğrafi işaret tescilinin yerel üreticiler ve çevre açısından beklenen sonuçları tek başına garanti etmediğine dikkat çekmektedir.

Gıda güvenliği açısından değerlendirildiğinde, coğrafi işaret sistemlerinin ürün şartnameleri, üretim yöntemlerinin tanımlanması, izlenebilirlik ve denetim yoluyla destekleyici bir işlev üstlenebileceği görülmektedir. Bununla birlikte, coğrafi işaret tescilinin tek başına gıda güvenliği garantisi olarak değerlendirilmemesi gerekir. De Rosa (2015) da coğrafi işaret ile gıda güvenliği arasındaki ilişkinin kendiliğinden ortaya çıkan bir ilişki olmadığını vurgulamaktadır. Bu nedenle coğrafi işaret sistemlerinin gıda güvenliği açısından sağlayabileceği katkıların etkin denetim ve üretim standartlarının uygulanmasıyla birlikte değerlendirilmesi daha uygun görünmektedir.

Gıda güvencesi bakımından ise ilişkinin daha çok dolaylı olduğu görülmektedir. Coğrafi işaretlerin yerel üretimin devamlılığına, üretici gelirlerine ve kırsal ekonomiye sağlayabileceği katkılar özellikle erişim ve istikrar boyutları açısından önem taşıyabilir. Bununla birlikte, coğrafi işaretlerin gıda bulunabilirliğini doğrudan artırdığı veya beslenme sonuçlarını iyileştirdiği yönünde genellenebilir bir sonuca ulaşmak mevcut literatür temelinde mümkün değildir. Bu nedenle coğrafi işaret–gıda güvencesi ilişkisinin özellikle üretici geliri, tüketici erişimi, beslenme ve yerel gıda sistemlerinin dayanıklılığı bakımından ampirik çalışmalarla incelenmesine ihtiyaç bulunmaktadır.

Bayburt örneği, bu genel çerçevenin yerel düzeydeki potansiyelini göstermektedir. İldeki coğrafi işaretli ürünlerin geleneksel üretim uygulamaları, yerel tarımsal kaynaklar ve yöresel bilgiyle ilişkili olması, coğrafi işaret sisteminin yerel üretim kültürünün korunmasında kullanılabilecek araçlardan biri olduğunu düşündürmektedir. Bununla birlikte, bu çalışma Bayburt'taki coğrafi işaretlerin üretici gelirleri, tüketici tercihleri veya gıda güvencesi üzerindeki etkilerini doğrudan ölçmemiştir. Bu nedenle Bayburt özelindeki değerlendirmeler nedensel bir etki göstergesi olarak değil, literatür ve mevcut kurumsal bilgiler temelinde belirlenen potansiyel katkılar olarak değerlendirilmelidir.

## Sonuç

Coğrafi işaretler, yerel üretim sistemlerinin ve geleneksel bilginin korunması, ürünlerin tanınırlığının ve izlenebilirliğinin desteklenmesi ve kırsal ekonomilerin güçlendirilmesi yoluyla sürdürülebilir tarım, gıda güvenliği ve gıda güvencesine katkı sağlayabilecek araçlardan biridir. Ancak bu katkılar yalnızca tescille ortaya çıkmamakta; etkin denetim, üretici örgütlenmesi, üretimin devamlılığı ve uygun pazarlama olanakları gibi tescil sonrası süreçlere bağlı bulunmaktadır. Bayburt'taki coğrafi işaretli ürünler bu açıdan önemli bir potansiyel taşımakla birlikte, söz konusu etkilerin ortaya konulabilmesi için üretici, tüketici ve ürün düzeyinde saha verilerine dayalı çalışmalara ihtiyaç vardır.

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Ümmügülsüm Erdoğan: Metodoloji, literatürün değerlendirilmesi, gözden geçirme ve düzenleme.

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# Passive fishing gears in ecosystem-approach fisheries: preliminary trials on shrimp fishing with simple trap systems in the Black Sea

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## Abstract

Passive fishing gears are increasingly considered important monitoring tools in ecosystem-approach fisheries because they may cause lower physical disturbance to the seabed compared with active fishing gears. This study presents preliminary trials on shrimp fishing using simple baited trap systems along the southern Black Sea coast. In the study, three simple traps were deployed in a seagrass-associated area and three traps in a nearby control area; the traps were set between November 2025 and April 2026 with soak times of 24, 48 and 72 hours. Captured organisms were recorded at species level, while rostrum length, carapace length, total length and body weight were measured for shrimp individuals. According to the available catch records, a total of 184 shrimp individuals were obtained, of which 46 were captured in the seagrass area and 138 in the control area. Standardized shrimp CPUE was calculated as 0.035 individuals/trap/hour in the seagrass area and 0.106 individuals/trap/hour in the control area. In morphometrically measured individuals, mean carapace length (CL) was determined as 12.48±0.51 mm in the seagrass area and 12.24±0.35 mm in the control area. When all measured individuals were evaluated together, mean CL was 12.29±0.29 mm. Although shrimp catch efficiency was higher in the control area, a higher diversity of benthic and epibenthic organisms was observed in traps associated with the seagrass habitat. Preliminary findings indicate that simple trap systems can be used for small-scale shrimp sampling and habitat-sensitive biodiversity monitoring. However, differences between areas also suggest that trap performance is affected by habitat structure, soak time and seasonal conditions.

***Keywords:*** *Black Sea, Shrimp, Passive Fishing Gears, Trap Fishing Gear, CPUE, Ecosystem-Approach Fisheries.*

**Ekosistem yaklaşımlı su ürünleri avcılığında pasif av araçları: Karadeniz'de basit tuzak sistemleri ile karides avcılığı üzerine ön denemeler**

## Özet

Pasif av araçları, aktif av araçlarına kıyasla deniz tabanı üzerinde daha düşük fiziksel etki oluşturabilmeleri nedeniyle ekosistem yaklaşımlı balıkçılık çalışmalarında giderek daha önemli izleme araçları olarak değerlendirilmektedir. Bu çalışma, Güney Karadeniz kıyılarında basit yemli sepet/tuzak sistemleriyle karides avcılığına yönelik ön deneme sonuçlarını sunmaktadır. Çalışmada deniz çayırıyla ilişkili bir saha ile yakınındaki kontrol sahasında üçer adet basit tuzak kullanılmış; tuzaklar Kasım 2025–Nisan 2026 döneminde 24, 48 ve 72 saatlik bekleme süreleriyle denize bırakılmıştır. Avlanan bireyler tür düzeyinde kaydedilmiş, karides bireylerinde ise rostrum boyu, karapaks boyu, toplam boy ve ağırlık ölçümleri alınmıştır. Mevcut av kayıtlarına göre toplam 184 karides bireyi elde edilmiş; bunların 46'sı deniz çayırı sahasında, 138'i ise kontrol sahasında yakalanmıştır. Standartlaştırılmış karides BÇAM değeri deniz çayırı sahasında 0,035 birey/tuzak/saat, kontrol sahasında ise 0,106 birey/tuzak/saat olarak hesaplanmıştır. Morfometrik olarak ölçülen bireylerde ortalama karapaks boyu (CL) deniz çayırı sahasında 12,48±0,51 mm, kontrol sahasında ise 12,24±0,35 mm olarak belirlenmiştir. Tüm ölçülen bireyler birlikte değerlendirildiğinde ortalama CL 12,29±0,29 mm olmuştur. Kontrol sahasında karides av verimi daha yüksek olmasına rağmen, deniz çayırıyla ilişkili tuzaklarda bentik ve epibentik organizma çeşitliliğinin daha yüksek olduğu gözlenmiştir. Bulgular, basit tuzak sistemlerinin küçük ölçekli karides örneklemesi ve habitat duyarlı biyoçeşitlilik izleme çalışmaları için kullanılabilir olduğunu göstermektedir. Bununla birlikte, saha farklılıkları tuzak performansının habitat yapısı, bekleme süresi ve mevsimsel koşullardan etkilendiğini ortaya koymaktadır.

***Anahtar Kelimeler:*** *Karadeniz, Karides, Tuzak Av Aracı, BÇAM, Ekosistem Yaklaşımlı Balıkçılık*

*ID: 878*

# In Vitro and In Silico Prediction of Syringic Acid, a Cichorium intybus-Derived Ligand, as a Potential Antimicrobial Scaffold and Adjuvant Enhancing β-Lactam Susceptibility in Multidrug- Resistant *Staphylococcus aureus*

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## Abstract

The emergence of multidrug-resistant (MDR) *Staphylococcus aureus* has created an urgent need for novel antimicrobial agents with improved pharmacological and safety profiles. The present study integrated *in vitro* antibacterial screening with comprehensive *in silico* molecular profiling to evaluate the therapeutic potential of *Cichorium intybus*-derived phytochemicals (CI-1 to CI-11) against MDR *S. aureus.* Among the medicinal plant extracts, ethanol extract of *C. intybus* demonstrated the strongest antibacterial activity with a 30 mm inhibition zone and highest antioxidant activity (68%), while *Mentha longifolia* exhibited 64% antioxidant activity. FTIR analysis confirmed the presence of bioactive functional groups including carboxylic acids, aromatic rings, alcohols, ethers, alkenes, and cyclohexyl groups associated with antibacterial potential. Molecular docking analysis revealed that CI-9 exhibited the strongest binding affinity against the selected target protein with a docking score of −11.1 kcal/mol, followed by CI-8 (−10.0 kcal/mol), CI-2 (−8.7 kcal/mol), and CI-5/CI-6 (−8.6 kcal/mol). The conceptual DFT of CI-6, CI-8, and CI-2 demonstrated lower HOMO–LUMO energy gaps, lower hardness, and higher softness values, indicating greater chemical reactivity and potential biological and electrophilicity index (0.7602), while CI-6 emerged as minimum hardness (0.021343) and maximum softness (23.427). PASS prediction further demonstrated that CI-9 possessed the highest predicted antibacterial activity (Pa = 0.677), antioxidant activity (Pa = 0.923), free radical scavenging activity (Pa = 0.988), and antineoplastic activity (Pa = 0.849). However, despite its superior docking affinity and biological activity, CI-9 demonstrated poor drug-likeness characteristics due to its very high molecular weight (610.52 g/mol), elevated TPSA (209.43 Å²), low predicted bioavailability (0.17), and multiple Lipinski rule violations, potentially limiting its oral therapeutic applicability. In contrast, CI-11 (syringic acid) exhibited comparatively moderate docking affinity but demonstrated the most balanced physicochemical, pharmacokinetic, and safety profile, characterized by favorable membrane permeability, balanced lipophilicity (Log P = 0.99), lower toxicity, excellent bioavailability radar characteristics, and strong drug-likeness properties. ADMET and STOPTOX analyses further identified CI-10 and CI-11 as the safest ligands, with CI-11 exhibiting 80% non-sensitization and 90% non-irritation confidence values. Additionally, CI-3 and CI-7 demonstrated the highest predicted bioavailability scores (0.85), whereas CI-1 showed the highest intestinal absorption (94.35%).

Conclusion, CI-9 may represent the biologically strongest antibacterial ligand, whereas CI-11 appears to be the most promising and safest lead compound for future anti-*S.aureus* drug development.

***Keywords:*** *Therapeutic potential, FTIR, Antibiotic susceptibility, Antioxidant activity Molecular docking*

*ID: 881*

# Influence of harvest time and cultivar type on the β-carotene content and quality of biofortified cassava in Ghana

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## Abstract

Vitamin A deficiency (VAD), the leading cause of preventable blindness, ranks second to anaemia in terms of global prevalence. Strategies developed to combat VAD include supplementation, food-to-food fortification and biofortification. However, biofortification is highly promising, as it uses staple crops of target populations to enhance intake of this micronutrient. Cassava is a major staple for millions of people worldwide and ranks second to maize as a major source of calories in Ghana and other West African countries. This research seeks to determine the influence of harvest time on the β-carotene content and flour quality four biofortified cassava cultivars (Adom Bankye, Taah bankye, Hewali Duade and Atseke Gbeli) released by the University of Cape Coast, Ghana, through mutagenesis of the IITA yellow cultivar. The five cassava cultivars were planted under standard agronomic practices and harvested after 8, 10, 12 and 14 months. Quality analysis of flours prepared from the cassava including pH, soluble sugars, colour, functional properties, amylose/amylopectin starch fractions and the levels of β-carotene was carried out. The pH of the flours remained fairly ranged 6.78 – 7.42, and was almost changed irrespective of cultivar or harvest time. The β-carotene peaked at the 10$^{th}$ month of harvest for all cultivars, with Taah Bankye having the highest value among the new cultivars. The yellowness index of the cultivars followed a similar trend to the β-carotene content, establish the fact that the colour of the cassava is mainly influenced by this micronutrient. The functional properties of the flours also varied with cultivar and harvest time, with the highest water absorption and swelling power observed for Hewali Duade and Taah Bankye after 14 and 10 months of harvest time, respectively.

***Keywords:*** *Biofortification; Cassava; β-Carotene; Harvest Time; Flour Quality*

*ID: 884*

# Nanocarrier-Assisted Foliar Applications in RNAi-Based Plant Protection Systems

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## Abstract

RNA interference (RNAi) is a highly effective gene-silencing mechanism that provides sequence-specific protection in plants against viruses, fungal pathogens, and insect pests. Based on this natural defense system, spray-induced gene silencing (SIGS) utilizes the exogenous application of nucleic acid molecules onto plant surfaces to suppress pathogen-derived genes. As this approach does not require the development of transgenic plants, it has emerged as a promising and environmentally friendly strategy for sustainable crop protection. Despite its considerable potential, the practical implementation of SIGS in agriculture remains challenging. The effectiveness of double-stranded RNA (dsRNA) is often constrained by environmental and physicochemical factors, including rainfall, ultraviolet radiation, temperature fluctuations, enzymatic degradation, and limited retention on leaf surfaces, all of which reduce RNA stability and biological efficacy under field conditions. These limitations represent major barriers to the successful application of RNA-based gene-silencing technologies in crop protection. In recent years, nanoparticle-based delivery systems have gained considerable attention as potential tools to address these challenges by enhancing dsRNA stability, prolonging its persistence on leaf surfaces, and improving its biological activity following application. Nevertheless, the successful implementation of RNA-based crop protection strategies requires a comprehensive understanding of the interactions among nanocarriers, dsRNA molecules, plant surfaces, and environmental factors, together with careful evaluation of their biosafety and agricultural applicability.

***Keywords:*** *RNA interference, spray-induced gene silencing, nanocarrier systems, sustainable agricultural practices*

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## RNAi Temelli Bitki Koruma Sistemlerinde Nanotaşıyıcı Destekli Yaprak Uygulamaları

## Özet

RNA interferans (RNAi), bitkilerde virüsler, fungal etmenler ve böcek zararlılarına karşı dizi-spesifik koruma sağlayan oldukça etkili bir gen susturma mekanizmasıdır. Bu doğal savunma sistemine dayanarak geliştirilen spray aracılı gen susturma (SIGS), patojen kökenli genleri baskılamak amacıyla nükleik asit moleküllerinin bitki yüzeylerine eksojen olarak uygulanmasını temel alır. Bu yaklaşım, transgenik bitkilerin geliştirilmesini gerektirmediği için sürdürülebilir bitki koruma stratejileri açısından umut vadeden ve çevre dostu bir yöntem olarak öne çıkmaktadır. Bununla birlikte, SIGS'in tarımsal uygulamadaki pratik kullanımı halen çeşitli zorluklarla karşı karşıyadır. Çift iplikli RNA (dsRNA)'nın etkinliği; yağmur, ultraviyole radyasyon, sıcaklık dalgalanmaları, enzimatik yıkım ve yaprak yüzeyinde sınırlı tutunma gibi çevresel ve fiziko-kimyasal faktörlerden sıklıkla kısıtlanmaktadır. Bu durum, RNA stabilitesini ve biyolojik etkinliği tarla koşullarında önemli ölçüde azaltmaktadır. Bu sınırlamalar, RNA temelli gen susturma teknolojilerinin bitki korumada başarılı şekilde uygulanmasının önündeki temel engelleri oluşturmaktadır. Son yıllarda nanopartikül tabanlı taşıma sistemleri, bu zorlukların aşılmasında potansiyel araçlar olarak önemli bir ilgi odağı haline gelmiştir. Bu sistemler, dsRNA stabilitesini artırarak, yaprak yüzeyinde kalıcılığını uzatarak ve uygulama sonrası biyolojik etkinliğini artırarak bu sorunların giderilmesine katkı sağlayabilir. Bununla birlikte, RNA temelli bitki koruma stratejilerinin başarılı şekilde uygulanabilmesi için nanotaşıyıcılar, dsRNA molekülleri, bitki yüzeyleri ve çevresel faktörler arasındaki etkileşimlerin kapsamlı şekilde anlaşılması ve bunların biyogüvenlik ile tarımsal uygulanabilirlik açısından dikkatle değerlendirilmesi gerekmektedir.

***Anahtar Kelimeler:*** *RNA interferans, sprey aracılı gen susturma, nanotaşıyıcı sistemler, sürdürülebilir tarım uygulamaları*

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# Cytotoxic Effects of Apple Peel Extract and Cisplatin Combination on Lung Cancer Cells

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## Abstract

Lung cancer is one of the most common malignancies worldwide and remains a leading cause of cancer-related deaths. Despite advances in diagnosis and treatment, patient survival rates remain unsatisfactory. The human lung adenocarcinoma cell line A549 is widely used as an *in vitro* model in lung cancer research. Cisplatin (Cis), a commonly used chemotherapeutic agent in lung cancer treatment, exhibits strong antitumor activity but is associated with serious side effects. Therefore, plant-derived bioactive compounds have attracted increasing attention for their potential to enhance the efficacy of chemotherapeutic agents while reducing adverse effects. Apple peel is rich in phenolic compounds, polyphenols, and triterpenes with strong antioxidant properties. Amasya Misket Apple is a geographically indicated local variety known for its unique aroma and bioactive composition. This study aimed to investigate the cytotoxic effects of the combination of Amasya Misket Apple peel extract obtained via supercritical $CO_2$ extraction and Cis on the A549 human lung adenocarcinoma cell line. The cytotoxic effects of the extract and Cis were first evaluated separately using the MTT assay, followed by combination treatments. According to the 24 h MTT assay results, dose-dependent cytotoxic effects were observed in the groups treated with the extract and Cis alone, and combination doses were selected based on these findings. Combination treatments significantly reduced cell viability compared with the control group ($p < 0.0001$). Cell viability decreased to 47% in the 0.1 mg/mL extract + 0.02 mg/mL Cis group ($p < 0.0001$), 50% in the 0.25 mg/mL extract + 0.01 mg/mL Cis group ($p < 0.0001$), 45% in the 0.25 mg/mL extract + 0.015 mg/mL Cis group ($p < 0.01$), and 47% in the 0.5 mg/mL extract + 0.01 mg/mL Cis group ($p < 0.01$). These findings suggest that Amasya Misket Apple peel extract may enhance the cytotoxic efficacy of Cis in A549 cells.

***Keywords:*** *A549, Cisplatin, Amasya Misket Apple, Supercritical $CO_2$ Extraction*

ID: 888

# A Comprehensive Assessment of Fertilization Strategies for Managing *Pectobacterium* and *Dickeya* Infections and Their Impacts on Plant Health and Productivity in Tomato

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## Abstract

Soft rot diseases caused by *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya solani* represent some of the most destructive bacterial diseases affecting vegetable production worldwide. Their extensive host range, remarkable capacity for tissue maceration, and ability to cause severe pre- and post-harvest losses make them major constraints to sustainable crop production. Furthermore, climate change-associated environmental conditions, particularly elevated temperatures and increased humidity, are expected to enhance pathogen aggressiveness and disease incidence, thereby posing growing threats to crop productivity, food security, and agricultural sustainability. In the present study, the effectiveness of six fertilizer treatments, including organomineral, mineral, micronutrient, organic, and biostimulant-based formulations, was investigated for their ability to suppress soft rot disease development and improve yield-related parameters in tomato plants artificially inoculated with the pathogens. Disease severity assessments revealed substantial reductions in all fertilizer-treated plants compared with the untreated control, with suppression rates reaching 80.92% and 84.27% for *P. carotovorum* subsp. *carotovorum* and *D. solani*, respectively. Disease severity values under fertilizer applications ranged from 32.13% to 34.29%, whereas significantly higher disease levels were observed in untreated plants. Among the evaluated treatments, micronutrient fertilization exhibited the most promising overall performance by simultaneously reducing disease severity and enhancing yield-associated characteristics. Statistical analyses confirmed that all fertilizer applications significantly decreased disease severity compared with the control treatment ($p \leq 0.05$). These findings demonstrate that sustainable fertilization strategies can serve as an effective component of integrated disease management programs by strengthening plant health and enhancing resistance against destructive bacterial pathogens. Beyond their role in disease suppression and yield improvement, such practices contribute to reducing dependence on synthetic chemical inputs, maintaining soil biological functionality, improving resource-use efficiency, and mitigating the environmental impacts of agricultural production. Consequently, the adoption of sustainable fertilization approaches offers a promising pathway toward climate-resilient, environmentally responsible, and economically viable tomato production systems capable of meeting future food security challenges.

***Keywords:*** *tomato, plant resistance, sustainability, environmental health, climate change*

## Domateste *Pectobacterium* ve *Dickeya* Bakteriyel Etmenlerine Karşı Gübreleme Stratejilerinin Bitki Sağlığı ve Verimlilik Üzerine Etkilerinin Bütüncül Olarak Değerlendirilmesi

## Özet

Yumuşak çürüklük hastalığına neden olan *Pectobacterium caratovorum* subsp. *caratovorum* ve *Dickeya solani* etmenleri, yüksek tahripkar potansiyelleri, geniş konukçu dizileri ve hasat öncesinden depolama aşamasına kadar devam eden ekonomik kayıpları nedeniyle dünya sebze üretiminde en kritik bakteriyel patojenler arasında yer almaktadır. Bu bakterilerin iklim değişikliğiyle ilişkili yüksek sıcaklık ve aşırı nem koşullarında daha agresif salgınlar oluşturabilmesi, yalnızca verim ve kalite kayıplarını değil, aynı zamanda gıda güvenliği, üretim sürdürülebilirliği açısından domateslerde stratejik bir tehdit oluşturmaktadır. Yürütülen çalışmada, farklı besinsel içeriklere sahip 6 ticari gübrenin (2 adet organomineral, 1 mineral, 1 mikroelement, 1 organik ve 1 biyostimülant içerikli) yumuşak çürüklük hastalıklarına karşı etkileri değerlendirilmiş, bu amaçla hastalık etmeni inokule edilmiş domates bitkilerinin yüzde hastalık şiddeti ve bazı verim parametrelerine etkileri belirlenmiştir. Elde edilen bulgulara göre; Pectobacterium ve Dickeya enfekteli bitkilerdeki yüzde hastalık şiddetleri kontrol bitkilerde sırasıyla %80,92 ve %84,27 iken en başarılı bulunan bir organomineral içerikli gübre uygulamasında yüzde hastalık şiddetleri sırasıyla %32,13 ve %34,29 olarak belirlenmiştir. Gerek yüzde hastalık şiddetleri gerekse bitki verim parametreleri dikkate alındığında yine en başarılı gübrenin etkinliği %40-60 arasında değişmiştir. Diğer tüm gübre uygulamalarında kontrollere kıyasla istatistiki olarak ($p \leq 0,05$) önemli düzeyde başarılı sonuçlar elde edilmiştir. Domates yetiştiriciliğinde farklı gübre türlerinin hastalık şiddeti ve verim parametreleri üzerindeki etkilerinin ortaya konulması, yalnızca daha yüksek ve kaliteli üretim hedeflerine hizmet etmekle kalmayıp; bitki sağlığını güçlendiren, kimyasal girdilere bağımlılığı azaltan, toprak ekosisteminin biyolojik işlevselliğini koruyan ve sera gazı emisyonlarını düşürerek iklim değişikliğine uyumu destekleyen sürdürülebilir tarım stratejilerinin geliştirilmesine önemli katkılar sağlayacak, böylece insan sağlığı, çevresel güvenlik ve gelecek nesiller için dirençli tarımsal üretim sistemlerinin oluşturulmasına bilimsel bir temel sunacaktır.

***Anahtar kelimeler****: domates, bitki direnci, sürdürülebilirlik, çevre sağlığı, iklim değişikliği*

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# Possibilities and Future Perspectives of Using Microorganisms Against Abiotic Stress Factors in Garden Plants

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## Abstract

Global climate change has placed increasing pressure on production systems in recent years; In particular, the distribution of garden flowers, which exhibits a high degree of detail, leads to significant yield and quality losses. Climate-related abiotic stress factors such as increasing temperature fluctuations, heat-induced rainfall, drought, salinity, flooding, and heavy metal levels lead to a decrease in the amount of homeostasis in plants, the establishment of photosynthetic activity, and intensified oxidative stress, resulting in a significant metabolic disruption. This situation has made the development of sustainable production models imperative; beneficial organisms, among environmentally friendly biological treatments, have emerged as an important research area in stress management.

This group of systematics is classified by summarizing the possibilities and future potential of using microorganisms against abiotic stress in garden plants. The first part of the article addresses the effects of global climate change on the production systems of garden flowers and the morphological, biochemical, and maintenance disruptions in plant physiology caused by abiotic stresses. Subsequently, a complex and detailed classification of fundamental abiotic stresses such as drought and flood-related water stress, salinity and osmotic stress, thermal stress from high and low temperatures, heavy metal damage, and nutrient imbalances was conducted.

The study also evaluated the stress tolerance of different microbial bacteria such as rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), endophytic microorganisms, and Trichoderma species that support plant growth. In the review, microorganism-induced tolerance loss was addressed within the framework of morphological and visible adaptations, such as root structure, stomatal regulation, water use efficiency, and mineral nutrient uptake. In addition, the contributions of biochemical changes to stress tolerance, such as the accumulation of osmolytes like proline, glycine betaine, and sugars, activation of antioxidant defense performance, and reduction of lipid peroxidation, were evaluated. Specifically, the reduction of stress-induced ethylene levels through ACC deaminase activity, the regulation of ABA and auxin signaling, and the level of changes in the expression of stress response genes such as HSP, ERF, WRKY, and DREB have been indicated. The application-focused section of the article examines stored microbial application examples in vegetable varieties, fruit types, viticulture, and ornamental plants; these effects on yield, quality, and stress tolerance are evaluated. Furthermore, multi-strain microbial consortia, metagenomics and transcriptomics computational omics technologies, nano-formulation systems, and "designer microbiota" methods, which have gained importance in recent years, are discussed within the framework of current literature. However, the reproducibility of laboratory results under field conditions, the effects on microbial colonization in the region, the stability of the formulations, and problems related to the administration of biological preparations are addressed within the scope of existing limitations.

In conclusion, beneficial microorganisms offer sustainable and advanced biological tools for abiotic stress management in horticultural plants under climatic conditions. It is predicted that microbiome engineering will become more effective in this field through AI-powered data analysis and the involvement of regionally specific microbial consortia.

***Keywords:*** *Abiotic stress, horticultural plants, beneficial microorganisms, PGPR, stress tolerance.*

## Bahçe Bitkilerinde Abiyotik Stres Faktörlerine Karşı Mikroorganizmaların Kullanım İmkanları ve Gelecek Perspektifi

## Özet

Küresel iklim değişikliği, son yıllarda tarımsal üretim sistemleri üzerinde giderek artan baskılar oluşturmakta; özellikle çevresel koşullara yüksek duyarlılık gösteren bahçe bitkileri üretiminde önemli verim ve kalite kayıplarına sebep olmaktadır. Artan sıcaklık dalgalanmaları, düzensiz yağış rejimleri, kuraklık, tuzluluk, su baskını ve ağır metal birikimi gibi iklimsel kaynaklı abiyotik stres faktörleri; bitkilerde hücresel homeostazın bozulmasına, fotosentetik aktivitenin azalmasına, oksidatif stresin artmasına ve metabolik süreçlerin sekteye uğramasına yol açmaktadır. Bu durum, sürdürülebilir üretim modellerinin geliştirilmesini zorunlu hale getirmiş; çevre dostu biyolojik yaklaşımlar arasında yer alan faydalı mikroorganizmalar, stres yönetiminde önemli bir araştırma alanı olarak öne çıkmıştır. Bu derleme makalede, bahçe bitkilerinde abiyotik stres faktörlerine karşı mikroorganizmaların kullanım imkânları ve geleceğe yönelik potansiyeli kapsamlı biçimde değerlendirilmiştir.

Makalenin ilk bölümünde küresel iklim değişikliğinin bahçe bitkileri üretim sistemleri üzerindeki etkileri ile abiyotik streslerin bitki fizyolojisinde meydana getirdiği morfolojik, biyokimyasal ve moleküler bozulmalar ele alınmıştır. Sonrasında kuraklık ve su baskınına bağlı su stresi, tuzluluk ve ozmotik stres, yüksek ve düşük sıcaklık kaynaklı termal stresler ile ağır metal toksisitesi ve besin elementi dengesizlikleri gibi temel abiyotik stres faktörleri ayrıntılı olarak

incelenmiştir. Değerlendirme çalışmasında ayrıca bitki gelişimini teşvik eden rizobakteriler (PGPR), arbusküler mikorizal funguslar (AMF), endofitik mikroorganizmalar ve *Trichoderma* türleri gibi farklı mikrobiyal grupların stres toleransındaki işlevleri değerlendirilmiştir. Derlemede, mikroorganizmalar aracılığıyla indüklenen tolerans mekanizmaları; kök mimarisinin iyileştirilmesi, stoma regülasyonu, su kullanım etkinliğinin artırılması ve mineral besin alımının desteklenmesi gibi morfolojik ve fizyolojik adaptasyonlar çerçevesinde ele alınmıştır. Bunun yanında prolin, glisin betain ve çözünür şekerler gibi ozmolitlerin akümülasyonu, antioksidan savunma sistemlerinin aktivasyonu ve lipid peroksidasyonunun azaltılması gibi biyokimyasal değişimlerin stres toleransına katkıları değerlendirilmiştir. Özellikle ACC deaminaz aktivitesi aracılığıyla stres kaynaklı etilen düzeylerinin azaltılması, ABA ve oksin sinyalizasyonunun düzenlenmesi ile HSP, ERF, WRKY ve DREB gibi stres yanıt genlerinin ekspresyonundaki değişimler moleküler düzeyde açıklanmıştır.

Makalenin uygulama odaklı bölümünde sebze yetiştiriciliği, meyve türleri, bağcılık ve süs bitkilerinde gerçekleştirilen mikrobiyal uygulama örnekleri incelenmiş; bu uygulamaların verim, kalite ve stres toleransı üzerindeki etkileri değerlendirilmiştir. Ayrıca son yıllarda önem kazanan çoklu suş içeren mikrobiyal konsorsiyumlar, metagenomik ve transkriptomik temelli omics teknolojileri, nano-formülasyon sistemleri ve "designer microbiota" yaklaşımları güncel literatür çerçevesinde tartışılmıştır. Bununla birlikte laboratuvar sonuçlarının saha koşullarında tekrarlanabilirliği, çevresel faktörlerin mikrobiyal kolonizasyon üzerindeki etkileri, formulasyon stabilitesi ve biyolojik preparatların tescil süreçlerine ilişkin sorunlar mevcut sınırlamalar kapsamında ele alınmıştır.

Sonuç olarak faydalı mikroorganizmaların, iklim değişikliği koşullarında bahçe bitkilerinde abiyotik stres yönetimine yönelik sürdürülebilir ve yenilikçi biyolojik araçlar sunduğu; gelecekte mikrobiyom mühendisliği, yapay zekâ destekli veri analizleri ve bölgeye özgü mikrobiyal konsorsiyumların geliştirilmesiyle bu alandaki uygulamaların daha etkin hale geleceği öngörülmektedir.

***Anahtar Kelimeler:*** *Abiyotik stres, bahçe bitkileri, faydalı mikroorganizmalar, PGPR, stres toleransı.*

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# Identification of Soil-Borne Fungal and Bacterial Pathogens in Cabbage Production Areas of Niğde Province

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## Abstract

Cabbage (*Brassica oleracea* var. *capitata* L.) is an important vegetable species on a global scale, and Türkiye, particularly the Niğde province, ranks among the top regions in white cabbage production. However, soil-borne fungal and bacterial pathogens cause significant yield and quality losses in cabbage production. The aim of this study is the isolation, morphological, and molecular identification of disease-causing soil-borne pathogens in the cabbage production areas of Niğde province. In this context, soil and infected plant samples were collected from cabbage production fields in Kaynarca village of the Bor district. Bacterial isolates were cultured on Nutrient Agar and subjected to pathogenicity tests, and molecular identification was carried out via PCR amplification targeting the 16S rRNA gene region. Fungal isolates were purified on Potato Dextrose Agar, morphologically characterized, and molecular studies targeting the Internal Transcribed Spacer (ITS) regions of ribosomal DNA were conducted. Based on morphological, pathogenicity, and molecular analyses, the bacterial isolates were identified as belonging to *Xanthomonas spp.* and *Pseudomonas* spp. Molecular analyses of the fungal isolates identified the pathogens as *Sclerotinia* spp., *Alternaria* spp., and *Fusarium* spp. This study revealed the presence of important bacterial and fungal pathogens associated with cabbage production soils in Niğde province and highlighted the importance of systematic investigations for the determination of soil-borne pathogens in the region.

***Keywords:*** *Brassica oleracea* var. *capitata*, soil-borne pathogens, *Xanthomonas* spp., *Pseudomonas* spp., *Fusarium* spp., pathogen identification, molecular characterization.

## Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is a vegetable crop of strategic importance for global nutrition and rural economies. With production exceeding 55 million tons annually across approximately 2.6 million hectares worldwide, cabbage is not only an essential food source but also a valuable plant in traditional medicine due to its bioactive compounds (Singh et al., 2006). In 2024, global cabbage production reached approximately 75.48 million tons in 2024, with China being the dominant producer, contributing about 36.82 million tons, representing nearly half of the total global production (FAOSTAT, 2024). Türkiye ranks 11th worldwide in cabbage production with an annual output of approximately 913 thousand tons according to FAOSTAT (2024). At the national level, TÜİK data for 2025 indicate that total white cabbage production is 634,900 tons, with Samsun (179,442 tons), Niğde (135,633 tons), İzmir (31,089 tons), Bursa (27,266 tons), and Afyonkarahisar (24,358 tons) being the leading production provinces (TÜİK, 2025). Among these, Niğde is the second-largest producer after Samsun. Despite this production potential, cabbage cultivation is significantly threatened by numerous soil-borne pathogens that can severely reduce yield and quality. Cabbage and other *Brassica* species are susceptible to a wide range of diseases caused by fungal and bacterial pathogens (Sharma et al., 2018). Major fungal pathogens include *Sclerotinia sclerotiorum* (white rot), *Plasmodiophora brassicae* (clubroot), *Fusarium oxysporum* f. sp. *conglutinans* (Fusarium wilt), and *Alternaria* spp. *Sclerotinia sclerotiorum* is a cosmopolitan necrotrophic fungus capable of infecting more than 500 host species and surviving in soil for many years through sclerotia. *Plasmodiophora brassicae* induces characteristic root galls, resulting in stunted growth, chlorosis, and yield losses, and can persist in soil as long-lived resting spores (Kageyama & Asano, 2009). *Fusarium oxysporum* f. sp. *conglutinans* colonizes vascular tissues, causing systemic wilt symptoms and long-term soil persistence (Liu et al., 2017; Ping et al., 2024). In addition, *Alternaria* spp. are common fungal pathogens of *Brassicaceae* crops and are responsible for leaf spot diseases characterized by dark necrotic lesions that reduce photosynthetic activity and market quality (Woudenberg et al., 2013).

Bacterial diseases can be equally destructive as fungal pathogens. *Xanthomonas* spp. are the causal agents of black rot disease, which typically begins with V-shaped lesions at leaf margins and leads to plant death through vascular blackening (Vicente & Holub, 2013). *Pseudomonas* spp. are also important bacterial pathogens of cabbage and other vegetable crops, causing leaf spot and bacterial blight symptoms characterized by water-soaked lesions and tissue necrosis. *Ralstonia solanacearum*, a pathogen with a broad host range, causes bacterial wilt by forming biofilms in vascular tissues and can survive in soil even under low temperatures (Ahmed et al., 2013). *Pectobacterium carotovorum*, first reported in Türkiye in 2016 in Samsun province (Aksoy et al., 2017), causes soft rot in plant tissues through the secretion of pectinolytic enzymes (Öztürk, 2022). This pathogen also poses a potential risk to cabbage cultivated in Niğde province, where intensive potato production occurs.

Although numerous studies on cabbage diseases exist in the literature, systematic investigations on the diversity and occurrence of soil-borne pathogens in intensively cultivated regions such as Niğde province are still limited. Therefore, the aim of this study was to isolate, identify, and characterize soil-borne fungal and bacterial pathogens causing yield and quality losses in cabbage production areas of Niğde province using morphological and microscopic approaches. The study confirmed *Xanthomonas* spp. and *Pseudomonas* spp. as bacterial pathogens, whereas fungal pathogens were identified as *Alternaria* spp., *Fusarium* spp., and *Sclerotinia* spp.

## Materials and Methods

### *Field Survey*

Approximately 15 soil and infected plant samples were collected from production areas with cabbage plants showing disease symptoms in Kaynarca village of the Bor district, Niğde province, and transported to the laboratory under aseptic conditions.

### *Bacterial Isolation*

One gram sample of material taken from the soil samples was suspended in 10 mL of sterile distilled water and vortexed; plant samples were cultured following surface sterilization. The suspensions were subjected to serial dilutions of $10^{-1}$–$10^{-6}$, inoculated using the spread plate method, and the plates were incubated at 37°C for 24 hours (Kannan et al., 2018). The developing colonies were purified on Nutrient Agar (NA) medium; pure cultures were propagated in Nutrient Broth medium and stored at 4°C for short-term preservation and at -80°C in 50% glycerol for long-term preservation.

### *Bacterial Pathogenicity Tests*

The pathogenicity potential of the bacterial isolates was evaluated using the hypersensitivity reaction (HR) test on tobacco leaves, inoculation studies on healthy cabbage seedlings, and the potato disk method. Sterile water was applied to the control group. Tissue degradation observed in the potato disk method was recorded as an indicator of pathogenicity.

### *Bacterial Molecular Identification*

Genomic DNA extraction from isolates showing pathogenic potential was performed using the Zymo Quick-DNA™ Fungal/Bacterial Microprep Kit (D-6007) according to the manufacturer's protocol. DNA purity and concentration were determined using a Nanodrop spectrophotometer. In molecular analyses, the 16S rRNA gene region was amplified by PCR using the 27F/1492R universal primers in a total reaction volume of 20 µL (Canene-Adams, 2013).

### *Fungal Isolation and Purification*

Pieces approximately 5 mm in size taken from plant tissues showing disease symptoms were subjected to surface sterilization, inoculated onto PDA medium, and incubated at 25°C for 5–7 days. Hyphal tips taken from the developing colonies were transferred to fresh PDA medium to obtain pure cultures, which were then stored at -80°C using the sterile filter paper method.

### *Fungal Morphological Characterization*

Fungal isolates were incubated on PDA medium at 25 ± 2°C for 7 days; colony characteristics, hyphal structures, and spore morphologies were evaluated under a light microscope.

### *Fungal Pathogenicity Test*

Pathogenicity tests were conducted on 8-week-old healthy white cabbage seedlings. Following inoculations performed using mycelial fragments or spore suspensions, the seedlings were kept under high humidity conditions, and symptom development was monitored regularly.

### *Fungal Molecular Identification*

Genomic DNA extraction from fungal isolates was performed using the Zymo Quick-DNA™ Fungal/Bacterial Microprep Kit (D-6007) from mycelia grown on PDA medium. Studies aimed at performing PCR amplification with ITS1F and ITS4 primers targeting the ITS region of nuclear rDNA from the obtained DNA samples, followed by sequencing analyses, are ongoing (Gardes and Bruns, 1993).

## Results

### *Bacterial Isolation and Identification*

As a result of isolations made from soil and infected plant samples, yellow-pigmented, round, smooth-edged colonies developed on Nutrient Agar medium. Representative colonies are shown in Figure 1. In the hypersensitivity reaction test applied to the purified isolates, necrosis formation was observed on tobacco leaves within 24-48 hours. In inoculations performed on cabbage seedlings, typical disease symptoms were recorded, including V-shaped yellowing at leaf

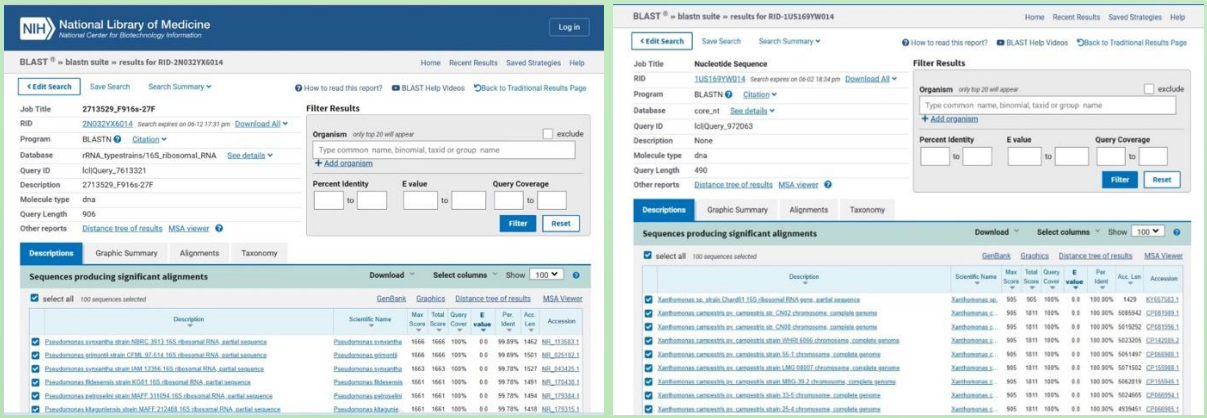
margins, vascular blackening, and leaf spot and blight symptoms. Tissue degradation in the potato disk method was also evaluated as a positive pathogenicity indicator.

As a result of PCR amplification targeting the 16S rRNA gene region and sequencing analysis of isolates that tested positive in pathogenicity assays, bacterial pathogens belonging to *Xanthomonas* spp. and *Pseudomonas* spp. were identified. The BLAST analysis results are presented in Figure 2.



A b C

Figure 1. (A-C) These are the colony morphologies of bacterial isolates obtained as a result of purification processes carried out using the streak plating method.

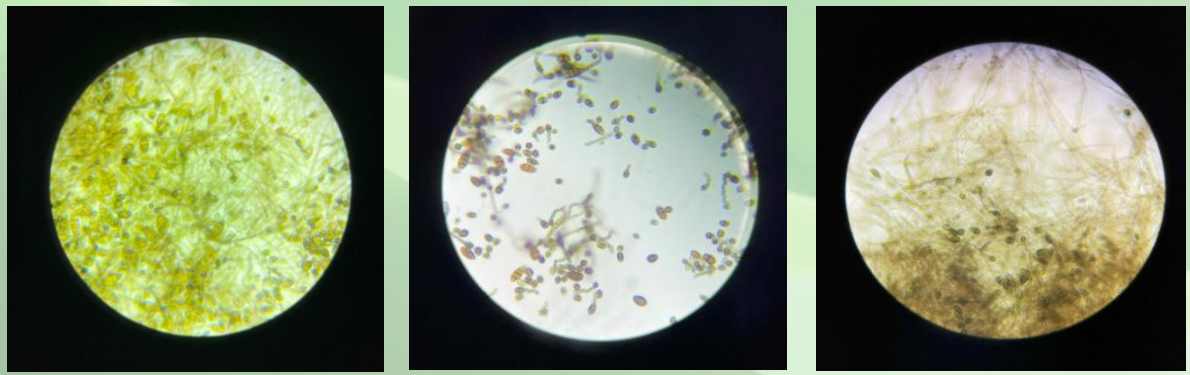



A B

Figure 2. NCBI BLAST analysis results of the 16S rRNA gene sequence of the isolated bacterial samples. (A) Pseudomonas spp. (B) Xanthomonas spp.

***Fungal Isolation and Morphological Characterization***

Fungal colonies were obtained a result of inoculations made from plant tissues showing disease symptoms onto PDA medium. In morphological examinations conducted under a light microscope, the hyphal structures and spore morphologies of the isolates were evaluated, and preliminary identifications were made. Microscopic images are presented in Figure 3.

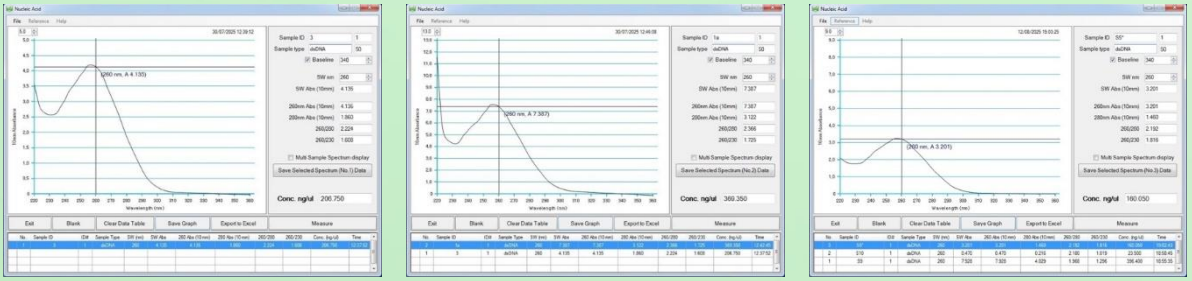


A B C

Figure 3. Light micrographs of fungal isolates cultured on PDA from samples. (A–C) Hyphal and spore morphologies of three distinct isolates recovered from the survey area.

***Fungal Molecular Identification***

PCR amplification using ITS1F and ITS4 primers targeting the ITS region of nuclear rDNA was performed on genomic DNA obtained from fungal isolates, followed by sequencing analysis. Based on ITS sequence analysis, the fungal isolates were identified as *Alternaria* spp., *Fusarium* spp., and *Sclerotinia* spp. DNA concentration and purity values of the extracted samples are presented in Figure 4.



A B C

Figure 4. NanoDrop results of extracted DNA showed varying concentrations among the samples, with values of 206.750 ng/µL (A), 369.350 ng/µL (B), 160.050 ng/µL (C).

## Discussion

The major diseases commonly reported in cabbage production areas in Türkiye include bacterial and fungal pathogens. Among bacterial diseases, black rot (*Xanthomonas campestris* pv. *campestris*), bacterial soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), bacterial leaf spot (*Pseudomonas syringae* pv. *maculicola*), Brassicaceae leaf spot disease (*Xanthomonas campestris* pv. *armoraciae*), and marginal leaf blight (*Pseudomonas marginalis*) are prevalent. Among fungal diseases, white rot (*Sclerotinia sclerotiorum*), Rhizoctonia root and crown rot (*Rhizoctonia solani*), Fusarium wilt (*Fusarium oxysporum*), Alternaria leaf spot (*Alternaria brassicae*), and blackleg disease (*Plenodomus lingam*, syn. *Leptosphaeria maculans*) are widely reported.

Survey studies conducted in the Central Anatolia Region also reveal a similar pathogen profile. Derviş et al. (2021) reported black rot as the most common bacterial pathogen in Niğde, while *Leptosphaeria maculans*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Fusarium oxysporum* were identified as important fungal pathogens in the region. Öztürk and Soylu (2022) reported *Pectobacterium carotovorum* subsp. *carotovorum* in Yozgat for the first time. Eroğlu and Elci (2026) confirmed the presence of *X. campestris* pv. *campestris* in Niğde using phenotypic and molecular approaches.

In the present study, *Xanthomonas* spp., *Pseudomonas* spp., *Alternaria* spp., *Fusarium* spp. and *Sclerotinia* spp. were identified. These genera represent black rot and leaf spot diseases, bacterial leaf spot/soft rot complexes, Alternaria leaf spot, vascular wilt, and white rot pathogens, respectively. The findings are consistent with previous studies, particularly regarding the dominance of *Xanthomonas* spp. and *Sclerotinia* spp. in the region, while the co-occurrence of *Pseudomonas* spp. and *Alternaria* spp. indicates the presence of a complex disease structure. Overall, the results demonstrate that the pathogen profile in Niğde is similar to those reported from other major cabbage production areas.

## Conclusion

This study confirmed the presence of major soil-borne bacterial and fungal pathogens in cabbage production areas of Niğde province. Bacterial isolates were identified as *Xanthomonas* spp. and *Pseudomonas* spp., while fungal isolates were identified as *Alternaria* spp., *Fusarium* spp. and *Sclerotinia* spp. For future studies, species-level identification of the isolates is required. Since universal primers (16S rRNA, ITS) gene analysis alone is not sufficient for accurate identification, more specific molecular procedures should be applied for precise taxonomic characterization.

## Acknowledgments

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under the 2209-A University Students Research Projects Support Program (Project No: 1919B012470865).

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ID: 891

# Detection of Soil-Borne Bacterial Diseases in Potato Cultivation in Niğde Province

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## Abstract

Potato (*Solanum tuberosum L.*) is a strategically important crop worldwide, and Türkiye's Niğde province has the largest potato cultivation area in the country. However, soil-borne bacterial diseases including blackleg, soft rot, bacterial wilt, ring rot and common scab cause substantial yield and quality losses. Direct detection of these pathogens from field soils has been rarely performed. This study aimed to isolate and characterize bacterial pathogens from soil samples collected from potato production fields in Niğde. Ten soil samples were collected from the vicinities of symptomatic plants in Hasaköy, Alay and Altunhisar districts. The serial dilution method was used to inoculate samples onto Nutrient Agar. Morphologically distinct bacterial isolates were obtained. Pathogenicity tests were performed on 21 isolates, and ten isolates tested positive in both the potato disc test and the tobacco hypersensitivity test. Genomic DNA was extracted from these ten isolates, followed by PCR amplification of the 16S rRNA gene region and Sanger sequencing. BLAST analysis identified isolates as members of bacterial genera commonly associated with potato diseases, including *Streptomyces* spp., *Bacillus* spp., *Chryseobacterium* spp., and *Pectobacterium* spp. These results demonstrate that diverse soil-borne bacterial pathogens are present in Niğde's potato production areas. Therefore, regular soil monitoring and integrated pest management are essential to reduce disease risks and sustain potato production in the region.

***Keywords:*** *Potato; Soil-borne diseases; Bacterial pathogens; Isolation and characterization; Pathogenicity test; Niğde province*

## Introduction

Potato (*Solanum tuberosum L.*) is a strategic crop from the *Solanaceae* family and stands as one of the world's primary food sources. Owing to its high yield potential and relatively low production costs, it contributes significantly to global food supply and ranks fourth among staple foods, after cereals (Yılmaz et al., 2006; Wang et al., 2021). In 2024, potato is cultivated in approximately 153 countries worldwide, with a total production of 390.4 million tonnes. The major producing countries include China, India, Ukraine, and the United States of America. In Türkiye, total potato production amounted to 6.9 million tonnes in 2024, cultivated across a harvested area of 195,767 hectares, with an average yield of approximately 35.3 t $ha^{-1}$ (FAO, 2025). Production is heavily concentrated in the provinces of Niğde, Konya, Afyonkarahisar, İzmir, and Nevşehir. According to 2025 data, Niğde ranked first with 884,023 tonnes, followed by Konya with 837,272 tonnes, Afyonkarahisar with 648,029 tonnes, İzmir with 339,920 tonnes, and Nevşehir with 295,089 tonnes (TÜİK, 2025)

Potato yield is strongly limited by both biotic and abiotic factors (Agrios, 2005). Among biotic factors, bacterial, fungal and viral diseases cause annual yield losses of up to about 22% worldwide (Czajkowski et al., 2011). Within these, soil-borne bacterial pathogens are particularly important because they severely affect both yield and quality. The main bacterial pathogens found in potato production include *Pectobacterium* spp. and *Dickeya* spp., which cause blackleg and soft rot, as well as *Ralstonia solanacearum*, the agent of bacterial wilt, *Clavibacter michiganensis* subsp. *sepedonicus*, the cause of bacterial ring rot, and *Streptomyces* spp., which cause common scab (Ünlü & Elçi, 2022).

*Pectobacterium* and *Dickeya* species are Gram-negative bacteria. They secrete pectinolytic enzymes, producing symptoms characterized by soft rot in plant tissues and blackening of the stem (Czajkowski et al., 2011; Pérombelon & Kelman, 1980). *Ralstonia solanacearum*, the causal agent of bacterial wilt, is a Gram-negative, soil-borne pathogen with a broad host range. It enters plants mainly through the roots, colonizes the vascular system, and disrupts water transport, leading to wilting, chlorosis, and eventual plant death (Ahmed et al., 2013). *Clavibacter michiganensis* subsp. *sepedonicus*, the causal agent of bacterial ring rot, is another highly significant pathogen, which causes typical symptoms including vascular tissue rot, stunting (dwarfing), leaf curling, and necrosis in infected plants (Whitworth et al., 2019). Pathogenic species belonging to the genus *Streptomyces*, which are commonly found in soil, cause common scab. This disease lowers the commercial value of the crop by forming superficial or deep lesions, especially on tubers. The severity of symptoms varies depending on pathogen density, environmental conditions and variety susceptibility (Bouchek-Mechiche et al., 2006; Charkowski et al., 2020; Uysal et al., 2025). *Chryseobacterium* spp. are yellow-pigmented bacteria, and some species have been reported to promote plant growth and suppress pathogens through various mechanisms (Morohoshi et al., 2014; Jeong et al., 2017). However, certain species within this genus act as opportunistic pathogens and have been associated with potato diseases; for instance, *Chryseobacterium mulctrae* has been reported to cause soft rot in potato tubers (Shrestha, 2025). *Bacillus* spp. are among the plant growth-promoting rhizobacteria (PGPR) and are

known to suppress plant pathogens through various mechanisms (Khan et al., 2022). However, some *Bacillus* species have also been reported to act as plant pathogens under certain conditions; for instance, *Bacillus amyloliquefaciens* has been reported to cause soft rot in potato tubers (Wang et al., 2017). These findings indicate that both *Bacillus* spp. and *Chryseobacterium* spp. may exhibit both beneficial and potentially pathogenic characteristics.

In the literature, studies on potato bacterial diseases have largely relied on isolations from diseased plant samples. However, with respect to the epidemiology of soil-borne pathogens, differing hypotheses exist as to whether the main source of inoculum is infected seed tubers or the soil itself (Czajkowski et al., 2011; De Boer, 2004). Although it has been proposed that the role of the soil may be more dominant, especially for pathogens such as *Streptomyces*, which are naturally present and persistent in the soil as saprophytic microorganisms, comprehensive studies on the prevalence of soil-borne inoculum in intensive production regions remain limited (Lerat et al., 2009).

This study was carried out in Niğde Province, a major potato production center in Türkiye. Soil samples collected from potato-growing areas were used for the isolation, identification, and characterization of bacterial populations associated with potato fields. The study focuses on determining the presence and diversity of soil-borne bacterial microorganisms in the region, with particular attention to potential plant pathogenic species.

## Materials and Methods

### *Sampling and Soil Collection*

A total of ten soil samples were taken from around symptomatic potato plants in Hasaköy, Alay and Altunhisar districts of Niğde province. The samples were collected carefully without disturbing the root zone of the plants. They were kept at +4°C until analysis. Reference pathogen isolates were obtained from the culture collection of the Plant Pathology Laboratory at Niğde Ömer Halisdemir University.

### *Bacterial Isolation*

One gram of each soil sample was homogenized in 10 ml of sterile distilled water. Serial dilutions were made from $10^{-1}$ to $10^{-5}$. From the $10^{-3}$ and $10^{-5}$ dilutions, 0.1 ml was spread onto Nutrient Agar and incubated at 29.5°C for 24-48 hours. Colonies showing different morphologies were purified and grown on Nutrient Agar and then transferred to Nutrient Broth. The isolates were finally kept at -80°C in 50% glycerol.

### *Pathogenicity Tests*

#### *Potato Disc Test*

Healthy potato tubers were surface-sterilized by soaking in 1% sodium hypochlorite for 3 minutes. They were then rinsed with sterile distilled water and cut into discs about 1 cm thick. The discs were placed in petri dishes. Bacterial cultures grown on NA for 24-48 hours were taken with a sterile toothpick and inoculated onto the disc surfaces. One milliliter of sterile water was added to each dish, and the dishes were sealed with parafilm before incubation. A reference isolate was used as a positive control, and sterile distilled water served as the negative control.

### *Hypersensitivity (HR) Test*

Bacterial suspensions were injected into wounds made on tobacco leaves (*Nicotiana tabacum*) with a sterile needle. Positive and negative controls were applied in the same way. The leaves were then observed for necrosis. Isolates that caused necrosis were recorded as HR positive.

### *Molecular Identification*

Genomic DNA was extracted from pathogenic bacterial isolates using the Zymo Quick-DNA™ Fungal/Bacterial Microprep Kit (D-6007) and stored at −20°C until further use. Molecular identification of the isolates was performed by amplification of the 16S rRNA gene using the universal primers 27F and 1492R. Polymerase chain reaction (PCR) was carried out under appropriate reaction conditions using a PCR Master Mix. PCR products were visualized by agarose gel electrophoresis. Subsequently, amplified DNA fragments were subjected to Sanger sequencing, and the obtained sequences were compared with those available in the NCBI-BLAST nucleotide database for species-level identification.

## Results

Bacterial isolation was performed using the serial dilution method, and colonies showing different morphological characteristics were purified. A total of 27 isolates were re-evaluated based on colony morphology, and after removing duplicates with similar features, 21 isolates were selected for further analyses. Images of petri dishes of some isolates are shown in Figure 1.

In the potato disc test, 10 isolates induced tissue degradation, whereas 17 isolates caused necrosis on tobacco leaves. Ultimately, the 10 isolates that exhibited positive reactions in both tests were confirmed as pathogenic. No symptoms were observed in the negative controls, while the positive control reference strains yielded the expected symptoms.

Genomic DNA was extracted from 10 bacterial isolates identified as potential pathogens. The quality and concentration of the obtained DNA samples were evaluated using spectrophotometric measurements and found to be suitable for molecular analyses (Figure 2). The extracted DNA samples were subsequently used for PCR amplification and molecular identification of the bacterial isolates.

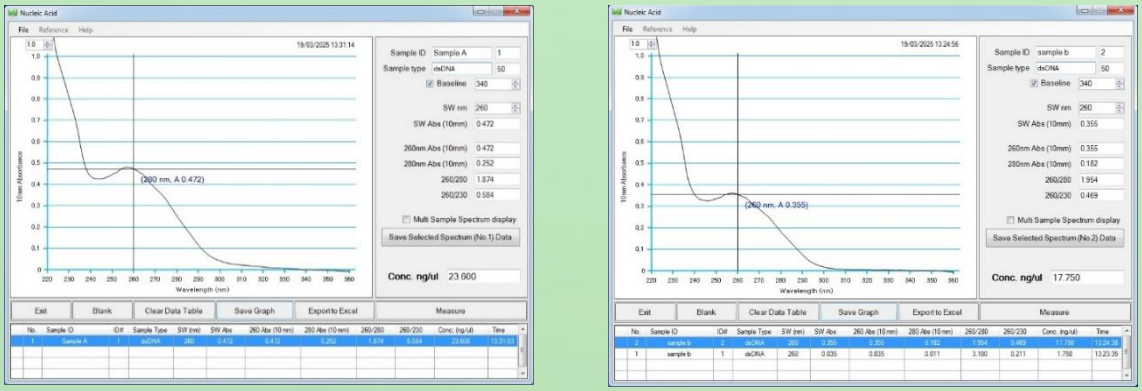
For molecular identification, the 16S rRNA gene region of bacterial isolates was amplified by PCR using genomic DNA and universal primers 27F/1492R. The PCR products were visualized by agarose gel electrophoresis, and the expected bands were observed (1568 bp). The selected PCR products were sent for Sanger sequencing analysis.

The obtained 16S rRNA sequence data were compared in the NCBI-BLAST database, and the isolates were identified at the genus level. The analysis revealed *Streptomyces* spp., *Bacillus* spp., *Chryseobacterium* spp., and *Pectobacterium* spp. BLAST results for *Chryseobacterium* spp. and *Pectobacterium* spp. isolates are presented in Figure 3.



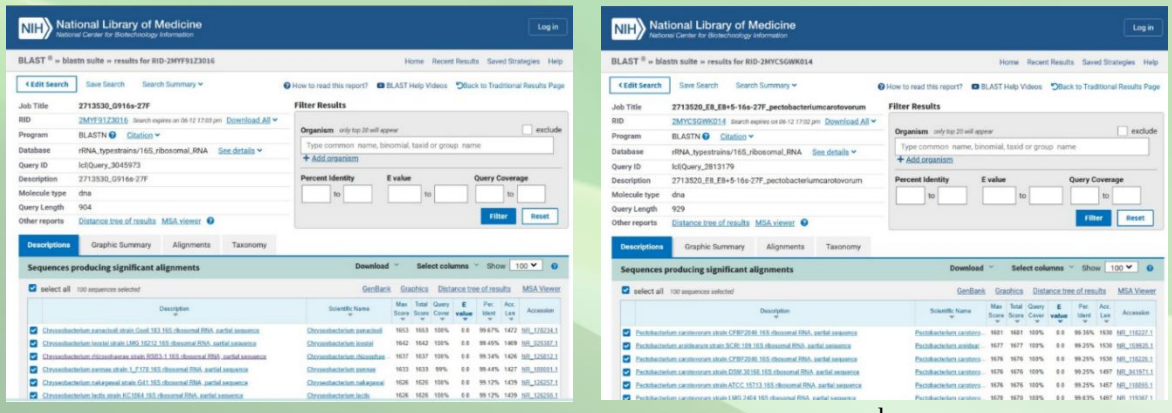
a b c

Figure 1. Images of petri dishes of some isolates (a, b, c).



a b

Figure 2. Concentration and purity analyses of genomic DNA from some bacterial isolates were performed using a NanoDrop spectrophotometer. DNA concentrations were determined as 23.6 ng/µL (a) and 17.75 ng/µL (b).



a b

Figure 3 NCBI-BLAST comparison results of 16S rRNA gene sequences from *Chryseobacterium* spp. (a) and Pectobacterium spp. (b) isolates.

## Discussion

Bacterial diseases of potato are caused by several important pathogens in production areas in Türkiye. Among these, *Pectobacterium* spp. and *Dickeya* spp. are responsible for blackleg and soft rot diseases, *Streptomyces* spp. causes common scab, *Ralstonia solanacearum* causes bacterial wilt, and *Clavibacter michiganensis* subsp. *sepedonicus* is the causal agent of bacterial ring rot. In particular, *Pectobacterium* and *Dickeya* species are considered among the most economically important bacterial pathogens worldwide due to their significant impact on yield and tuber quality (Mansfield et al., 2012).

Previous studies conducted in the Central Anatolia Region have demonstrated the widespread occurrence of bacterial pathogens in potato fields. Benlioğlu (1991) reported blackleg and soft rot agents in Niğde and Nevşehir as *Erwinia carotovora* subspecies. Öztürk et al. (2018) identified *Pectobacterium carotovorum* subsp. *brasiliense*, *Pectobacterium parmentieri* and *Pectobacterium atrosepticum* in the Central Anatolia and Black Sea regions. In addition, Ünlü and Elçi (2022) confirmed the presence of *Streptomyces scabiei* as the causal agent of common scab in Niğde province at the molecular level. These findings indicate the long-term presence of bacterial pathogens in potato production areas of the region.

In the present study, *Pectobacterium* spp. (blackleg and soft rot), *Streptomyces* spp. (common scab), *Bacillus* spp., *Chryseobacterium* spp., and *Clavibacter michiganensis* subsp. *sepedonicus* were identified. The results are consistent with previous reports, particularly regarding the presence of *Pectobacterium* and *Streptomyces* species. Moreover, the detection of *Bacillus* spp. and *Chryseobacterium* spp. suggests that, in addition to well-known pathogenic groups, other bacterial groups are also present in the region's potato fields.

## Conclusion

Bacteria belonging to the genera *Pectobacterium* spp., *Streptomyces* spp., *Bacillus* spp., and *Chryseobacterium* spp. were detected in soil samples collected from potato fields in Niğde Province. These findings highlight the presence and diversity of soil-borne bacterial communities associated with potato-growing areas in the region and suggest a potential risk for bacterial diseases under field conditions. Species-level identification is required to better understand the pathogenic potential of the isolates, as 16S rRNA gene analysis alone is insufficient for precise taxonomic resolution, and species-specific approaches are necessary for accurate characterization.

## Acknowledgments

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under the 2209-A University Students Research Projects Support Program (Project No: 1919B012465499).

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# Comparison of Wintering Performance of Honey Bee (Apis mellifera L.) Colonies in Traditional and Mobile Systems in Niğde Province

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## Abstract

With a mobile beekeeping system, it is possible to plan operations based on the flowering periods of nectar-producing plants and to utilize foraging areas that maximize the benefits derived from nectar and pollen sources. Recently developed innovative equipment and precision apiculture practices have helped mobile systems evolve from mere means of transport into integrated production platforms. However, for these innovative mobile beekeeping practices to be considered a viable model, they must be applicable not only to the production of bee products but also to other critical periods—such as overwintering—which serve as essential links in a chain. In this study, a total of 20 honeybee colonies of the Anatolian bee breed (Apis mellifera anatoliaca) were examined under the ecological conditions of Niğde province. The study groups consisted of 10 traditional (fixed) hives and 10 mobile multi-shelf hives. The research evaluated the proportional (%) changes in the number of frames covered with bees before and after overwintering, as well as the rates of colony collapse or queen loss (%). The results demonstrated an equal colony survival rate of 80% for both overwintering methods. Among the colonies that successfully completed the overwintering period, the average loss of frames covered with bees per colony was 1.25 ± 1.16 in the traditional overwintering group and 1.00 ± 1.07 in the mobile overwintering group. Statistical analysis revealed that the difference in population loss between the two methods was not statistically significant (P = 0.701). Although colonies in the mobile system entered winter with numerically fewer frames compared to traditional hives (5.00 ± 0.93 and 5.88 ± 1.36, respectively), they performed as successfully as the traditional hives during the overwintering period. In conclusion, the mobile beekeeping system presents no disadvantages compared to the traditional method under the winter conditions of Niğde province.

***Keywords:*** *Apis mellifera L., Mobile beekeeping, Innovation, Precision beekeeping, Wintering performance*

## Introduction

Migratory beekeeping is based on seasonal changes in the plant flora across different geographical areas (Official Gazette of the Republic of Türkiye, 2011). However, it also involves challenging factors such as the individual loading and unloading of hives, transportation costs, security concerns, and direct exposure to undesirable external environmental conditions (Küçüktekin and Yelboğa, 2022; Erkan and Aşkın, 2021; Tew, 2002; Yeninar et al., 2010).

In modern beekeeping, the development of innovative equipment and the concept of precision apiculture have enabled mobile systems, which have become increasingly utilized in migratory beekeeping in recent years, to evolve beyond being merely a practical means of transportation. This approach encompasses innovative practices such as the implementation of decision support systems for colony transportation, the remote monitoring of biological needs and environmental parameters of bee colonies, the utilization of alternative energy sources such as solar and wind power, and the transition toward autonomous, data-driven colony management (Zacepins et al., 2015).

Modern mobile beekeeping systems—such as multi-rack trailer systems with hydraulic units and climate-controlled mobile platforms, which increasingly drive development in the sector and are considered the heart of this transformation—are equipped with Internet of Things (IoT)-based sensor technologies that monitor temperature, weight, humidity, and vibration, as well as integrated insulation materials (Şirin & Sevin, 2025; Zacepins et al., 2015).

In addition, through artificial intelligence and machine learning, high-accuracy predictions can be made by analyzing the behavior and health status of bee colonies using audio and image processing techniques. Thanks to sensitive sensors, queen bee and disease detection can be achieved with an accuracy of up to 95–99%, providing early warnings against potential adverse conditions (Şirin & Sevin, 2025; De Simone et al., 2024; Iqbal et al., 2024).

Using systems based on image processing and deep learning, valuable data such as the density around the hive entrance and the proportion of forager bees carrying pollen can be monitored in real time (Şirin & Sevin, 2025; Bilik et al., 2024; Rozenbaum et al., 2024).

For these models to be considered beneficial, they must be integrated into all interconnected phases of beekeeping. Without integration across all phases, the fundamental goals of the innovations used in mobile beekeeping—minimizing resource consumption and maximizing bee health and productivity—cannot be fully

achieved. The most critical phase demanding these innovative approaches is overwintering, a period when colonies are highly vulnerable to external environmental stress.

Niğde province, with the typical continental climate characteristics of the Central Anatolia Region and its cold, harsh winters, presents a challenging overwintering environment for bee colonies, thus constituting a suitable model for this research. The aim of this study is to quantitatively determine the survival rates and population changes of honeybee colonies overwintered in traditional fixed hives and in mobile multi-rack systems equipped with innovative features under the ecological conditions of Niğde, and to assess the sustainability of mobile systems under harsh winter conditions.

## Materials and Methods

The research was conducted under the ecological conditions of Niğde province. A total of 20 healthy honey bee colonies belonging to the Anatolian honey bee subspecies (*Apis mellifera anatoliaca*) with queens of similar age were utilized in the study. Following the completion of colony equalization procedures (brood area, honey reserves, etc.), the colonies were randomly divided into two equal groups.

The first group consisted of colonies housed in standard wooden Langstroth hives, which were secured on bases in an open area using traditional methods. These hives were directly exposed to external weather conditions (wind, rain, etc.). The second group, on the other hand, consisted of colonies managed in accordance with the concept of mobile beekeeping, utilizing a multi-shelf transport platform where the hives were grouped together. In this system, the colonies were not directly exposed to the open air; instead, the hives were elevated off the ground and arranged side by side.

Prior to overwintering, the number of frames covered with bees in both colony groups was determined, and *Varroa* mite control procedures were conducted and standardized. The average number of frames covered with bees upon entering the overwintering period was recorded as 5.88 ± 1.36 in the traditional group and 5.00 ± 0.93 in the mobile group.

During the early spring maintenance conducted after the overwintering period, the viability of the colonies (specifically, checking for colony collapse or queen loss) was evaluated. Population loss was calculated for the colonies that successfully completed the overwintering phase. A statistical analysis of the difference in population loss between the two methods was performed using an independent samples t-test, with the significance level set at $p < 0.05$.

## Results And Discussion

The parameters regarding colony viability and changes in the number of frames covered with bees, obtained from the research conducted under the winter conditions of Niğde province, are presented in Table 1.

| *Performance Parameter* | *Traditional* | *Mobile* | *p* |
|---|---|---|---|
| **Colony survival (%)** | 80.0 | 80.0 | - |
| **Queen loss / colony collapse (%)** | 20.0 | 20.0 | - |
| **Frames covered with bees before overwintering** | 5.88 ± 1.36 | 5.00 ± 0.93 | - |
| **Average loss of frames covered with bees** | 1.25 ± 1.16 | 1.00 ± 1.07 | 0.701 |

According to the research findings, the colony survival rate was calculated at 80% for both overwintering methods. In both groups, two colonies experienced either colony collapse or queen loss during the overwintering period. The fact that the survival rate in the mobile system was identical to that of the traditional system, despite the harsh winter conditions of a continental climate, indicates that mobile hive platforms do not introduce any additional stress factors affecting these aspects of colony physiology.

When examining the colonies that successfully completed the overwintering period without queen loss or colony collapse, the average loss of frames covered with bees per colony was calculated as 1.25 ± 1.16 in the traditional group and 1.00 ± 1.07 in the mobile group. Statistical analysis revealed that the difference in population loss was not statistically significant ($p = .701$).

One of the most striking findings of the study is that, although colonies in the mobile system entered the overwintering period with a lower number of frames covered with bees (5.00 ± 0.93), they completed this phase with a lower average population loss (1.00 frame). This success is attributed to the innovative design features of the mobile systems. Compared to traditional methods, the arrangement of hives in a compact block within multi-rack mobile systems is believed to reduce the total surface area exposed to the external environment, thereby minimizing the direct impact of wind.

It is thought that placing the hives adjacent to each other may have allowed the heat radiated from neighboring hives to create a shared thermal mass. Similarly, in their study modeling heat distribution within winter clusters, Eskov and Toboev (2009) reported that thermal stability slows down the food consumption of honey bees, consequently delaying their physiological aging.

It has been emphasized that thermal mass, which is considered a key factor in minimizing temperature fluctuations, proportionally reduces the rate of colony collapse. Kaya (2007) supports this assumption by reporting that colony collapse is most prevalent during February and March, a period characterized by extreme temperature fluctuations.

## Conclusion And Recommendations

Through this study, it was observed that in a region like Niğde, which experiences harsh continental climate conditions, the technologically innovative mobile beekeeping system presents no disadvantages compared to the traditional fixed method in terms of colony survival and the number of frames covered with bees during the overwintering period. On the contrary, the shared thermal mass believed to be facilitated by the mobile systems enabled even those colonies that entered the overwintering period with numerically fewer bees to successfully survive until spring.

Mobile systems, which have emerged from efforts to modernize beekeeping and improve efficiency, present an opportunity to ensure a healthy overwintering period with minimal losses by reducing labor and transportation costs and providing stable temperatures inside the hives. In future studies, the effects of precision apiculture innovations on overwintering physiology can be examined in greater detail through the integration of IoT (Internet of Things)-based temperature and humidity sensors into mobile platforms. Furthermore, conducting similar studies in coastal regions—where temperature fluctuations are more pronounced during the overwintering period—is expected to provide valuable additional insights into the subject.

### *Conflict of Interest*

Please declare conflicts of interest or state “The authors declare no conflict of interest.” Authors must identify and declare any personal circumstances or interests that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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# ITS2 Metabarcoding and Open Data Approaches in Honey Bee Pollen Research

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## Abstract

Honey bees (*Apis mellifera*) are among the most important pollinating agents supporting the sustainability of terrestrial ecosystems and agricultural production. Determining the botanical origin of pollen collected by honey bees provides critical information for melissopalynology, pollination biology, colony development, and ecological monitoring studies. Conventional microscopic pollen identification methods have been widely used for many years; however, due to their time-consuming nature and the requirement for advanced expertise in pollen taxonomy, molecular-based identification methods have increasingly been preferred in recent years. In DNA metabarcoding studies, the Internal Transcribed Spacer 2 (ITS2) region has emerged as a powerful molecular marker effectively used for discriminating plant taxa. Large amounts of ITS2 sequence data have accumulated in open-access molecular databases such as NCBI GenBank. Nevertheless, the potential for re-evaluating these open-access datasets in honey bee pollen research and melissopalynological studies has not yet received sufficient attention. Re-analysis of open-access molecular datasets has the potential not only to generate new biological interpretations but also to contribute to reproducible and sustainable scientific research through the re-evaluation of existing data. In this context, interdisciplinary analysis pipelines integrating open-source analytical software workflows, BLAST-based taxonomic matching systems, metabarcoding analysis tools, and network analysis approaches are widely used. This presentation aims to highlight the importance of integrating ITS2 metabarcoding with open-data approaches in honey bee pollen research. For this purpose, a bioinformatic re-evaluation based on the re-analysis of ITS2 datasets obtained from publicly available research data is presented as an example, demonstrating the potential of such approaches for advancing melissopalynological studies through the testing of different hypotheses. The proposed approach is expected to contribute to the molecular identification of bee flora, the evaluation of pollen resources in urban landscapes, and the testing of new hypotheses in pollination research.

***Keywords:*** *Apis mellifera, ITS2 metabarcoding, melissopalynology, open data, bioinformatics, pollen analysis, DNA metabarcoding*

## Bal Arısı Polen Araştırmalarında ITS2 Metabarkodlama ve Açık Veri Yaklaşımları

## Özet

Bal arısı (*Apis mellifera*), karasal ekosistemlerin sürdürülebilirliğini ve tarımsal üretimi destekleyen önemli tozlayıcı ajanlardan biridir. Bal arılarının topladığı polenlerin botanik kökeninin belirlenmesi; melissopalinoloji, polinasyon biyolojisi, koloni gelişimi ve ekolojik izleme çalışmaları açısından kritik bilgiler sunmaktadır. Geleneksel mikroskobik polen tanımlama yöntemleri uzun yıllardır kullanılmaktadır; ancak bu yöntemlerin zaman alıcı olması ve ileri düzey polen taksonomisi uzmanlığı gerektirmesi nedeniyle son yıllarda moleküler tabanlı tanımlama yöntemlerinin kullanımı giderek daha fazla tercih edilmektedir. DNA metabarkodlama çalışmalarında "Internal Transcribed Spacer 2 (ITS2)" bölgesi, bitki taksonlarının ayırt edilmesinde etkin biçimde kullanılan güçlü bir moleküler belirteç olarak öne çıkmaktadır. NCBI GenBank gibi açık erişimli moleküler veri tabanlarında büyük miktarda ITS2 dizisi birikmiş durumdadır. Ancak bu açık erişimli veri setlerinin bal arısı polen araştırmaları ve melissopalinoloji çalışmaları bakımından yeniden değerlendirilme potansiyeli henüz yeterince dikkat çekmemiştir. Açık erişimli moleküler veri setlerinin yeniden analiz edilmesi, mevcut verilerin yeniden değerlendirilmesi yoluyla tekrarlanabilir ve sürdürülebilir bilimsel araştırmaların yürütülmesinin yanı sıra yeni biyolojik yorumların geliştirilmesine de katkı sağlama potansiyeline sahiptir. Bu kapsamda açık kaynak kodlu analiz yazılımlarına dayalı iş akışları, BLAST tabanlı taksonomik eşleştirme sistemleri, metabarkod analiz araçları ve ağ analiz yöntemlerini bir araya getiren disiplinlerarası analiz hatları yaygın olarak kullanılmaktadır. Bu sunumda, bal arısı polen araştırmalarında ITS2 metabarkodlama ile açık veri yaklaşımlarının bütünleştirilmesinin öneminin ortaya konulması amaçlanmaktadır. Bu amaç doğrultusunda, bir bilimsel araştırmaya ait açık veri kaynaklarından elde edilen ITS2 verilerinin yeniden analizi ile farklı hipotezlerin test edilmesine olanak sağlayan biyoinformatik bir değerlendirme örnek olarak sunulmuş, böylece melissopalinoloji çalışmalarının geliştirilmesindeki söz konusu potansiyel ortaya konulmuştur. Önerilen yaklaşımın; arı florasının moleküler düzeyde tanımlanması, şehir peyzajındaki polen kaynaklarının değerlendirilmesi ve polinasyon araştırmalarında yeni hipotezlerin test edilmesine katkı sağlayacağı düşünülmektedir.

***Anahtar Kelimeler:*** *Apis mellifera, ITS2 metabarkodlama, melissopalinoloji, açık veri, biyoinformatik, polen analizi, DNA metabarkodlama*

*ID: 895*

# Predictive Modeling of Perishable Supply Chains under Infrastructure Constraints: A Comprehensive Review of Data Scarcity, Intermittent Connectivity, and Informal Distribution Integration

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## Abstract

Perishable food supply chains in developing economies face severe challenges affecting global food security, with post-harvest losses reaching 40% of agricultural produce in India and one-third of agri-fresh products annually. This literature review examines predictive modeling research for supply chains operating under infrastructure constraints, focusing on data scarcity, intermittent connectivity, and informal distribution networks. The review analyzes mathematical optimization, machine learning applications, and integrated decision-support frameworks across diverse geographic contexts where infrastructure deficits create food insecurity rates up to 46.4% in remote communities. A critical finding reveals a significant gap between theoretical modeling advances and practical implementation capabilities. While sophisticated mathematical optimization and machine learning techniques show promising theoretical results, real-world application remains limited due to persistent infrastructure deficits. Inadequate cold chain facilities contribute to massive losses, including 370 million tons of fruits and vegetables deteriorating annually in China alone in 2012. Current research inadequately addresses the complex interplay between formal and informal distribution networks, despite evidence that intermediary syndicates substantially influence market dynamics through price manipulation and artificial scarcity. However, emerging integration strategies demonstrate quantifiable benefits. Documented improvements include transport cost reductions up to 29% and operational savings reaching 78 million BDT through optimized distribution center placement. Advanced hybrid distribution models show that plants can directly meet 63% of mango demand and 53% of jackfruit demand, significantly reducing intermediary reliance. However, there is lack of comprehensive frameworks simultaneously addressing infrastructure limitations, data constraints, connectivity challenges, and informal market dynamics. This review identifies critical theoretical gaps and proposes a research agenda emphasizing context-adaptive modeling approaches, empirical validation methodologies, and holistic integration strategies. The findings contribute to supply chain theory by highlighting the need for paradigm shifts in modeling approaches for resource-constrained environments and establishing priorities for sustainable food system transformation research.

***Keywords:*** *Developing Economies, Infrastructure Constraints, Perishable Supply Chains, Post-Harvest Losses, Predictive Modeling*

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# Growth Performance and Body Condition Score of Pregnant and Lactating West African Dwarf Goats Fed Microbial-Treated Rice Husk-Based Diets

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## Abstract

Rice husk is an abundant agricultural by-product with limited use in ruminant feeding due to its low protein, and high fibre contents. This study evaluated the effects of microbial-treated rice husk-based diets on growth performance and body condition score of pregnant and lactating West African Dwarf goats. Rice husk was subjected to biological treatment using *Alcaligenes faecalis*, *Aspergillus flavus*, and a combination of both microorganisms before inclusion in experimental diets as a substitute for twenty-five percent maize. Forty healthy nulliparous West African Dwarf does were randomly assigned to four dietary treatments in a completely randomized design and managed throughout gestation and seven weeks after parturition. Nutritional analysis revealed that microbial treatment significantly improved the quality of rice husk, increasing crude protein from 4.43% in the raw sample to 10.68–11.14% in treated samples, while maintaining comparable ash and fibre contents. Growth performance of pregnant goats was significantly influenced by dietary treatment, with average daily weight gain ranging from 68.03 to 68.98 g/day in treated groups compared with 76.67 g/day in the maize-based control. Similarly, lactating goats fed treated rice husk diets recorded average daily weight gains of 18.78–22.02 g/day compared with 26.33 g/day in the control group. Despite these reductions, treated diets sustained satisfactory growth and maintained body condition throughout the experimental period. Body condition score was not significantly affected by treatment and ranged from 4.45 to 4.90 across diets. The results demonstrate that microbial bioconversion substantially enhanced the feeding value of rice husk and supports its valorization as a sustainable alternative feed resource capable of partially replacing conventional feed ingredients in diets of breeding West African Dwarf goats.

***Keywords:*** *Rice husk, WAD goat, Pregnant, Lactating, Fungi, Bacteria*

## Introduction

West African Dwarf (WAD) goats are a small, trypanotolerant breed important to many rural West African farming regions, providing meat, milk, and income (Afolayan *et al.,* 2025), however, production is limited by scare, expensive and low-quality forage, especially during the long dry season (Ogunbosoye *et al.* 2022). To address these gaps, agro-industrial by-products have been explored as alternative ruminant feeds. One promising, abundant but underutilized feed resource is rice husk (RH). According to Nnadiukwu *et al.,* 2023, raw RH is very low in crude protein (~ 1.9%) and high in ash (~ 23.4%) and crude fibre (~ 25.7%). These properties, high crude fiber and ash content mean rice husk provides little nutritional value in its untreated form (Kolade *et al.,* 2024). To improve the nutrient content of rice husk, white-rot fungal fermentation by *Pleurotus ostreatus* has been reported to improve rice husk crude protein (CP) by a staggering 44.17%, and also notable reductions in fiber (NDF, ADF) and lignin (Dairo *et al.,* 2017). Afolayan *et al.* (2025) found that solid-state fermentation (SSF) (bacteria + fungus) increased RH crude protein from ~9.9% (untreated) to 11.1% and lowered crude fibre from 66.79% to 62.04%. *Trichoderma viride* treatment similarly reduced RH NDF and ADF markedly after 4–6 weeks (Aderolu *et al.,* 2007).

Pregnancy and lactation impose very high nutritional demands on does (Chen *et al.,* 2024) with any energy and or protein deficiency resulting to slow growth, longer kidding intervals and higher kidding mortality (Ogunbosoye *et al.,* 2022). Microbial-treated RH diets can sustain satisfactory goat growth at lower cost, provided RH inclusion is kept moderate (often ≤50%) and the overall nutrient balance is maintained (Afolayan *et al.,* 2024, Afolayan *et al.,* 2025). However, limited studies have investigated microbial-treated RH in breeding does. The current experiment evaluated diets in which 25% maize was substituted by rice husk fermented with bacteria (*Alcaligenes faecalis*), fungi (*Aspergillus flavus*) or a 1:1 of both, and measured pregnant and lactating WAD doe growth and body condition. The hypotheses were that (1) microbial treatment will raise RH's CP, and (2) does fed treated-RH diets will perform comparably to the control diet, maintaining weight gain and Body Condition Score.



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## Materials and Methods

***Experimental site***

This experiment was carried out at the small ruminant unit, Afolayan Farms, Oro, Irepodun Local Government Area, Kwara State, Nigeria which lies between the latitude of 8.2258° N and longitude 4.8923° E.

***Experimental Materials and Inoculation***

Rice husk samples were sourced, cleaned, and sterilized in an autoclave at 121°C for 15 minutes. The sterilized rice husk was inoculated with each microbe separately at 1ml of spores to 9mL saline water and 1kg of substrate to 1 litre of water. Incubation lasted 10 days, after which the fermented substrates were subsequently dried to safe storage moisture level, packaged, labeled, and stored prior to diet formulation.

***Experimental Animals and Diets***

Forty healthy, nulliparous West African Dwarf does were randomly assigned to four dietary treatments in a completely randomized design (CRD), with 10 does per treatment serving as replicates. Animals were approximately 20-24 months old, estimated from dentition and farmers records. After a 30-day adaptation period, does were bred by proven bucks and managed throughout gestation and 7 weeks postpartum. Growth parameters were recorded over an average of approximately 147 days. The entire experiment, including adaptation and breeding management was conducted over a period of 7.5 months. Four diets (Table 1) were formulated using common ingredients, variation existing only in the 25% maize fraction. In the control (T1) this fraction was maize, in T2-T4 it was substituted by rice husk treated with; bacteria (*Alcaligenes faecalis*), fungi (*Aspergillus flavus*) or a 1:1 bacterium + fungi cocktail, respectively. All diets were formulated to contain ~15% crude protein and meet the nutrient requirements of pregnant does. Feed ingredients were analyzed by standard methods (Horwitz & Latimer, (2005) for proximate composition; Van Soest, (1991) for fiber fractions)

Table 1. Composition of Treatment Diets

| | *Diets (kg)* | | | |
|---|---|---|---|---|
| Ingredients | T1 (Control) | T2 (B-RH) | T3 (F-RH) | T4 (B+F-RH) |
| Treated rice husk | - | 25 | 25 | 25 |
| Maize | 25 | - | - | - |
| Soya beans meal | 15 | 15 | 15 | 15 |
| Palm kernel cake | 10 | 10 | 10 | 10 |
| Groundnut cake | 10 | 10 | 10 | 10 |
| Cassava peel | 20 | 20 | 20 | 20 |
| Wheat bran | 15 | 15 | 15 | 15 |
| Bone meal | 2.5 | 2.5 | 2.5 | 2.5 |
| Salt | 1 | 1 | 1 | 1 |
| Premix | 1.5 | 1.5 | 1.5 | 1.5 |
| Total | 100 | 100 | 100 | 100 |

Keys: Control= Maize diet, B-RH=Bacterial treated rice husk, F-RH =Fungi treated rice husk, B+F-RH = Bacterial + Fungi treated rice husk

***Animal Feeding and Management***

The does were fed weighed treatment diets twice daily, and water was provided ad libitum. The initial body weights of the animals were recorded at the commencement of the feeding trial, and weekly recordings were taking subsequently by estimation using body measurements (Afolayan *et al.*, 2006). Feed intake (FI) was recorded daily, total weight gain, and average daily weight gain were calculated using the weekly records. Feed conversion ratio (FCR) was also calculated.

BW (kg)= $HG^2$(cm)×BL (cm) / 10840

Body condition score (BCS) was assessed at regular intervals using a 1–5 scoring scale, where 1 represented skinny and 5 represented obese animals. Scores were assigned by visual appraisal and palpation of the lumbar region, sternum and ribs. Mean BCS values obtained during the trial period were subjected to statistical analysis.

***Statistical analysis***

Data recorded were subjected to one-way Analysis of Variance (ANOVA) using the DSAASTAT version 1.101 (Onofri, 2011). Where significant treatment effects were detected, means were separated using the Least Significant Difference (LSD) test at $P < 0.05$.

## Results

### *Nutritional Analysis of Treated Rice husk Samples*

The nutritional analysis, as presented in Table 2 revealed significant (P< 0.05) improvement in the nutritional values of treated rice husk. Crude protein (CP) jumped from 4.43% in raw rice husk all the way to 11.14% in the cocktail treatment, while dry matter also saw an increase compared to the maize control (93.19 - 95.25%). Crude fibre, and ash remained comparable across treatments, showing no significance.

The results revealed all proximate and fibre fraction parameters were significantly (P< 0.05) different, except for CP which remained comparable across treatments (15.86 to 16.55%) confirming the diets were isonitrogenous. Microbial-treated rice husk increased the crude fibre from 24.02% in the maize control diet, to ~ 25.2% in the bacterial-treated diet, with a similar trend observed in the carbohydrate content. Similarly, NDF, ADF and lignin content followed the same pattern with the control diet presenting the lowest value compared to the treated diets. On the other hand, total ash decreased from 13.92% in control to the extreme end again in the bacterial-treated diet.

Table 2: Nutritional Analysis of Treated Rice husk Samples

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters (%) | (RRH) | (BTRH) | (FTRH) | (B+FTRH) | SEM | p-Value |
| Dry matter | 93.19$^{a}$ | 95.25$^{c}$ | 94.39$^{b}$ | 94.40$^{b}$ | 0.163 | 0.0001** |
| ASH | 18.46 | 18.55 | 18.91 | 14.48 | 3.001 | 0.7027 |
| Crude fibre | 66.79 | 66.85 | 65.62 | 62.04 | 1.198 | 0.0670 |
| Crude fat | 4.59$^{a}$ | 4.09$^{b}$ | 4.19$^{b}$ | 4.14$^{b}$ | 0.106 | 0.0395* |
| Crude protein | 4.43$^{b}$ | 10.71$^{a}$ | 10.68$^{a}$ | 11.14$^{a}$ | 0.209 | 3.7E-08** |
| Carbohydrate | 57.29$^{ab}$ | 58.08$^{a}$ | 56.37$^{bc}$ | 55.25$^{c}$ | 0.395 | 0.0050** |

Keys: a,b,c = means within the same row with different superscripts are significantly (P<0.05) different. RRH: raw rice husk, BTRH: Bacteria treated rice husk, FTRH: fungi treated rice husk, B+FRH: Bacteria + Fungi treated rice husk (cocktail).

Table 3: Proximate and Fibre Fraction Composition of the Experimental Diets.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters (%) | T1 | T2 | T3 | T4 | SEM | p-value |
| Dry matter | 89.62$^{d}$ | 88.83$^{a}$ | 89.03$^{b}$ | 89.31$^{c}$ | 1.07E-02 | 1.06E-10** |
| Total Ash | 13.92$^{a}$ | 9.89$^{d}$ | 12.18$^{b}$ | 11.30$^{c}$ | 4.02E-02 | 1.28E-11** |
| Crude Fibre | 24.02$^{d}$ | 25.21$^{a}$ | 24.26$^{c}$ | 24.96$^{b}$ | 2.77E-02 | <0.01** |
| Crude Fat | 5.17$^{a}$ | 4.27$^{d}$ | 4.89$^{b}$ | 4.45$^{c}$ | 4.66E-02 | 2.97E-06** |
| Crude Protein | 15.97 | 15.86 | 15.86 | 16.55 | 0.34 | 0.0667 |
| CHO | 50.56$^{c}$ | 53.63$^{a}$ | 52.69$^{b}$ | 52.83$^{b}$ | 9.46E-02 | 8.58E-08** |
| Neutral detergent soluble | 23.01$^{a}$ | 21.40$^{c}$ | 22.25$^{b}$ | 21.4$^{c}$ | 0.175 | 0.000488** |
| Neutral detergent fibre | 74.99$^{c}$ | 78.43$^{a}$ | 77.76$^{b}$ | 78.6$^{a}$ | 0.154 | 0.00029** |
| Acid detergent fibre | 52.83$^{c}$ | 54.13$^{b}$ | 53.91$^{b}$ | 54.79$^{a}$ | 0.115 | 1.56E-05** |
| Acid detergent Lignin | 10.24$^{d}$ | 11.46$^{a}$ | 10.97$^{c}$ | 11.38$^{b}$ | 2.369E-02 | 1.28E-09** |
| Silica | 8.24$^{d}$ | 8.62$^{b}$ | 8.71$^{a}$ | 8.49$^{c}$ | 1.093E-02 | 4.64E-07** |
| Cellulose | 22.16$^{c}$ | 24.3$^{a}$ | 23.85$^{b}$ | 23.81$^{b}$ | 7.23E-02 | 0.003319** |
| Hemicellulose | 42.67$^{b}$ | 42.67$^{b}$ | 42.95$^{ab}$ | 43.31$^{a}$ | 0.139 | 0.025237* |

Keys: a,b,c = means within the same row with different superscripts are significantly (P<0.05) different TI = Diet with maize (control), T2 = diet with *Alcaligenes faecalis* (Bacteria), T3 = Diet with *Aspergillus flavus*, T4 = *Diet with Alcaligenes faecalis + Aspergillus flavus*

### *Growth Performance Response in Pregnant and Lactating WAD Goats to Diets*

Dietary treatment showed a significant (P < 0.05) influence on growth parameters of pregnant WAD goats. The control had the highest (76.67 g/day) average daily weight gain (ADWG) and declined to 68.03–68.98 g/day in goats fed microbial-treated rice husk diets. In a similar trend, total weight gain dropped from 11.27 kg in the control diet to 10.00–10.14 kg in the treated groups (P = 0.024) with the bacterial-treated rice husk recording the lowest value. Feed intake was also affected, with total feed intake of 70.46 to 72.89 kg in treated diets compared with 74.22 kg in the control, while average daily feed intake also saw a declined from 503.97 to 480.49–495.89 g/day (P < 0.05). Dietary treatment had no significant (P > 0.05) effect on the initial and final body weights, as well as feed conversion ratio 6.59–7.19.

The significant (P < 0.05) effects of the dietary treatment on growth performance extended till lactation. With average daily weight gain (ADWG) ranging from the lowest in fungi treated husk, 18.78 to 22.02 g/day in the mixture as compared with 26.33 g/day in the control group. Similarly, total weight gain declined from 1.29 kg in the control group to 0.94–1.11 kg in the treated groups (P < 0.05). Extending the significance trend, total feed intake and average daily feed intake were also affected, decreasing from 39.99 kg and 816.09 g/day in the control

group to 34.74–39.15 kg and 708.89–799.06 g/day, respectively, in the treated groups. Feed conversion ratio was also significantly affected (P = 0.022), ranging from 35.27 to 37.73 in goats fed treated rice husk diets compared with 31.00 in the control group. However, similar to the pregnant does results, initial and final body weights were not significantly influenced by dietary treatment (P > 0.05) in the lactating does.

The body condition score results presented no significant (P = 0.14) dietary effect on body condition score (BCS) of pregnant and lactating WAD goats. Mean BCS values ranged from 4.45 to 4.90 across treatments, with goats fed the control diet recording the highest score (4.90) and those fed the bacteria + fungi-treated rice husk diet at the lower extreme (4.45). Despite the numerical differences observed, body condition scores remained high and comparable across all treatment groups.

Table 4: The Growth Performance of Pregnant WAD Goats Fed Experimental Diets.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-value |
| Initial weight (kg) | 15.43 | 15.42 | 15.44 | 15.47 | 1.26 | 0.99 |
| Final weight (kg) | 26.70 | 25.52 | 25.44 | 25.61 | 1.38 | 0.91 |
| ADWG (g) | $76.67^{a}$ | $68.71^{b}$ | $68.03^{b}$ | $68.98^{b}$ | 2.16 | 0.024* |
| Total Weight Gain (kg) | $11.27^{a}$ | $10.10^{b}$ | $10.00^{b}$ | $10.14^{b}$ | 0.32 | 0.024* |
| Total Feed Intake (kg) | $74.22^{a}$ | $72.09^{ab}$ | $70.46^{b}$ | $72.89^{a}$ | 0.71 | 0.0058** |
| ADFI (g) | $503.97^{a}$ | $490.43^{ab}$ | $480.49^{b}$ | $495.89^{a}$ | 4.73 | 0.01* |
| FCR | 6.59 | 7.14 | 7.05 | 7.19 | 0.21 | 0.23 |

Keys: a,b,c = means within the same row with different superscripts are significantly (P<0.05) different, TWG = Total weight gain, ADWG = Average daily weight gain, TDFI = Total daily feed intake, ADFI = Average daily feed intake, FCR = Feed conversion ratio. TI = Diet with maize (control), T2 = diet with *Alcaligenes faecalis* (Bacteria), T3 = Diet with *Aspergillus flavus*, T4 = *Diet with Alcaligenes faecalis* + *Aspergillus flavus*

Table 5: The Growth Performance of Lactating WAD Goats Fed Experimental Diets.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-value |
| Initial weight (kg) | 20.51 | 18.07 | 18.05 | 19.16 | 1.05 | 0.32 |
| Final weight (kg) | 21.81 | 19.07 | 18.97 | 20.27 | 1.07 | 0.22 |
| ADWG (g) | $26.33^{a}$ | $20.21^{b}$ | $18.78^{b}$ | $22.02^{b}$ | 1.08 | 0.0001** |
| Total Weight Gain (kg) | $1.29^{a}$ | $1.00^{bc}$ | $0.94^{c}$ | $1.11^{b}$ | 5.1E-02 | 0.0001** |
| Total Feed Intake (kg) | $39.99^{a}$ | 37.73b | $34.74^{c}$ | $39.15^{a}$ | 0.49 | 2.4E-08** |
| ADFI (g) | $816.09^{a}$ | $770.02^{b}$ | $708.89^{c}$ | $799.06^{a}$ | 9.96 | 2.3E-08** |
| FCR | $31^{b}$ | $37.73^{a}$ | $36.96^{a}$ | $35.27^{ab}$ | 1.86 | 0.022* |

Keys: a,b,c = means within the same row with different superscripts are significantly (P<0.05) different, TWG = Total weight gain, ADWG = Average daily weight gain, TDFI = Total daily feed intake, ADFI = Average daily feed intake, FCR = Feed conversion ratio. TI = Diet with maize (control), T2 = diet with *Alcaligenes faecalis* (Bacteria), T3 = Diet with *Aspergillus flavus*, T4 = *Diet with Alcaligenes faecalis* + *Aspergillus flavus*

Table 6: Body Condition Scoring in Pregnant and Lactating WAD Goats Fed Experimental Diets.

| Treatments | Body Condition Score |
|---|---|
| T1 | 4.90 |
| T2 | 4.80 |
| T3 | 4.70 |
| T4 | 4.45 |
| SEM | 0.16 |
| p-Value | 0.14 |

T1 = control maize diet, T2 = bacteria-treated rice husk, T3 fungi-treated rice husk =, T4 = bacteria + fungi-treated rice husk)

**Discussion**

The substantial jump in crude protein content after microbial fermentation confirms the efficiency in enhancing the nutritive quality of rice husk. Similar findings have been reported following microbial treatment of rice husk and other lignocellulosic residues (Aderolu *et al.*, 2007; Afolayan *et al.*, 2025 Dairo *et al.*, 2017; Nnadiukwu *et al.*, 2023). Improvements such as this have been attributed to microbial biomass synthesis and partial breakdown of carbohydrates, leading to improved nutritional value of the substrate (Van Soest, 1994; Chen *et al.*, 2024).

Although the performance of the maize-based control diet outweighs the other treatments, goats placed on microbial-treated rice husk diets sustained satisfactory growth during pregnancy and lactation, thereby suggesting that the treated rice husk was able to support heightened nutrient demands attributed with these physiological

periods (Chen *et al.*, 2024). This finding agrees with reports that biologically treated agro-industrial by-products can partially replace conventional feed ingredients in small-ruminant diets without severely compromising animal performance and with economic benefits (Afolayan *et al.*, 2024; Ogunbosoye *et al.*, 2022).

The lack of dietary effects on body condition score suggests the experimental diets adequately maintained body reserves throughout pregnancy and lactation. Since body condition is an important indicator of nutritional status and reproductive efficiency in breeding does, this result demonstrates the potential of microbial-treated rice husk as a sustainable alternative feed resource for WAD goat production systems, especially in regions of forage scarcity and expensive feed materials (Ogunbosoye *et al.*, 2022).

## Conclusion

Treatment rice husk with microbes substantially enhanced the nutritional quality, particularly through increased crude protein content, demonstrating its potential for value addition and utilization in ruminant nutrition. Despite the maize-based control diet produced higher growth rates, diets containing microbial-treated rice husk maintained satisfactory growth performance, especially in body condition score of pregnant and lactating WAD goats. The lack of detrimental consequences on body condition score further indicates that treated rice husk can support the high nutritional demands of breeding does. Therefore, microbial bioconversion presents a potential for the valorization of rice husk, changing an otherwise waste, and abundant agricultural by-product into a useful feed resource capable of partially replacing conventional feed ingredients in small-ruminant production systems.

### *Conflict of Interest*

The authors declare no conflict of interest.

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*ID: 898*

# Evaluation of the Antiproliferative and Antiviral Potential of *Equisetum arvense* L. Extract

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## Abstract

Plant-derived natural products represent an important area of research in pharmaceutical and biotechnological applications due to their bioactive compounds. *Equisetum arvense*, widely found in the America, Europe, and Asia, has a long history of use in traditional medicine. The aim of this study was to determine the composition and biological activities of the ethanolic extract of *E. arvense*. The aerial parts of *E. arvense* were ground and subjected to ultrasound-assisted extraction using 70% ethanol. The total flavonoid content of this extract was determined colorimetrically using the aluminum chloride method. Cytotoxic effects were analyzed using the MTT assay on two different cancer cell lines. In addition, antiviral activity potential was analyzed *in ovo* using embryonated chicken eggs against the Infectious Bronchitis virus. The extract contained 1.421±0.11 mg catechin equivalents (CE)/g of total flavonoids. Cytotoxicity analyses showed that the extract reduced cell viability in a dose- and time-dependent manner. In particular, the extract exhibited the strongest antiproliferative effect in HeLa cells, with an $IC_{50}$ of 554.32±28.89 µg/mL at 72 hours. Similarly, in T24 cells, the $IC_{50}$ was 582.57±24.03 µg/mL at 72 hours. The extract demonstrated significant activity, achieving 73.43%–88.28% viral inhibition at concentrations ranging from 2.5 to 5 mg/kg. The results show that *E. arvense* ethanol extract is a significant natural bioactive source with antiproliferative and antiviral potential. The findings suggest that it may be evaluated for pharmaceutical and biotechnological applications.

***Keywords:*** *Equisetum arvense, total flavonoid content, cytotoxicity, antiviral activity*

*ID: 900*

# Climate Change, Deep Learning, and Plant Disease: A Bibliometric Analysis (2000-2025)

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## Abstract

Climate change is reshaping environmental conditions in ways that influence the occurrence, spread, and severity of plant diseases, creating growing challenges for agricultural productivity and global food security. At the same time, advances in artificial intelligence, particularly deep learning, have provided new opportunities for improving plant disease detection, diagnosis, and prediction. Although research on climate change and plant health, as well as deep learning applications in agriculture, has expanded considerably in recent years, studies combining these topics remain limited and dispersed. This study presents a bibliometric analysis of the scientific literature linking climate change, deep learning, and plant disease between 2000 and 2025. Bibliographic data were collected from the Scopus database using a structured search strategy. After screening and applying the inclusion criteria, 59 relevant publications were selected for analysis. The dataset was examined using Bibliometrix (R package) and VOSviewer to evaluate publication trends, influential authors, leading journals, productive countries and institutions, citation patterns, collaboration networks, and keyword relationships. Based on the results, the first publication linking climate change, deep learning, and plant disease was identified in 2019, showing the recent emergence of this research field. However, publication output has increased rapidly since then, with an annual growth rate of 51.31%, reflecting growing interest in the use of artificial intelligence to address climate-related agricultural challenges. The average document age of 2.76 years further confirms the recent and emerging nature of this field. Citation analysis demonstrated that the earliest publications have had a strong influence on subsequent research, particularly studies published in 2019 and 2020. Despite the increasing number of studies, important gaps remain, especially regarding the integration of climate data with advanced deep learning models for large-scale disease prediction. By providing a comprehensive overview of research trends, influential contributions, and emerging themes, this study offers valuable insights for researchers and policymakers working to develop climate-resilient and data-driven approaches for plant disease management.

***Keywords:*** *Deep learning, plant disease prediction, climate change, bibliometric analysis, Precision agriculture*

## İklim Değişikliği, Derin Öğrenme ve Bitki Hastalıkları: Bibliyometrik Analiz (2000-2025)

### Özet

İklim değişikliği, bitki hastalıklarının ortaya çıkışını, yayılımını ve şiddetini etkileyen çevresel koşulları yeniden şekillendirerek tarımsal üretkenlik ve küresel gıda güvenliği açısından giderek artan zorluklar yaratmaktadır. Aynı zamanda, yapay zekâ alanındaki gelişmeler, özellikle derin öğrenme teknikleri, bitki hastalıklarının tespiti, teşhisi ve tahmin edilmesinin geliştirilmesi için yeni fırsatlar sunmaktadır. Son yıllarda iklim değişikliği ve bitki sağlığı ile tarımda derin öğrenme uygulamalarına yönelik araştırmalar önemli ölçüde artmış olsa da, bu konuları bir araya getiren çalışmalar hâlâ sınırlı ve dağınık durumdadır. Bu çalışma, 2000-2025 yılları arasında iklim değişikliği, derin öğrenme ve bitki hastalıklarını ilişkilendiren bilimsel literatürün bibliyometrik bir analizini sunmaktadır. Bibliyografik veriler, yapılandırılmış bir arama stratejisi kullanılarak Scopus veri tabanından elde edilmiştir. Tarama ve dahil edilme kriterlerinin uygulanmasının ardından, analiz için 59 ilgili yayın seçilmiştir. Veri seti; yayın eğilimleri, etkili yazarlar, önde gelen dergiler, üretken ülkeler ve kurumlar, atıf örüntüleri, iş birliği ağları ve anahtar kelime ilişkilerini değerlendirmek amacıyla Bibliometrix (R paketi) ve VOSviewer yazılımları kullanılarak incelenmiştir. Sonuçlara göre, iklim değişikliği, derin öğrenme ve bitki hastalıklarını birlikte ele alan ilk yayın 2019 yılında tespit edilmiş olup, bu araştırma alanının yakın zamanda ortaya çıktığını göstermektedir. Bununla birlikte, yayın sayısı o tarihten itibaren hızla artmış ve yıllık büyüme oranı %51,31'e ulaşmıştır. Bu durum, iklim kaynaklı tarımsal sorunların çözümünde yapay zekâ kullanımına yönelik artan ilgiyi yansıtmaktadır. Ortalama doküman yaşının 2,76 yıl olması da bu alanın yeni ve gelişmekte olan yapısını doğrulamaktadır. Atıf analizi, özellikle 2019 ve 2020 yıllarında yayımlanan çalışmaların sonraki araştırmalar üzerinde güçlü bir etkiye sahip olduğunu göstermiştir. Çalışma sayısındaki artışa rağmen, özellikle iklim verilerinin geniş ölçekli hastalık tahmini için gelişmiş derin öğrenme modelleriyle bütünleştirilmesi konusunda önemli araştırma boşlukları bulunmaktadır. Araştırma eğilimleri, etkili katkılar ve yükselen temalar hakkında kapsamlı bir değerlendirme sunan bu çalışma, iklim değişikliğine dayanıklı ve veri odaklı bitki hastalığı yönetim stratejilerinin geliştirilmesine yönelik çalışan araştırmacılar ve politika yapıcılar için değerli bilgiler sağlamaktadır.

***Anahtar Kelimeler:*** Derin öğrenme, bitki hastalığı tahmini, iklim değişikliği, bibliyometrik analiz, hassas tarım

*ID: 903*

# AI-enhanced synthesis and characterization of bioinspired chitosan-ZnO based polysaccharides gums with calendula leaves extract as a biodegradable food packaging material

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## Abstract

Back ground: Bioinspiring synthesis and characterization of chitosan–ZnO (C–ZnO) form-based smart food packaging using machine learning approach has been developed with incorporation of Calendula officinalis extract into Acacia senegal gum, almond gum and mastic gum (Pistacia lentiscus). A simple, green and ecofriendly synthesis method was used to prepare zinc oxide nanoparticles (ZnO NPs) using naturally occurring polysaccharide gums as stabilizing and chelating agents.

Methods: The synthesized ZnO nanoparticles using the selected gums (Acacia senegal gum, almond gum and mastic gum) were characterized with their improved physicochemical properties and tunable morphology which were found to be effective and sustainable for the controlled synthesis of the nanoparticles.Further, the synthesized ZnO nanoparticles were embedded within a biodegradable chitosan hydrogel matrix, which was also functionalized with the Calendula officinalis extract for enhancing the antimicrobial, antioxidant and cytotoxicity of the active and intelligent food packaging systems. In the present study we develop zinc oxide nanoparticles sutures for the accelerated for food application.The formation and structure of ZnO nanoparticles and chitosan-ZnO based hydrogel composites were verified by Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), UV-Visible spectroscopy, Scanning Electron Microscopy (SEM),zeta sizer.

Results: It was observed that the Acacia gum solution had a transparent white yellow colour, the Almond gum solution an milky white colour, and the mastic gum solution an yellowish brown color. The XRD analysis revealed sharp peaks which indicates the crystalline structure of biogenic nanoparticles.These gum based nanoparticles coated stutures show excellent antioxidant activities confirmed by DPPH radical seavenging assay.The agar well diffusion method was used to check the antibacterial potential against selected bacterial strain such as Escherichia coli, Bacillus subtilis,staph aureus and pseudomonas aeruginosa and their zones of inhibitions were measured indicated excellent antibacterial activity hence the potential value is high for the shelf life extension and to prevent the microbial contamination in packaged foods.

***Keywords:*** *Chitosan–ZnO nanocomposites; Smart food packaging; Zinc oxide nanoparticles; Calendula officinalis; Antibacterial activity*

*ID: 906*

# Synthesis And Characterization Of Samarium Doped Nickel Cobalt Ferrite For Photocatalysis Application

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## Abstract

Back ground: Spinel ferrite nanomaterials - $NiFe_2O_4$, $CoFe_2O_4$, Cu–Ni ferrite, and Ni–Zn–Co ferrite - were successfully doped with $Sm^{3+}$ using a co-precipitation route, followed by calcination to achieve the desired phase and properties. The main objective of this research was to investigate the structural, morphological, and photocatalytic properties of ferrite nanomaterials with samarium doping

Methods: The synthesis and characterization of samarium-doped spinel ferrite nanomaterials were carried out using a systematic experimental approach designed to evaluate both their structural properties and photocatalytic performance.Spinel ferrite nanomaterials including $NiFe_2O_4$, $CoFe_2O_4$, Cu–Ni ferrite, and Ni–Zn–Co ferrite were prepared via the co-precipitation method. Co-precipitation was selected for its ability to produce homogeneous, nanoscale particles with controlled stoichiometry. Stoichiometric amounts of metal nitrates corresponding to the desired ferrite composition were dissolved in deionized water. An aqueous solution of $Sm^{3+}$ was then introduced to achieve samarium doping. The mixed metal solution was subjected to controlled precipitation by dropwise addition of a precipitating agent under continuous stirring and maintained pH conditions. The resulting precipitate was collected, thoroughly washed to remove residual ions, dried, and subsequently subjected to calcination at an optimized temperature. Calcination was performed to enhance crystallinity, promote phase formation, and stabilize the spinel structure, thereby ensuring the desired magnetic and photocatalytic properties.The synthesized ferrite nanomaterials were subjected to multiple analytical techniques to confirm phase purity, chemical bonding, morphology, optical properties, and particle size distribution of these composites were verified by Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), UV-Visible spectroscopy, Scanning Electron Microscopy (SEM),zeta sizer.

Results: SEM analysis revealed agglomerated nanostructured particles with almost uniform morphology. The degradation percentage of Rhodamine B increased significantly after samarium incorporation, attributed to better charge separation and enhanced surface activity of the nanomaterials. Comparative study of graphs, spectra, and calculated values showed that Sm-Fe ferrite exhibited higher photocatalytic activity compared to undoped ferrite. Samarium-doped spinel ferrites can be considered potential multifunctional materials for environmental remediation and wastewater treatment processes.

***Keywords:*** *Samarium-doped spinel ferrites; Ferrite nanomaterials; Photocatalytic degradation; Rhodamine B; Wastewater treatment*

*ID: 839*

# Trace Element Concentrations and Health Risk Assessment of Veined Rapa Whelk (*Rapana venosa*) from the Southern Black Sea (Sinop, Türkiye)

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## Abstract

(1) Background: The veined rapa whelk (*Rapana venosa*), an invasive alien species introduced to the Black Sea in the 1940s, has evolved from a significant marine pest threatening native bivalves into a highly valuable, globally exported commercial commodity. However, because mollusks possess a high capacity for bioaccumulating trace elements, monitoring their chemical profile is essential to ensure food safety for human consumers. (2) Methods: This study evaluates the concentrations of key trace elements, specifically Zn, Cu, Pb, As, Hg, and Cd, in the edible soft tissues of *R. venosa* collected from the Akliman coasts of Sinop in the southern Black Sea. Sampling was conducted from depths of 1–2 meters during May and June, utilizing specimens with shell lengths ranging between 6 and 8 cm. The element concentrations within the soft tissues were quantified using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) system. (3) Results: The results demonstrated that absolute mass concentrations followed a consistent abundance pattern, with Zn exhibiting the highest levels and Cd showing the lowest. Crucially, to prevent overestimating toxicological hazards, As risk characterization was refined by focusing on its hazardous inorganic fraction (iAs) based on established literature consensus. The human health risk assessment via target hazard quotient (THQ) and carcinogenic risk index (RI) analyses revealed that all values fell significantly below international safety thresholds. (4) Conclusions: Consequently, the commercial consumption of these mollusks poses no systemic or oncological health risks to consumers, though continuous monitoring of trace element dynamics remains crucial for long-term food safety management.

***Keywords:*** *Veined rapa whelk, Trace element bioaccumulation, Target Hazard Quotient, Invasive alien species, Marine gastropods, Consumer safety.*

## Introduction

The global landscape of fisheries and aquaculture has experienced unparalleled expansion over the last decade (FAO 2024). In Türkiye, which is surrounded by four seas on three sides, the Black Sea has historically been the most productive marine environment with the highest capture fisheries potential, owing to its unique biological dynamics and massive nutrient inputs (Bat et al. 2011). However, in recent years, due to overfishing, climate change (Bat et al. 2024a), and various anthropogenic pressures (Bat et al. 2018a), the volume of seafood obtained through capture fisheries in the Black Sea has steadily declined, falling below the production levels generated by modern aquaculture activities (Bat et al. 2024b), Amidst this structural transformation, the historical fishing city of Sinop (Bat et al. 2013 and 2018b)-with its hydrobiological advantages such as sheltered bays, optimal water temperatures, and favorable current patterns-has emerged as the primary hub where marine aquaculture is most intensively and efficiently practiced across the entire Black Sea region (Bat et al. 2024b). By dominating the decline in traditional fisheries with sustainable aquaculture investments, the coastal waters of Sinop not only contribute millions of dollars in export revenues to the regional economy but also serve as a critical stronghold for the seafood supply security of the Black Sea (Bat et al. 2024b).

As a rich source of high-quality animal protein, the safe consumption of seafood is essential for sustaining global food security. Among marine seafood commodities, mollusks are one of the most widely produced and consumed groups. The veined rapa whelk (*Rapana venosa*) is a commercially valuable gastropod (Sağlam et al. 2015a, 2015b; Gönener and Özsandıkçı 2017) characterized by its high productivity and rich nutritional profile, being high in protein, low in fat, and rich in essential elements (Bayraklı et al. 2016; Panayotova et al. 2019; Bayraklı 2024). However, the carnivorous feeding habits and benthic nature of certain mollusks facilitate the bioaccumulation of environmental contaminants from both their prey and the surrounding aquatic medium (Bat et al. 2023). Humans' dietary exposure to these pollutants through mollusk consumption has historically led to poisoning incidents, indicating that the food safety of these species deserves critical attention since few decades (Bat et al. 2000; Bat and Öztekin 2016). The Black Sea, a semi-enclosed basin receiving massive river discharges, is highly susceptible to anthropogenic pollution (Bat et al. 2018a). Within marine environments, trace elements constitute one of the most serious pollutant categories due to their toxicity, persistence, and pronounced tendency to bioaccumulate in aquatic organisms. This issue is compounded by the dynamics of invasive alien species within the basin. First introduced to the Black Sea in the 1940s (Dapkin 1953), *R. venosa* has successfully colonized

benthic habitats. Its success as an invasive species is primarily attributed to its rapid growth rates, early reproductive maturity, high fecundity, and remarkable adaptability to diverse habitats (Mutlu et al. 2022; Başusta et al. 2025). Owing to its high fertility, versatile nature, and tolerance to hypoxia and pollution, *R. venosa* has exerted significant predatory pressure on native bivalves, such as the Mediterranean mussel (*Mytilus galloprovincialis*) (Janssen et al. 2014). Although initially classified strictly as a marine pest, *R. venosa* has transformed into a high-value commercial commodity, representing million-dollars export market for the Black Sea littoral states. Nevertheless, because they are selective predatory gastropods positioned high in the benthic food web, rapa whelks can bioaccumulate trace elements to levels that may exceed food safety thresholds (Bat and Arici 2018). Consequently, continuous assessment of the chemical body burden of this species is crucial for ensuring consumer safety and evaluating the ecological status of the region. To address this need, the objective of the present study was to determine the concentrations of key trace elements (Zn, Cu, Pb, As, Hg, and Cd) in the soft tissues of sized (6-8 cm) wild *R. venosa* collected from the Akliman coasts of Sinop (Southern Black Sea, Türkiye) in May and June of year 2024. Furthermore, a comprehensive health risk assessment was performed using the Target Hazard Quotient (THQ), Hazard Index (HI), and Carcinogenic Risk Index (RI) to evaluate (USEPA 2024; RAIS 2024) whether the consumption of these wild gastropods poses any potential threat to human health.

## Materials and Methods

### *Sampling and Sample Preparation*

Wild specimens of the veined rapa whelk were collected from the Akliman coasts of the Sinop Peninsula (Southern Black Sea, Türkiye) during May and June 2024 (Figure 1). Sampling was restricted to individuals with a shell length ranging between 6 and 8 cm to maintain size uniformity. A total of 30 individuals were randomly divided into three replicate groups, each containing 10 specimens.

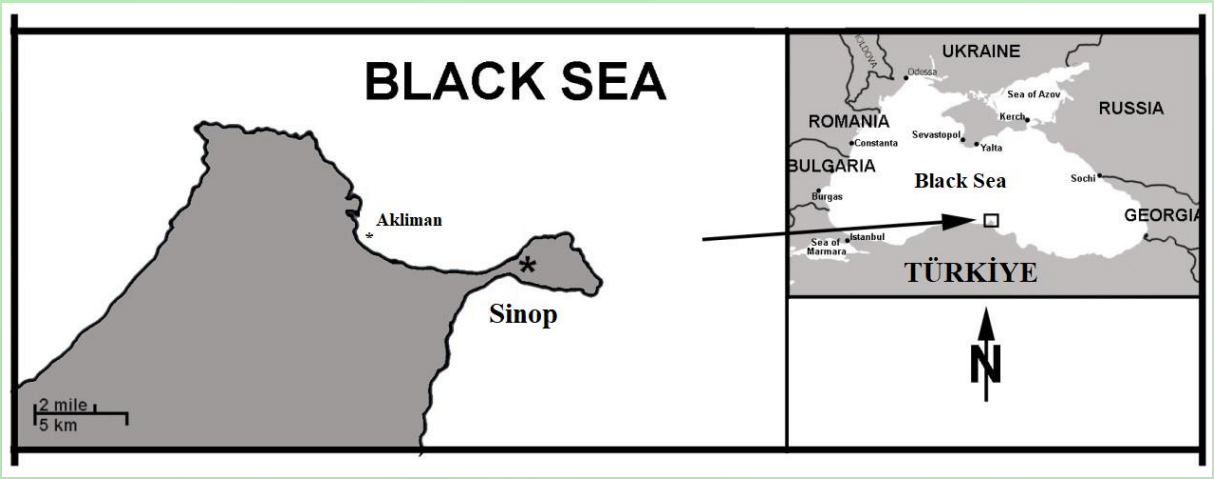



**Figure 1.** Sampling area

The samples collected were promptly transported to the laboratory under cold chain conditions. The shell surfaces were thoroughly cleaned using a synthetic brush to remove extraneous materials, labelled according to the sampling month, and stored in plastic bags at -21°C until further biometric measurements and chemical analyses. Prior to dissection, the whelks were thawed at room temperature. The soft tissues (including internal organs up to the furthest spiral fold) were carefully extracted from the shells, rinsed thoroughly with deionized water, and homogenized for trace element determination.

### *Analytical Procedures and Instrumentation*

The concentrations of trace elements, specifically zinc (Zn), copper (Cu), lead (Pb), arsenic (As), mercury (Hg), and cadmium (Cd), in the soft tissues of *R. venosa* were determined using an Inductively Coupled Plasma Mass Spectrometry system (Agilent Technologies 7700X ICP-MS). Sample digestion and instrumentation was described as below (Bat et al. 2023, 2026):

The chemical analysis was conducted following the principles of EPA Method 200.3 for biological tissue preparation. Tissue samples of *R. venosa* (approximately 1.5 g) were placed into Teflon vessels and digested using a microwave digestion system (Milestone SK10). The digestion matrix consisted of an ultra-pure (suprapur) mixture of concentrated $HNO_3$ (65%) and $H_2O_2$ (30%) at a 7:1 (v/v) ratio, adhering to the HPR-FO-67 temperature and pressure operational program. The sealed vessels were brought to 200°C over 15 minutes and held at this thermal plateau for an additional 15 minutes. Once cooled, the digested extracts were transferred into 50 mL

polypropylene falcon tubes and diluted to volume with ultra-pure water. Analytical quality assurance and control protocols were verified by analyzing triplicate samples alongside a Certified Reference Material (CRM), specifically Lobster Hepatopancreas TORT-2. Multi-element calibration curves were established using Agilent standard solutions (27-element mix 2A, 8500-6940, and Hg, 8500-6940). The precision of the analytical method remained within ±10%. For continuous instrumental drift monitoring, a 1 ppm internal standard (Agilent 5188-6525) was run systematically throughout the analysis. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) values established for the validated analytical method are summarized in Table 1.

**Table 1.** Instrumental Limits of Detection (LOD) and Quantitation (LOQ) for the Analyzed Trace Elements.

| *Trace elements* | *LOD (ppb)* | *LOQ (ppb)* |
|---|---|---|
| Zn | 0.979 | 2.937 |
| Cu | 0.030 | 0.090 |
| Pb | 0.008 | 0.024 |
| As | 0.007 | 0.021 |
| Hg | 0.039 | 0.117 |
| Cd | 0.007 | 0.021 |

***Consumer Health Risk Assessment***

To evaluate the potential health risks associated with the dietary intake of trace elements through *R. venosa* consumption, the Estimated Daily Intake (EDI) and the Target Hazard Quotient (THQ) were calculated in accordance with the United States Environmental Protection Agency guidelines (USEPA, 2000, 2001).

***Estimated Daily Intake (EDI)***

The EDI (mg $kg^{-1}day^{-1}$) represents the theoretical daily exposure dose of a specific contaminant per unit of body weight and was calculated using the following equation guidelines (USEPA, 2000, 2001):

$$EDI = \frac{FIR \times C}{BW}$$

Where:

- **FIR:** Food intake rate for adult mollusks consumption (g $day^{-1}$), adapted as <1 g $day^{-1}$ based on FAO (2023) criteria.
- **C:** Concentration of the target trace element in the soft tissue (mg $kg^{-1}$ wet weight).
- **BW:** Average adult body weight, set at 70 kg (USEPA, 2000).

***Target Hazard Quotient (THQ) and The Hazard Index (HI)***

The THQ characterizes the ratio between the exposure dose and the reference dose (Rf. D.), serving as a metric for non-carcinogenic health risk assessment guidelines (USEPA, 2000, 2001):

$$THQ = \frac{EF \times ED \times FIR \times C}{Rf.\,D. \times BW \times AT}$$

Where:

- **EF:** Exposure frequency (365 days $year^{-1}$).
- **ED:** Exposure duration (70 years for adults).
- **Rf. D.:** Oral reference dose (mg $kg^{-1}$ $day^{-1}$) for each element established by USEPA (2024).
- **AT:** Averaging time for non-carcinogenic effects, calculated as the product of EF x ED (365 days $year^{-1}$ x 70 years).

An HI value exceeding the safety threshold of 1.0 indicates potential non-carcinogenic health risks for the consuming population, whereas HI equal to or below this baseline implies that consumption is safe and devoid of significant health risks (USEPA, 2001, 2024).

***Carcinogenic Risk Index (RI)***

To estimate the potential lifetime carcinogenic risk associated with exposure to specific trace elements via *R. venosa* consumption, the Risk Index (RI) was calculated using the following equation (USEPA 2024; RAIS 2024):

$RI = EDI \times SF$

- **SF:** Oral Slope Factor (mg $kg^{-1}$ $day^{-1}$) $^{-1}$ established by the USEPA for carcinogenic contaminants

The calculated RI values were interpreted based on the regulatory safety thresholds defined by the USEPA (2024):

- $RI < 10^{-6}$ indicates a negligible or insignificant risk, meaning the probability of developing cancer from dietary exposure is exceptionally low.

- An RI value ranging between $10^{-6}$ and $10^{-4}$ ($10^{-6}$ <RI<$10^{-4}$) is classified as an acceptable or tolerable risk level.
- An RI>$10^{-4}$ is considered a significant health concern, indicating an unacceptable carcinogenic risk that requires immediate attention.

***Statistical Analysis***

All experimental data were expressed as mean values ± standard deviation (SD). Prior to parametric hypothesis testing, the datasets were evaluated for normality using the Shapiro-Wilk test, and homogeneity of variances was verified via Levene's test. To determine whether the temporal variations in trace element concentrations, target hazard quotients (THQ), and carcinogenic risk indices (RI) between May and June were statistically significant, an independent-samples *t*-test (or Mann-Whitney U test for non-normally distributed data) was performed. Additionally, Pearson correlation analysis (or Spearman's rank correlation) was utilized to assess the strength and direction of the linear relationships among the verified trace elements in the soft tissues of *R. venosa*. All statistical evaluations were conducted using the SPSS software package (Version 27, IBM Corp., Armonk, NY, USA), and the threshold for statistical significance was strictly established at a p-value of less than 0.05 ($p < 0.05$).

**Results**

***Trace Element Accumulation Profiles***

The mean concentrations of six analyzed trace elements (Zn, Cu, Pb, As, Hg, and Cd) in the soft tissues of the veined rapa whelk collected from the Akliman coasts of Sinop were summarized in Table 2.

**Table 2.** Mean concentrations (mg. $kg^{-1}$ wet weight ± standard deviation, n=3) of trace elements in the soft tissues of *R. venosa* collected from Akliman coasts (Sinop, Southern Black Sea) during May and June 2024.

| *Months* | *Zn* | *Cu* | *Pb* | *As* | *Hg* | *Cd* |
|---|---|---|---|---|---|---|
| May | 7.80±0.16 | 3.81±0.28 | 0.040±0.002 | 0.034±0.004 | 0.022±0.002 | 0.012±0.001 |
| June | 7.34±0.43 | 3.13±0.15* | 0.045±0.009 | 0.028±0.004* | 0.020±0.001 | 0.013±0.001 |

* Indicates a statistically significant difference between May and June ($p < 0.05$).

Statistical analysis revealed distinct monthly variations for specific elements between the two sampling periods. The decrease in Cu and As concentrations from May to June was found to be statistically significant ($p < 0.05$), indicating a shifting bioaccumulation dynamic or a change in environmental availability as the season progressed into early summer. Conversely, no statistically significant temporal differences were observed for Zn, Pb, Hg, and Cd ($p > 0.05$), suggesting that the bioaccumulation of these elements remained stable throughout the studied months. While total As (tAs) concentrations stood at 0.034 (May) and 0.028 (June), the hazardous inorganic As (iAs) fraction-estimated at 10% of tAs (Marengo et al. 2018; Souza-Araujo et al. 2021; da Silva et al. 2025)-was remarkably lower (0.0034 mg/kg and 0.0028 mg/kg).

***Dietary Exposure Risk Table***

The estimated dietary exposure levels, expressed as EDI and EWI through the consumption of *R. venosa*, are presented in Table 3. The dietary exposure profile for both daily and weekly intake mirrors the established soft tissue bioaccumulation trend, with Zn and Cu values remaining noticeably higher than the rest of the analyzed elements. In contrast, the exposure rates calculated for non-essential toxic elements (Pb, As, Hg, and Cd) were considerably minimal across both sampling months. Temporal comparisons indicated a general slight reduction in the intake levels for most elements from May to June, except for Pb and Cd, which demonstrated stable or marginally higher values in the early summer period.

**Table 3.** Estimated Daily Intake (EDI, mg $kg^{-1}$ $day^{-1}$) and Estimated Weekly Intake (EWI, mg $kg^{-1}$ $week^{-1}$) of trace elements from the consumption of *R. venosa* collected in May and June 2024.

| *Risk Index* | *Month* | *Zn* | *Cu* | *Pb* | *As* | *Hg* | *Cd* |
|---|---|---|---|---|---|---|---|
| EDI | May | $1.11 \times 10^{-3}$ | $5.44 \times 10^{-4}$ | $5.8 \times 10^{-6}$ | $4.9 \times 10^{-6}$ | $3.2 \times 10^{-6}$ | $1.8 \times 10^{-6}$ |
| | June | $1.05 \times 10^{-3}$ | $4.47 \times 10^{-4}$ | $6.4 \times 10^{-6}$ | $4.0 \times 10^{-6}$ | $2.9 \times 10^{-6}$ | $1.8 \times 10^{-6}$ |
| EWI | May | $7.80 \times 10^{-3}$ | $3.81 \times 10^{-3}$ | $4.0 \times 10^{-5}$ | $3.4 \times 10^{-5}$ | $2.2 \times 10^{-5}$ | $1.2 \times 10^{-5}$ |
| | June | $734 \times 10^{-3}$ | $3.13 \times 10^{-3}$ | $4.5 \times 10^{-5}$ | $2.8 \times 10^{-5}$ | $2.0 \times 10^{-5}$ | $1.3 \times 10^{-5}$ |

***Health Risk Assessment and Trace Elements Index Evaluation***

The non-carcinogenic THQ and carcinogenic RI calculated for trace elements exposures during May and June are summarized in Table 4. To avoid the overestimation of health risks, As toxicity was specifically evaluated

based on its inorganic form (iAs), if iAs constitutes approximately 10% of the total As (tAs) concentration as widely established in the literature (Marengo et al. 2018; Souza-Araujo et al. 2021; da Silva et al. 2025).

The calculated THQ values for all elements, including iAs, ranged from $1.35 \times 10^{-6}$ to $1.80 \times 10^{-5}$. In May, Cd exhibited the highest THQ ($1.80 \times 10^{-5}$), followed by Cu ($1.36 \times 10^{-5}$) and Hg ($1.10 \times 10^{-5}$), while iAs showed a lower non-carcinogenic potential ($1.62 \times 10^{-6}$). A similar trend was observed in June, where Cd remained the dominant contributor ($1.80 \times 10^{-5}$) and iAs decreased slightly to $1.35 \times 10^{-6}$.

Carcinogenic RI was performed for Pb and iAs. The RI values for iAs were determined as $7.29 \times 10^{-7}$ in May and $6.07 \times 10^{-7}$ in June. For Pb, the RI values were $4.90 \times 10^{-8}$ and $5.46 \times 10^{-8}$ for May and June, respectively.

**Table 4**. Target Hazard Quotient (THQ) and Carcinogenic Risk Index (RI) values of trace elements across monitoring periods.

| | *Period* | *Zn* | *Cu* | *Pb* | *tAs* | *iAs* | *Hg* | *Cd* |
|---|---|---|---|---|---|---|---|---|
| THQ | May | $3.71 \times 10^{-6}$ | $1.36 \times 10^{-5}$ | $1.90 \times 10^{-6}$ | $1.62 \times 10^{-5}$ | $1.62 \times 10^{-6}$ | $1.10 \times 10^{-5}$ | $1.80 \times 10^{-5}$ |
| | June | $3.50 \times 10^{-6}$ | $1.12 \times 10^{-5}$ | $2.10 \times 10^{-6}$ | $1.35 \times 10^{-5}$ | $1.35 \times 10^{-6}$ | $9.70 \times 10^{-6}$ | $1.80 \times 10^{-5}$ |
| RI | May | - | - | $4.90 \times 10^{-8}$ | $7.29 \times 10^{-6}$ | $7.29 \times 10^{-7}$ | - | - |
| | June | - | - | $5.46 \times 10^{-8}$ | $6.07 \times 10^{-6}$ | $6.07 \times 10^{-7}$ | - | - |

tAs= As (Total); iAs= As (inorganic)

## Discussion

### *Trace Element Accumulation Profiles*

The concentrations of the analyzed trace elements in the soft tissues of *R. venosa* exhibited distinct variations depending on the specific element and the sampling month (Bat et al. 2023). In the present study, for both sampling periods, a consistent and robust bioaccumulation hierarchy was observed across the specimens, establishing a definitive decreasing profile. Zn was predominantly the most abundant element accumulated within the soft tissues, followed closely by Cu. This prominent dominance of Zn and Cu reflects their biological roles as essential physiological elements, which gastropods actively regulate for metabolic processes and enzymatic functions.

Conversely, the non-essential and highly toxic heavy metals, along with metalloids, displayed remarkably lower bioaccumulation levels throughout the study. Among these regulated contaminants, Pb and As occupied the intermediate segment of the accumulation spectrum, showing minor fluctuations between the late spring and early summer periods. Hg and Cd were sequestered in the least amounts, with Cd consistently remaining the least accumulated element in the soft tissues of the veined rapa whelk. The minimal accumulation of these hazardous trace metals suggests a low environmental exposure level at the studied habitat or a specific metabolic exclusion mechanism by the organism during the sampling months.

### *Comparison of Trace Element Concentrations with Food Safety Standards*

To evaluate the commercial viability and food safety compliance of the analyzed matrix, the measured trace element concentrations during May and June were compared against established national and international regulatory thresholds for mollusks.

A critical observation from the regulatory framework is that no specific permissible limit or criterion has been established for As across any of the evaluated guidelines, including the Ministry of Agriculture, Forestry and Fisheries (MAFF 1995), Turkish Food Codex (Official Gazette of Republic of Türkiye 2002 and 2009), and European Commission Regulations (Council of Europe 2001; Commission Regulation 2006). This absence of an As standard highlights the complexity of metalloid regulation in marine organisms and underscores the importance of utilizing risk indices like THQ and RI to independently verify its safety. For the remaining regulated elements, the concentration levels in this study were found to be exceptionally low and well within all safe legal limits:

**Zn and Cu**: The maximum concentrations detected in this study (Zn: 7.80 mg/kg; Cu: 3.81 mg/kg in May) were profoundly below the most stringent guidance limits. Zn levels were safely lower than the 50 mg/kg threshold specified by both MAFF (1995) and the Turkish Food Codex (Official Gazette of Republic of Türkiye 2002). Similarly, Cu levels did not exceed the strict 20 mg/kg maximum permissible limit set by the same authorities.

**Pb**: The maximum measured Pb concentration (0.045 mg/kg in June) was significantly lower than the maximum allowable level of 1.5 mg/kg stipulated for mollusks by the Commission Regulations (Council of Europe 2001; Commission Regulation 2006) and the Turkish Food Codex (Official Gazette of Republic of Türkiye 2002 and 2009), as well as the 10 mg/kg screening limit of MAFF (1995).

**Hg**: The recorded Hg concentrations (0.022 mg/kg in May and 0.020 mg/kg in June) were substantially below the uniform European and Turkish regulatory limit of 0.5 mg/kg (Council of Europe, 2001; Official Gazette of Republic of Türkiye 2002 and 2009; Commission Regulation 2006).

**Cd**: Despite having the lowest physiological tolerance ceiling among the metals, the measured Cd values (0.012 - 0.013 mg/kg) exhibited a vast safety margin when compared to the legal threshold of 1.0 mg/kg strictly enforced for molluscan products by European and Turkish food codices, and the 0.2 mg/kg limit of MAFF (1995).

In conclusion, the comparative assessment demonstrates that the concentrations of all regulated heavy metals in the sampled mollusks are safely below the maximum permissible limits set by both Turkish (Official Gazette of Republic of Türkiye 2002 and 2009) and international food safety authorities (Council of Europe, 2001; Commission Regulation 2006). These results confirm that the monitored matrix poses no toxicological threat regarding commercial food safety regulations.

***Health Risk Assessment and Trace Elements Index Evaluation***

The non-carcinogenic health risk coefficients (THQ) for all investigated heavy metals were significantly below the US EPA threshold of 1 (THQ<1), indicating no substantial non-carcinogenic health hazard to the consumer population during the monitoring periods.

A critical refinement was applied to the carcinogenic risk evaluation of As. Raw risk estimates based on total As (tAs) often overemphasize the actual threat, as organic As compounds possess minimal toxicity. By adopting the conservative literature consensus (Marengo et al. 2018; Souza-Araujo et al. 2021; da Silva et al. 2025) that inorganic As (iAs) represents roughly 10% of tAs, the realistic carcinogenic RI for iAs were calculated as 7.29 $x10^{-7}$ (May) and 6.07 $x10^{-7}$ (June).

According to the US EPA risk characterization guidelines, an RI value below $10^{-6}$ indicates a negligible or insignificant carcinogenic risk. Prior to speciation correction, tAs values marginally fell into the $10^{-6}$ - $10^{-4}$ acceptable/tolerable risk range. However, focusing precisely on the toxicologically relevant iAs fraction revealed that the true carcinogenic risk of As in this study is entirely negligible (RI < $10^{-6}$). Similarly, the RI values for Pb (approx. $5 \times 10^{-8}$) were well below the critical baseline. Consequently, it can be concluded that the long-term consumption or exposure to the investigated matrix does not pose any alarming oncological or systemic health risks regarding Pb and As dynamics.

The minor temporal fluctuations observed between May and June can be attributed to environmental factors such as variation in hydrodynamic conditions, seasonal precipitation changes altering dilution effects, or localized shifts in anthropogenic discharges. The subtle increment in Pb indices during June underscores the possibility of minor seasonal traffic / tourism activity or agricultural run-off impacts, which should be verified with extended longitudinal data.

## Conclusion

This study provided a comprehensive temporal evaluation of trace element concentrations (Zn, Cu, Pb, As, Hg, and Cd) and associated human health risks in the investigated molluscan matrix during May and June. The concentration hierarchy consistently followed the pattern of Zn > Cu > Pb > As > Hg > Cd across both monitoring periods. Comparative assessments against national and international food safety standards (including Turkish Food Codex, European Commission Regulations, and MAFF guidelines) demonstrated that all regulated heavy metals were exceptionally low and remained safely below the maximum permissible thresholds. Notably, while global food safety guidelines lack specific criteria for As, target health risk indexes effectively bridged this regulatory gap.

By implementing a toxicologically refined speciation approach-evaluating As risks based on the toxic inorganic fraction (iAs= 10% of tAs)-the study demonstrated that the realistic non-carcinogenic THQ and carcinogenic RI < $10^{-6}$) for all elements were well within safe physiological limits. Minor temporal fluctuations between May and June, particularly the subtle elevation of Pb in June, highlight the seasonal dynamics of environmental factors and potential localized anthropogenic inputs. In conclusion, the analyzed mollusks are commercially viable and completely safe for human consumption, presenting no systemic or oncological health risks. Nonetheless, establishing localized longitudinal monitoring programs is recommended to maintain comprehensive oversight of trace elements dynamics and long-term food safety security in the region.

## Declarations

***Ethical Approval Certificate***

Ethical approval was not required for this study. This article does not contain any studies involving human subjects. We confirm that this manuscript complies with all Ethical Rules applicable to the journal.

***Author Contribution Statement***

Levent Bat: Conceptualization, validation, data curation, writing-original draft preparation, supervision, writing-review and editing.

Ayşah Öztekin: Investigation, resources, visualization, software, methodology, formal analysis.

Dilara Kaya Öztürk: Data collection, investigation, methodology, formal analysis.

Zeynep Hasançavuşoğlu: Data collection, investigation, methodology, formal analysis.

All authors have read and agreed to the published version of the manuscript.

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***Conflict of Interest***

The authors declare no conflict of interest.

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# Haematology and Serum Biochemistry of Pregnant West African Dwarf Goats Fed Diets Containing Microbial Treated Rice Husk as a Replacement for Maize

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## Abstract

The study was conducted to evaluate the effects of microbial treated rice husk as a replacement for Maize on the haematology and serum biochemical parameters of West African Dwarf (WAD) goats. Raw rice husk was subjected to solid-state fermentation using *Alcaligenes faecalis*, *Aspergillus flavus*, or a mixture of both. Forty West African Dwarf goats were randomly placed on four dietary treatments with ten goats per treatment. The treatments were 0% treated rice husk, 25% bacterial-treated, 25% fungi-treated and 50:50 bacterial + fungi treated rice husk diet. The animals were fed at 5% of their body weight. Blood samples of 10 ml were collected before morning feeding at the end of each trimester of gestation to analyze for haematology and biochemical components. Blood samples were taken before morning feeding via jugular vein puncture into a plain and an anticoagulant bottle. Packed Cell Volume, Haemoglobin, red blood cell and total white Blood Cells were determined. Mean Corpuscular volume, Mean Corpuscular haemoglobin and Mean Corpuscular haemoglobin concentration was calculated from Packed Cell Volume, haemoglobin and Red Blood Cell. Serum biochemical parameters measured include total protein, albumin, blood urea nitrogen aspartate aminotransferase and alanine aminotransferase parameters were determined. Goat fed the cocktail diet had better red blood cell, Packed Cell Volume, hemoglobin, platelet, eosinophil and aspartate aminotransferase but high in cholesterol level. Goat on bacterial-treated diet had the highest Total protein and better hemoglobin, Packed Cell Volume, platelet lymphocytes, aspartate aminotransferase and globulin content. The treated rice husk had positive impact on the blood parameters, the values are within the normal range for healthy goat. Hence, with the results of this study, the cocktail microbial treated rice husk replacement for Maize is suitable for better performance of WAD goats.

***Keywords:*** *Haematology, Serum, Rice husk, Microbial, Maize, Pregnant WAD goats*

## Introduction

Small ruminant production represents a vital segment of livestock agriculture across sub-Saharan Africa, particularly in Nigeria where goats are integral to rural livelihoods, household economy, and food security (Olanrewaju *et al.*, 2025).Among indigenous goat breeds, the West African Dwarf (WAD) goat is highly valued due to its tolerance to trypanosomiasis, adaptability to diverse climatic conditions, resistance to endemic diseases, small body size, and good reproductive performance (Daramola et al., 2005; Ibhaze et al., 2021).. These attributes make WAD goats especially suitable for low-input systems where conventional feeds are not always accessible or affordable. However, the availability and the quality of feed resources for WADGs during pregnancy can significantly impact their heamatological and serum biochemical and overall health. Rice husk is one of the most abundant agricultural by-products produced in rice-growing regions around the world. Due to the rising cost and limited availability of conventional feed ingredients, there is growing interest in utilizing rice husk as an alternative feed resource for livestock, particularly ruminants. However, its use is often limited by its high fibre, lignin, and silica contents, which reduce digestibility and restrict the amount of nutrients that animals can obtain from it. To improve its feeding value, microbial treatment has been explored as an effective and environmentally friendly approach. This process involves the use of beneficial microorganisms, such as bacteria and fungi, which ferment the rice husk and break down its complex fibrous structure. As a result, the treated rice husk becomes more digestible, nutrients become more readily available, and the protein content may increase due to microbial growth during fermentation. These improvements can enhance the nutritional quality of rice husk, making it a more suitable, affordable, and sustainable feed ingredient for ruminant production. The haematology and serum biochemical parameters of WAD goats fed diets containing treated rice husk as a replacement for maize have generated interest among researchers and farmers alike. Understanding the effects of these dietary modifications on the health status and metabolic responses of WAD goats is essential for ensuring optimum productivity, physiological well-being, and overall animal welfare. For instance, Afolayan et al. (2025) observed that the combined bacteria and fungi-treated rice husk diet (T4) improved economic efficiency by lowering feed cost, although it resulted in a marginal reduction in weight gain compared to the maize-based control diet. This indicates its potential as a cheaper alternative feed ingredient in small ruminant production systems. Similarly, Ibhaze et al. (2021) investigated the blood chemistry of West African Dwarf goats fed microbial-treated maize cob and husk diets and reported that the diets supported normal erythropoiesis and maintained serum electrolyte balance without

adverse effects on liver function, suggesting that they are safe for inclusion in goat diets at levels of up to 30%. However, limited research has been conducted on the effects of microbial treatment of rice husk specifically on WADGs during pregnancy and lactation. Despite the growing interest in the use of treated rice husk as an alternative feed resource, information on its effects on the health and physiological status of West African Dwarf (WAD) goats remains limited. Therefore, there is a need to address this knowledge gap by evaluating the potential advantages and possible limitations associated with the inclusion of treated rice husk in goat diets. This study was designed to assess the effects of diets containing microbially treated rice husk on the haematological and serum biochemical characteristics of WAD goats. The haematological parameters evaluated include red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) concentration, and packed cell volume (PCV), while the serum biochemical indices include total protein, albumin, globulin, urea, creatinine, and liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Evaluation of these parameters will provide valuable information on the nutritional adequacy, physiological responses, and overall health status of goats fed diets containing treated rice husk, thereby contributing to the development of sustainable and cost-effective feeding strategies for small ruminant production.

## Materials and Methods

### *Experimental Site*

The study was conducted at the Small Ruminant Unit of Afolayan Farms, located in Oro, Irepodun Local Government Area of Kwara State, Nigeria. Oro lies within the Southern Guinea Savannah agro-ecological zone of Nigeria, which is characterized by distinct wet and dry seasons. The area receives an average annual rainfall ranging from 1,000 to 1,500 mm and experiences mean ambient temperatures between 27 °C and 33 °C.

### *Experimental materials and collection*

*Aspergillus flavus* (fungus) and *Alcaligenes faecalis* (bacterium) were obtained from the Microbiology culture laboratory, University of Ilorin, Ilorin, Nigeria. The organisms were cultured on appropriate media and maintained under optimum conditions (25–37°C) through regular sub-culturing to ensure purity and viability.

The culture media were prepared according to standard microbiological procedures and sterilized by autoclaving at 121°C for 15 minutes. After cooling and solidification in sterile Petri dishes, the organisms were inoculated onto the media using the streak plate technique. The inoculated plates were incubated at 37°C for 24–48 hours until distinct colonies were obtained. Pure cultures were subsequently maintained and used for the preparation of inocula for rice husk fermentation.

*Inoculation of Rice husk* reduce moisture content to a safe storage level. Rice husks samples were sourced, cleaned and sterilized in an autoclave at 121. A microbial suspension was prepared by diluting 1mL of inoculum in 9 mL sterile saline solution and uniformly applied to the sterilized rice husk."The incubation lasted for 10days, after which the fermented substrate was subsequently dried to reduce moisture content to a safe storage level, packaged, labeled and stored prior to diet formulation.

### *Experimental Animals*

Forty (40) clinically healthy pregnant West African Dwarf (WAD) goats with average weight of 15 ± 0.5 kg were purchased from Ajase Local Market, Irepodun Local Government Area, Kwara State, Nigeria for this experiment. The animals were treated against ecto and endo-parasites using Ivomec® and vaccinated against Peste des Petits Ruminants (PPR) using Tissue Culture Rinderpest Vaccine and were fed ad libitum with formulated experimental diets with adequate supply of drinking water.

### *Experimental Diets*

Four experimental diets were formulated for the study as presented in Table 1. The treatments consisted of T1 (diet containing 25% maize, serving as the control), T2 (diet containing 25% bacteria-treated rice husk), T3 (diet containing 25% fungi-treated rice husk), and T4 (diet containing 25% combined bacteria- and fungi-treated rice husk). The experimental animals were randomly assigned to the four dietary treatments using a Completely Randomized Design (CRD), with ten animals allocated to each treatment.

### *Procedures for blood Profile*

Blood samples were collected from the jugular vein before morning feeding into EDTA-treated and plain tubes. The EDTA samples were used for haematological analysis, while serum obtained from the plain tubes was used for biochemical and immunological analyses.

Haematological parameters determined included Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), and White Blood Cell (WBC) counts, Lymphocyte, Eosinophil. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from PCV, Hb, and RBC values.

Serum biochemical parameters analyzed were glucose, total protein, albumin, blood urea nitrogen (BUN), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using standard laboratory procedures.

Antibody and cytokine concentrations were determined using enzyme-linked immunosorbent assay (ELISA) techniques.

***Statistical Analysis***

Data collected was subjected to Analysis of Variance (ANOVA) and differences between treatment means were separated using Least Significant Difference (LSD) using General Linear Model procedure of Statistical Analysis System (DSAASTAT, 2011).

Table 1: Composition of Treatment Diets

| | *Diets (kg)* | | | |
|---|---|---|---|---|
| Ingredients | TI (Control) | T2 (BTRH) | T3 (FtRH) | T4 (B+F-TRH) |
| Treated rice husk | - | 25 | 25 | 25 |
| Maize | 25 | - | - | - |
| Soya beans meal | 15 | 15 | 15 | 15 |
| Palm kernel cake | 10 | 10 | 10 | 10 |
| Groundnut cake | 10 | 10 | 10 | 10 |
| Cassava peel | 20 | 20 | 20 | 20 |
| Wheat bran | 15 | 15 | 15 | 15 |
| Bone meal | 2.5 | 2.5 | 2.5 | 2.5 |
| Salt | 1 | 1 | 1 | 1 |
| Premix | 1.5 | 1.5 | 1.5 | 1.5 |
| Total | 100 | 100 | 100 | 100 |

Keys: Control = maize diet, BTRH=Bacterial treated rice husk, FTRH =Fungi treated rice husk, B+FTRH = Bacterial + Fungi treated rice husk)

**Results**

The haematological profile of pregnant West African Dwarf goats during the first trimester is presented in Table 2. White blood cell count (WBC) ranged from 13.36 to 16.71 $\times 10^9$/L across the dietary treatments. Red blood cell count (RBC) varied significantly ($P < 0.05$), with values of 12.27, 11.63, 13.42 and 13.23 $\times 10^{12}$/L recorded for T1, T2, T3 and T4, respectively. Packed cell volume (PCV) ranged from 31.33 to 33.33%, while haemoglobin concentration (HGB) varied between 10.87 and 11.43 g/dl.

Lymphocyte counts ranged from 7.26 to 8.06 $\times 10^9$/L, whereas neutrophil values ranged from 4.63 to 5.44 $\times 10^9$/L. Monocyte counts varied between 0.26 and 0.42 $\times 10^9$/L, eosinophils ranged from 0.34 to 0.53 $\times 10^9$/L, and basophils ranged from 0.03 to 0.06 $\times 10^9$/L. Mean corpuscular volume (MCV) values were between 18.97 and 20.03 fl, while mean corpuscular haemoglobin concentration (MCHC) ranged from 30.00 to 31.20 g/dl.

Red cell distribution width expressed as coefficient of variation (RDW-CV) ranged from 14.93 to 16.80%, whereas red cell distribution width standard deviation (RDW-SD) ranged from 24.60 to 26.50 fl. Apart from RBC, all measured haematological parameters showed no significant differences ($P > 0.05$) among the dietary treatments during the first trimester of pregnancy.

Table 2: 1st Trimester Haematology Study of Pregnant WAD Goats Fed Experimental Diet.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-Value |
| WBC ($\times 10^9$/L) | 16.71 | 15.07 | 13.46 | 13.36 | 1.368 | 0.330 |
| RBC ($\times 10^{12}$/L) | $12.27^{ab}$ | $11.63^{b}$ | $13.42^{a}$ | $13.23^{a}$ | 0.414 | 0.049* |
| PCV (%) | 31.33 | 31.67 | 33.33 | 33.33 | 1.607 | 0.826 |
| HGB (g/dl) | 11.43 | 11.13 | 10.87 | 10.97 | 14.374 | 0.396 |
| LYM ($\times 10^9$/L) | 8.06 | 7.26 | 7.62 | 7.69 | 0.856 | 0.929 |
| NEUT ($\times 10^9$/L) | 4.63 | 4.69 | 4.92 | 5.44 | 0.736 | 0.859 |
| MONOCYTE ($\times 10^9$/L) | 0.33 | 0.26 | 0.42 | 0.35 | 0.117 | 0.796 |
| EOS ($\times 10^9$/L) | 0.53 | 0.34 | 0.35 | 0.39 | 0.128 | 0.696 |
| BAS0. ($\times 10^9$/L) | 0.06 | 0.05 | 0.03 | 0.05 | 1.384 | 0.455 |
| MCV (fl) | 20.03 | 18.97 | 19.26 | 19.67 | 1.192 | 0.925 |
| MCHC (g/dl) | 30.23 | 30.00 | 30.07 | 31.20 | 1.629 | 0.947 |
| RDW-CV (%) | 14.93 | 16.00 | 16.80 | 16.77 | 1.388 | 0.758 |
| RDW-SD (FL) | 26.43 | 25.80 | 26.50 | 24.60 | 1.106 | 0.614 |

a,b,c = means within the same row with different superscripts are significantly ($P<0.05$) different, WBC = White blood cell, RBC = Red blood cell, PCV = Packed cell volume, HGB = Haemoglobin, Lymphocyte, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MCV = Mean corpuscular, MCHC = Mean corpuscular haemoglobin concentration, RDW = Red cell distribution width, RDW-CV = Red cell distribution width- coefficient of variation, RDW-SD = Red cell distribution width- standard deviation.

The serum biochemical profile of pregnant West African Dwarf goats during the first trimester is presented in Table 3. Urea concentration ranged from 6.17 to 9.00 mmol/l, with the highest value observed in T2 and the lowest in T1. Cholesterol values varied between 2.10 and 2.61 mmol/l, while high-density lipoprotein cholesterol (HDL-C) ranged from 1.01 to 1.16 mmol/l. Low-density lipoprotein cholesterol (LDL-C) values were similar across treatments, ranging from 0.62 to 0.71 mmol/l. Triglyceride concentrations varied from 0.17 to 0.29 mmol/l. Liver enzyme activities showed values ranging from 23.72 to 26.38 U/L for alanine aminotransferase (ALT), 93.52 to 115.86 U/L for aspartate aminotransferase (AST), 42.63 to 50.98 U/L for gamma-glutamyl transferase (GGT), and 133.67 to 160.67 U/L for alkaline phosphatase (ALP). Total protein concentrations ranged from 67.03 to 69.58 g/l, whereas albumin values ranged from 33.48 to 35.54 g/l. Total bilirubin concentrations varied between 0.19 and 0.34 mg/dl, while direct bilirubin ranged from 0.01 to 0.07 mg/dl. Significant differences ($P < 0.05$) were observed only for direct bilirubin, with T1 and T2 recording the highest values (0.07 mg/dl) and T3 the lowest value (0.01 mg/dl). All other serum biochemical parameters were not significantly affected ($P > 0.05$) by dietary treatments during the first trimester of pregnancy.

Table 3: 1st Trimester Serum Blood Profile of Pregnant WAD Goats Fed Experimental Diet.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-Value |
| Urea (mmol/l) | $6.17^{b}$ | $9.00^{a}$ | $8.13^{ab}$ | $7.4^{ab}$ | 0.622 | 0.061 |
| Cholesterol (mmol/l) | 2.10 | 2.15 | 2.61 | 2.29 | 0.238 | 0.475 |
| HDL-C (mmol/l) | 1.16 | 1.01 | 1.06 | 1.09 | 0.186 | 0.956 |
| LDL-C (mmol/l) | 0.62 | 0.64 | 0.71 | 0.67 | 0.133 | 0.968 |
| Triglyceride (mmol/l) | 0.24 | 0.29 | 0.17 | 0.19 | 5.228 | 0.432 |
| ALT (U/L) | 26.38 | 24.43 | 23.72 | 26.38 | 2.195 | 0.052 |
| AST (U/L) | 93.52 | 115.86 | 95.38 | 100.97 | 8.713 | 0.325 |
| GGT (U/L) | 42.63 | 43.86 | 50.98 | 44.41 | 5.619 | 0.723 |
| ALP (U/L) | 156.0 | 149.0 | 160.67 | 133.67 | 31.419 | 0.933 |
| Total Protein (g/l) | 68.15 | 68.17 | 67.03 | 69.58 | 4.622 | 0.984 |
| Albumin (g/l) | 33.79 | 33.48 | 35.54 | 34.45 | 2.787 | 0.954 |
| Total Bilirubin(mg/dl) | 0.31 | 0.34 | 0.31 | 0.19 | 0.119 | 0.830 |
| Direct Bilirubin(mg/dl) | $0.07^{a}$ | $0.07^{a}$ | $0.01^{b}$ | $0.03^{ab}$ | 1.453 | 0.052 |

a,b,c = means within the same row with different superscripts are significantly ($P<0.05$) different, HDL-C = High density lipoprotein, LDL-C = Low-density lipoprotein cholesterol, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma-glutanmyl transferase, ALP = Alkaline phosphatase

The haematological indices of pregnant West African Dwarf (WAD) goats fed microbial-treated rice husk-based diets during the second trimester are presented in Table 4. The results showed that dietary treatments did not significantly ($P > 0.05$) influence most of the haematological parameters evaluated, including WBC, RBC, PCV, lymphocyte, neutrophil, monocyte, eosinophil, basophil, MCV, RDW-CV, and RDW-SD. This suggests that the inclusion of microbial-treated rice husk in the diets did not adversely affect the physiological status or blood-related functions of the animals, as the haematological and biochemical responses remained within normal physiological ranges reported for West African Dwarf goats.

The similar white blood cell (WBC) values observed across dietary treatments suggest that the experimental diets did not induce infections, inflammatory responses, or physiological stress in the animals. Likewise, the comparable lymphocyte and neutrophil counts indicate that immune function remained within normal physiological limits throughout the feeding period. These findings suggest that microbial treatment may improve the nutritive value and safety of rice husk-based diets without compromising the health status of West African Dwarf goats. This result is supported by established haematological reference frameworks for goats, which indicate that stable leukocyte profiles are consistent with normal physiological and immune function (Daramola et al., 2005; Weiss & Wardrop, 2022).

Red blood cell count, packed cell volume, and haemoglobin concentration are important indicators of oxygen transport and general health status. Although haemoglobin values differed numerically among treatments, goats fed T2 and T3 recorded marginally higher values than those fed T4. The maintenance of normal RBC, PCV, and HGB values across treatments suggests that the diets provided adequate nutrients required for erythropoiesis and prevented the occurrence of anaemia during pregnancy (Weiss & Wardrop, 2022).

Among all the parameters measured, mean corpuscular haemoglobin concentration (MCHC) was significantly ($P < 0.05$) affected by dietary treatment. Goats fed T4 recorded a lower MCHC value (29.00 g/dl) compared with those fed T1, T2, and T3. However, the value remained within the normal physiological range reported for healthy goats, indicating that the reduction was not severe enough to impair oxygen-carrying capacity or animal performance. The lower mean corpuscular haemoglobin concentration (MCHC) observed in T4 may indicate slight variation in erythrocytic haemoglobin distribution, which is often influenced by nutritional and physiological factors affecting red cell indices (Thrall *et al.*, 2019).

Table 4: 2nd Trimester Haematology Study of Pregnant WAD Goats Fed Experimental Diet.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-Value |
| WBC ($\times10^9$/L) | 17.55 | 17.35 | 15.93 | 18.65 | 0.953 | 0.317 |
| RBC ($\times10^{12}$/L) | 11.23 | 12.33 | 12.74 | 13.30 | 0.678 | 0.252 |
| PCV (%) | 28.67 | 30.00 | 29.67 | 29.00 | 0.849 | 0.926 |
| HGB (g/dl) | $10.83^{ab}$ | $11.10^{a}$ | $11.07^{a}$ | $10.01^{b}$ | 0.256 | 0.078 |
| LYM ($\times10^9$/L) | 8.08 | 8.03 | 6.89 | 7.13 | 0.578 | 0.397 |
| NEUT ($\times10^9$/L) | 0.37 | 0.30 | 0.47 | 0.38 | 0.084 | 0.596 |
| MONOCYTE ($\times10^9$/L) | 0.43 | 0.3 | 0.47 | 0.50 | 0.13 | 0.730 |
| EOS ($\times10^9$/L) | 0.02 | 0.02 | 0.01 | 0.02 | 7.8E-03 | 0.781 |
| BAS0. ($\times10^9$/L) | 0.05 | 0.07 | 0.04 | 0.05 | 1.9E-02 | 0.545 |
| MCV (fl) | 20.00 | 22.07 | 21.80 | 21.85 | 0.837 | 0.335 |
| MCHC (g/dl) | $33.27^{a}$ | $31.83^{a}$ | $31.47^{a}$ | $29.00^{b}$ | 0.574 | 0.005** |
| RDW-CV (%) | 17.53 | 17.47 | 17.50 | 17.90 | 0.606 | 0.951 |
| RDW-SD (FL) | 27.13 | 28.23 | 27.30 | 27.10 | 0.995 | 0.832 |

a,b,c = means within the same row with different superscripts are significantly (P<0.05) different, WBC = White blood cell, RBC = Red blood cell, PCV = Packed cell volume, HGB = Haemoglobin, Lymphocyte, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MCV = Mean corpuscular, MCHC = Mean corpuscular haemoglobin concentration, RDW = Red cell distribution width, RDW-CV = Red cell distribution width- coefficient of variation, RDW-SD = Red cell distribution width- standard deviation.

The serum biochemical profile of pregnant West African Dwarf (WAD) goats fed microbial-treated rice husk-based diets during the second trimester is presented in Table 11. The results showed that most serum biochemical parameters, including cholesterol, HDL-C, LDL-C, triglycerides, ALT, AST, GGT, ALP, total protein, and total bilirubin, were not significantly (P > 0.05) affected by dietary treatments. This indicates that the inclusion of microbial-treated rice husk did not adversely influence lipid metabolism, protein utilization, or liver function of the pregnant goats.

Urea concentration showed significant variation among treatments, with goats fed T2 and T4 recording higher values than those fed T3. The differences in serum urea levels may reflect variations in dietary protein degradation and nitrogen utilization among the treatment groups. However, the values obtained remained within the normal physiological range for goats, suggesting efficient protein metabolism and adequate utilization of dietary nitrogen.This interpretation is consistent with recent findings showing that serum urea levels in goats are influenced by dietary protein intake and rumen nitrogen metabolism, but values within physiological limits indicate effective nitrogen utilization without metabolic stress (Ibhaze et al., 2021; Olanrewaju et al., 2025).

Similarly, albumin concentration differed among treatments, with T2 recording the highest value and T4 the lowest. Albumin is a major indicator of protein nutritional status and liver synthetic activity. The higher albumin concentration observed in T2 may suggest improved protein availability and utilization resulting from microbial treatment of rice husk. Nevertheless, all values remained within normal physiological limits, indicating that liver function was not compromised (Thrall et al., 2019).

Table 5: 2nd Trimester Serum Blood Profile of Pregnant WAD Goats Fed Experimental Diet.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-Value |
| Urea (mmol/l) | $7.43^{ab}$ | $8.13^{a}$ | $6.67^{b}$ | $8.17^{a}$ | 0.399 | 0.087 |
| Cholesterol (mmol/l) | 1.91 | 2.05 | 2.21 | 1.93 | 0.179 | 0.638 |
| HDL-C (mmol/l) | 0.99 | 1.05 | 1.05 | 0.67 | 0.144 | 0.261 |
| LDL-C (mmol/l) | 0.77 | 0.67 | 0.65 | 0.72 | 6.344 | 0.581 |
| Triglyceride (mmol/l) | 0.39 | 0.35 | 0.29 | 0.42 | 5.739 | 0.472 |
| ALT (U/L) | 29.12 | 20.20 | 21.05 | 19.36 | 3.031 | 0.164 |
| AST (U/L) | 76.01 | 89.44 | 86.13 | 80.04 | 4.16 | 0.186 |
| GGT (U/L) | 34.45 | 42.92 | 38.68 | 39.16 | 8.56 | 0.918 |
| ALP (U/L) | 180.11 | 218.49 | 217.81 | 187.01 | 35.565 | 0.809 |
| Total Protein (g/l) | 65.50 | 67.95 | 68.08 | 63.85 | 3.631 | 0.813 |
| Albumin (g/l) | $29.93^{ab}$ | $32.57^{a}$ | $30.61^{ab}$ | $28.95^{b}$ | 0.925 | 0.113 |
| Total Bilirubin(mg/dl) | 0.48 | 0.44 | 0.31 | 0.44 | 5.102 | 0.172 |
| Direct Bilirubin(mg/dl) | $0.08^{ab}$ | $0.10^{a}$ | $0.01^{b}$ | $0.03^{ab}$ | 5.265 | 0.131 |

a,b,c = means within the same row with different superscripts are significantly (P<0.05) different, HDL-C = High density lipoprotein, LDL-C = Low-density lipoprotein cholesterol, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma-glutanmyl transferase, ALP = Alkaline phosphatase

The absence of significant differences in ALT, AST, GGT, and ALP activities suggests that the experimental diets did not induce hepatic damage or metabolic disorders. Likewise, the similarity in total protein, cholesterol fractions, triglycerides, and bilirubin concentrations among treatments indicates stable metabolic and physiological conditions throughout the feeding period. These findings demonstrate that microbial-treated rice husk can be incorporated into diets of pregnant WAD goats without detrimental effects on serum biochemical characteristics during the second trimester.

The haematological profile of pregnant West African Dwarf (WAD) goats during the third trimester is presented in Table 6. The results revealed that dietary treatments had no significant ($P > 0.05$) effect on all the haematological parameters measured, including WBC, RBC, PCV, HGB, lymphocytes, neutrophils, monocytes, eosinophils, basophils, MCV, MCHC, RDW-CV, and RDW-SD. This indicates that the inclusion of microbial-treated rice husk in the diets did not adversely affect the health status or physiological functions of the pregnant goats.

The similar WBC and differential leukocyte counts across treatments suggest that the experimental diets did not induce stress, infection, or inflammatory responses. The values obtained indicate that the immune system of the animals remained stable throughout the third trimester of pregnancy (Olafadehan, 2011). Likewise, the absence of significant differences in lymphocyte and neutrophil counts further confirms that microbial-treated rice husk did not compromise immune competence.

Red blood cell count, packed cell volume, and haemoglobin concentration were also unaffected by dietary treatments. The maintenance of comparable RBC, PCV, and HGB values across treatments suggests adequate oxygen-carrying capacity and normal erythropoietic activity in the goats. These findings indicate that the experimental diets supplied sufficient nutrients required for blood formation and prevented the occurrence of anaemia during late pregnancy (Daramola et al., 2005).

Similarly, MCV, MCHC, RDW-CV, and RDW-SD values did not differ significantly among treatments, indicating normal red blood cell morphology and uniformity in erythrocyte size distribution. The stability of these indices suggests that microbial treatment improved the utilization of rice husk without causing abnormalities in red blood cell production or function (Weiss & Wardrop, 2022).

This results demonstrate that microbial-treated rice husk-based diets supported normal haematological status during the third trimester of pregnancy. The absence of significant differences among treatments indicates that the diets were safe and capable of maintaining adequate health and physiological functions in pregnant WAD goats.

Table 6: 3rd Trimester Haematology Study of Pregnant WAD Goats Fed Experimental Diet.

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-Value |
| WBC ($\times10^9$/L) | 21.48 | 21.76 | 21.40 | 23.01 | 2.057 | 0.938 |
| RBC ($\times10^{12}$/L) | 10.40 | 11.03 | 10.83 | 10.63 | 0.721 | 0.932 |
| PCV (%) | 28.00 | 27.33 | 28.00 | 28.00 | 2.279 | 0.996 |
| HGB (g/dl) | 9.33 | 9.10 | 9.17 | 10.03 | 1.029 | 0.912 |
| LYM ($\times10^9$/L) | 6.00 | 7.13 | 6.37 | 7.13 | 0.459 | 0.279 |
| NEUT ($\times10^9$/L) | 9.67 | 9.36 | 9.99 | 8.21 | 1.261 | 0.772 |
| MONOCYTE ($\times10^9$/L) | 0.43 | 0.3 | 0.47 | 0.50 | 0.13 | 0.730 |
| EOS ($\times10^9$/L) | 0.24 | 0.20 | 0.29 | 0.28 | 7.7E-02 | 0.817 |
| BAS0. ($\times10^9$/L) | 0.05 | 0.07 | 0.04 | 0.06 | 1.8E-02 | 0.627 |
| MCV (fl) | 21.96 | 21.38 | 23.40 | 23.07 | 1.526 | 0.769 |
| MCHC (g/dl) | 34.43 | 31.9 | 32.13 | 32.23 | 1.246 | 0.484 |
| RDW-CV (%) | 18.77 | 18.50 | 18.50 | 18.80 | 0.894 | 0.991 |
| RDW-SD (FL) | 29.07 | 29.37 | 29.80 | 30.07 | 1.563 | 0.969 |

a,b,c = means within the same row with different superscripts are significantly ($P<0.05$) different, WBC = White blood cell, RBC = Red blood cell, PCV = Packed cell volume, HGB = Haemoglobin, Lymphocyte, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MCV = Mean corpuscular, MCHC = Mean corpuscular haemoglobin concentration, RDW = Red cell distribution width, RDW-CV = Red cell distribution width- coefficient of variation, RDW-SD = Red cell distribution width- standard deviation.

The serum biochemical profile of pregnant West African Dwarf (WAD) goats during the third trimester is presented in Table 7. The results showed that most serum metabolites and liver function indices were not significantly ($P > 0.05$) affected by dietary treatments, indicating that the inclusion of microbial-treated rice husk did not adversely influence metabolic activities or organ function in the pregnant goats.

Serum urea concentration showed a treatment effect, with goats fed T4 recording the highest value and those fed T3 the lowest. Variations in serum urea are often associated with differences in dietary protein degradation and nitrogen utilization efficiency. Nevertheless, all values remained within the normal physiological range for goats, suggesting efficient protein metabolism and adequate utilization of dietary nitrogen across treatments.This observation is consistent with recent reports that serum urea levels in goats are influenced by dietary nitrogen intake and rumen microbial activity, while values within physiological limits indicate proper protein utilization without metabolic stress (Ibhaze et al., 2021; Olanrewaju et al., 2025).

Among the liver enzymes evaluated, alanine aminotransferase (ALT) was significantly ($P \leq 0.05$) affected by dietary treatment. Goats fed T4 recorded the highest ALT value (43.43 U/L), while those fed T2 and T3 had lower values. Although elevated ALT activity is commonly associated with increased hepatic cellular activity or mild tissue stress, the values observed remained within acceptable physiological limits for healthy goats and were not accompanied by corresponding increases in AST, GGT, or bilirubin concentrations. This suggests that the diets did not induce hepatic damage or compromise liver integrity (Thrall et al., 2019).

The absence of significant differences in cholesterol, HDL-C, LDL-C, and triglyceride concentrations indicates that lipid metabolism was not altered by the dietary treatments. Similarly, total protein, albumin, total bilirubin, and direct bilirubin values remained comparable among treatments, suggesting adequate protein nutrition and normal liver synthetic function (Kaneko et al., 2008).

Furthermore, the non-significant AST, GGT, and ALP activities across treatments provide additional evidence that microbial-treated rice husk did not exert adverse effects on hepatic function or general metabolic status. These findings agree with reports that serum biochemical indices within normal physiological ranges are indicative of good nutritional status and healthy organ function in goats (Daramola et al., 2005).

Table 7: 3rd Trimester Serum Blood Profile of Pregnant WAD Goats Fed Experimental Diet.

| | *Treatments* | | | | | |
|---|---|---|---|---|---|---|
| Parameters | T1 | T2 | T3 | T4 | SEM | p-Value |
| Urea (mmol/l) | 8.1$^{ab}$ | 8.47$^{ab}$ | 6.67$^{b}$ | 9.17$^{a}$ | 0.58 | 0.081 |
| Cholesterol (mmol/l) | 1.84 | 1.88 | 2.21 | 1.56 | 0.222 | 0.306 |
| HDL-C (mmol/l) | 0.83 | 0.92 | 0.87 | 0.76 | 0.127 | 0.824 |
| LDL-C (mmol/l) | 0.69 | 0.54 | 0.71 | 0.57 | 9.1E-02 | 0.468 |
| Triglyceride (mmol/l) | 0.46 | 0.59 | 0.63 | 0.53 | 9.9E-02 | 0.662 |
| ALT (U/L) | 36.45$^{ab}$ | 27.78$^{b}$ | 30.72$^{b}$ | 43.43$^{a}$ | 3.429 | 0.050 |
| AST (U/L) | 176.49 | 256.10 | 219.47 | 250.58 | 45.017 | 0.600 |
| GGT (U/L) | 54.51 | 54.11 | 51.12 | 50.65 | 5.495 | 0.939 |
| ALP (U/L) | 353.11 | 258.49 | 407.81 | 287.60 | 49.596 | 0.219 |
| Total Protein (g/l) | 62.17 | 64.81 | 62.74 | 63.85 | 3.994 | 0.965 |
| Albumin (g/l) | 26.41 | 26.20 | 26.28 | 24.95 | 0.426 | 0.130 |
| Total Bilirubin(mg/dl) | 0.53 | 0.49 | 0.48 | 0.48 | 8.2E-02 | 0.973 |
| Direct Bilirubin(mg/dl) | 0.11 | 0.05 | 0.08 | 0.14 | 2.9E-02 | 0.225 |

a,b,c = means within the same row with different superscripts are significantly ($P<0.05$) different, HDL-C = High density lipoprotein, LDL-C = Low-density lipoprotein cholesterol, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma-glutamyl transferase, ALP = Alkaline phosphatase

## Conclusion

This study evaluated the effects of microbial-treated rice husk as a partial replacement for maize in the diets of pregnant West African Dwarf (WAD) goats. The findings revealed that inclusion of bacterial-treated, fungal-treated, and combined bacteria–fungi treated rice husk did not negatively affect the haematological and serum biochemical parameters of the experimental animals across the three trimesters of pregnancy.

Most haematological indices, including white blood cells, red blood cells, packed cell volume, haemoglobin, and differential leukocyte counts, remained within normal physiological ranges, indicating stable immune status, adequate oxygen-carrying capacity, and absence of anaemia or physiological stress. Similarly, serum biochemical parameters such as total protein, albumin, cholesterol fractions, liver enzymes (ALT, AST, ALP, GGT), and bilirubin were largely unaffected by dietary treatments, suggesting normal metabolic function, protein utilization, lipid metabolism, and liver integrity.

Among the treatments, the combined bacteria–fungi treated rice husk (T4) generally showed comparable or slightly improved haematological and biochemical performance, indicating its potential superiority in enhancing nutrient utilization. Overall, the results demonstrate that microbial fermentation improves the feeding value of rice husk, making it a safe and effective alternative feed ingredient for replacing maize in diets of pregnant WAD goats without compromising health status.

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*ID: 915*

# A comparative study of the nutritional profile of three Pigeon pea (*Cajanus cajan)* cultivars to enhance food security in sub-Saharan Africa

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## Abstract

The pigeon pea (*Cajanus cajan*) is an important but underutilised legume in Côte d'Ivoire. This study aims to establish the nutritional profile of three pigeon pea cultivars grown in Côte d'Ivoire. Several analyses were carried out on three pigeon pea cultivars distinguished by their colour (white, brown and purple). These included the determination of pH and the content of reducing sugars and fibre. In addition, the profiles of vitamins, minerals and essential amino acids were determined. Antinutritional compounds were also identified. The results showed that the pH of the three cultivars is practically neutral (6.9–6.8) and the reducing sugar content ranges from 1.5 to 2.62 mg/100 g. As for fibre content, it varies from 18.5 to 22.42 mg/100 g, with a high content in the purple cultivar. The results also showed that the cowpea cultivars contain several vitamins, notably vitamins A, D, C, K1 and the B vitamins. The brown-coloured cultivar has a high vitamin content compared to the others (Vitamin A: 41.13 µg/100 g; vitamin B1: 61.27 µg; vitamin B5: 291.71 µg). Furthermore, the analyses revealed the presence of minerals such as phosphorus, potassium, calcium, iron, magnesium and sodium. The results also showed that the cowpea cultivars contain all nine essential amino acids in varying proportions. The phytate content of the three cultivars ranges from 16.65 mg (brown cultivar) to 20.08 mg (purple cultivar), whilst the oxalate content ranges from 12.02 (purple cultivar) to 15.03 mg (white cultivar). Although the cultivars studied contain antinutritional compounds, these can be significantly reduced by various techniques, including cooking, soaking and germination. In summary, all three cultivars analysed offer enormous potential, particularly the brown variety. This legume can be incorporated into local dietary habits to improve the nutritional status of vulnerable communities.

***Keywords:*** *Pigeon pea, cultivar, nutritional compounds, food security*

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# Dams, Recreation Areas, Climate Change and Water Security: An Assessment Using the Çoruh River as an Example

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## Abstract

Water is an essential resource for the sustainability of human and societal life. Although two-thirds of the world is covered with water, our planet is not rich in usable water resources. The sustainability of usable resources is threatened by problems such as global warming, drought, and climate change. The Çoruh River originates in the Mescit Mountains, flows through the provinces of Bayburt and Erzurum, then through Georgia before emptying into the Black Sea. Of its total length of 431 km, 21 km is within Georgian borders, making it one of Turkey's five transboundary rivers. The Çoruh Basin, one of Turkey's 26 hydrological basins, covers a total area of 20,259,900,000 m². The Çoruh River is Turkey's fastest-flowing river and is utilized for numerous purposes, from energy production and irrigation to providing drinking water and creating recreational areas. With its existing dams and hydroelectric power plants, the river and its basin contribute 6% of Turkey's total energy production, positively impacting the economy. However, the river and basin are also experiencing negative environmental changes due to decreasing water levels and the degradation of the river ecosystem. The increasing scarcity of usable water resources worldwide due to human actions has led to water, as a geopolitical resource, having a significant impact on international power balances and being considered a national security issue by nations due to competition in scarce regions. Transboundary waters, in particular, can lead to serious disputes, tensions, and even water wars between the states sharing them. The Çoruh River Basin is a water system of strategic importance for our country and constitutes a significant portion of its hydroelectric power generation capacity. This study examines the interaction of dams and recreational areas constructed in the basin with climate change and their effects on water security. Data obtained through a literature review address hydrological regime changes, greenhouse gas emissions, ecosystem pressures, and transboundary water management issues. The findings reveal that despite the economic and social contributions of dams and recreational areas, they will lead to problems in the long term in terms of water security, climate change, and environmental sustainability, thus requiring multi-dimensional planning.

***Keywords:*** *Çoruh River, Hydroelectric Energy, Recreation Areas, Climate Change, Water Security, Sustainable Water Management*

## Barajlar, Rekreasyon Alanları, İklim Değişikliği ve Su Güvenliği: Çoruh Nehri Örneğinde Bir Değerlendirme

## Özet

Su insan ve toplum yaşamının sürdürülebilirliği için elzem bir kaynaktır. Dünyanın üçte ikisi sularla kaplı olmakla birlikte dünyamız kullanılabilir su kaynakları açısından zengin değildir. Kullanılabilir kaynakların sürdürülebilirliği ise küresel ısınma, kuraklık, iklim değişikliği gibi sorunların tehditi altındadır. Çoruh Nehri Mescit dağlarında doğan, Bayburt ve Erzurum illeri sonrasında Gürcistan içinden geçerek Karadeniz'e dökülen bir nehirdir. Toplam 431 km uzunluktaki nehrin 21 km'si Gürcistan sınırları içinde olup Çoruh, ülkemizin sınıraşan beş suyundan biridir. Türkiye'nin 26 hidrolojik havzasından biri olan Çoruh Havzası toplamda 20.259.900.000 m² lik bir alana sahiptir. Çoruh Nehri ülkemizin en hızlı akan nehri olup kendisinden, enerji üretiminden sulamaya, içme suyu sağlanmasına, rekreasyonel alanlar oluşturulmasına birçok amaçla yararlanılmaktadır. Üzerindeki enerji üretimine yönelik mevcut barajlarındaki HES'lerle Türkiye'de üretilen toplam enerjinin %6'sını karşılayan ve ekonomiye olumlu katkı sağlayan nehir ve havzası, yatağındaki su seviyesinin azalması ve nehir ekosisteminin bozulmasına dayalı olarak çevresel açıdan olumsuz değişimler de yaşamaktadır. Dünyadaki kullanılabilir su kaynaklarının, insanoğlunun eylemleriyle giderek azalması, jeopolitik bir kaynak olarak suyun; uluslararası güç dengeleri üzerinde etkili olmasına ve kıt olduğu bölgelerde oluşan rekabetle, uluslarca ulusal güvenlik sorunu olarak değerlendirmesine yol açmıştır. Özellikle sınıraşan sular onu paylaşan devletler arasında ciddi uyuşmazlıklara, gerginliklere hatta su savaşlarına yol açabilmektedir. Çoruh Nehri Havzası, ülkemiz için stratejik önemde ve hidroelektrik üretim kapasitesinin önemli bir bölümünü oluşturan bir su sistemidir. Bu çalışmada, havzada inşa edilen barajların, rekreasyon alanlarının iklim değişikliğiyle etkileşimi ve su güvenliği üzerindeki etkileri incelenmektedir. Bu kapsamda yapılan literatür taramasıyla sağlanan verilerle; hidrolojik rejim değişimleri, sera gazı emisyonları, ekosistem üzerindeki baskılar ve sınır aşan su yönetimi sorunları ele alınmıştır. Ulaşılan sonuçlar, barajların, rekreasyon alanlarının ekonomik, sosyal katkılarına karşın, uzun vadede su güvenliği iklim değişikliği ve çevresel sürdürülebilirlik açısından sorunlara yol açacağını ve bu bağlamda çok boyutlu planlama gerektirdiğini ortaya koymaktadır.

***Anahtar Kelimeler:*** *Çoruh Nehri, Hidroelektrik Enerji, Rekreasyon Alanları, Iklim Değişikliği, Su Güvenliği, Sürdürülebilir Su Yönetimi*

*ID: 918*

# Multivariate Evaluation of the Relationships Between Egg and Chick Traits in Denizli, Gerze and Sultan Native Chicken Genotypes

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## Abstract

This study aimed to evaluate the relationships between egg morphometric traits, incubation characteristics and chick quality parameters in Denizli, Gerze and Sultan native chicken genotypes using univariate and multivariate statistical approaches. A total of 410 eggs obtained from Denizli, Gerze and Sultan native chicken genotypes were used in the study. Egg weight, egg width, egg length, shape index, transfer egg weight and weight loss were determined. After hatching, hatch time, chick weight, chick yield, activity score, navel score, chick score and chick length were measured. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were applied to determine differences among genotypes. Principal component analysis (PCA), correlation heatmap analyses and linear discriminant analysis (LDA) were performed to evaluate multivariate relationships among traits. Significant differences were detected among Denizli, Gerze and Sultan genotypes for most egg and chick traits ($P<0.05$). Denizli chickens exhibited higher egg weight, transfer egg weight, chick weight, chick yield and chick length values compared to the other genotypes. Sultan chickens showed lower growth-related parameters but higher activity scores. Correlation analyses revealed strong positive relationships among egg weight, transfer egg weight, chick weight and chick length. PCA results demonstrated a clear phenotypic separation of the Sultan genotype from Denizli and Gerze chickens. In the combined linear discriminant analysis based on egg and chick traits, the overall classification accuracy was determined as 73.5%. The findings demonstrated strong relationships between egg morphometric characteristics and chick quality parameters in Denizli, Gerze and Sultan native chicken genotypes. Multivariate analyses revealed clear phenotypic differentiation among genotypes and highlighted the importance of maternal egg characteristics on embryonic development and post-hatch chick performance.

***Keywords:*** *Native Chicken, Denizli, Gerze, Sultan, Egg Quality, Chick Quality*

*ID: 919*

# Effect of crossbreeding on production performances and egg quality traits between commercial ISA Brown, Blue Holland hybrid cocks, and local hens

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## Abstract

In Niger, poultry farming has not yet developed to the same extent as in other countries of the sub-region. Local breeds constitute 54.7% of the flock. However, the yield of local chickens remains low due to their limited genetic potential and challenging conditions; local hens have lower productivity than commercial breeds. Therefore, this study aimed to investigate the production performances and egg quality characteristics of local improved ISA Brown hens (obtained by crossbreeding between the local hens and commercial ISA Brown hybrid cocks) and improved local Blue Holland hens (obtained by crossbreeding between the local hens and commercial Blue Holland hybrid cocks) in comparison with local hens (parents and young hens). The experiment was arranged in a completely randomized design with three-layer genotypes (40 hens per genotype) used in this study. These three genotypes were monitored under the same rearing conditions for 51 weeks. Feed was measured and distributed to the birds twice daily, and the collected eggs were used to assess internal and external quality for four weeks. The results revealed a significant difference ($P < 0.05$) between genotypes in laying rate, with 33.73% in improved local ISA Brown hens, 25.53% in improved local Blue Holland hens, 25.43% in local parent hens, and 11.37% in young local hens. The feed conversion ratio was lower in improved local ISA Brown (6.40) and Blue Holland (8.25) hens compared to local parent hens (9.16) and young local hens (14.01). The average egg weight was higher in improved local Blue Holland (49.65 g) and ISA Brown (49.22 g) hens than in local parent hens (40.94 g) and young local hens (39.66 g). The eggs from improved local ISA Brown hens also showed higher values ($P<0.05$) in egg index (77.00%), albumen height (4.06 mm), yolk height (7.74 mm), albumen proportion (55.71%), yolk diameter (39.07 mm), albumen length (70.91 mm), and albumen index (5.87) compared to the eggs of young local hens. Haugh units and yolk index did not show a significant difference between all genotypes ($P > 0.05$). The local improved ISA Brown hens exhibited the best zootechnical performance and laid better-quality eggs. These results indicate that genetic improvement of local hens is most effective with ISA Brown hybrid cocks. In conclusion, this study showed that crossbreeding between ISA Brown and Blue Holland cocks and local hens might be an alternative method for improving Niger's local chickens.

***Keywords:*** *improved local hens, zootechnical performance, hybrid Cocks, genetic improvement, ISA Brown, Blue Holland, egg quality*

ID: 920

# Effects of Short-Term Temperature Manipulations During Storage on Embryonic Development and Hatchability of Hatching Eggs

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## Abstract

In the poultry industry, long-term storage of hatching eggs is a common practice due to market demands, logistical planning, and breeding company operations. However, prolonged storage of eggs beyond seven days has been associated with increased cellular apoptosis in the blastoderm, deterioration of albumen quality (including changes in Haugh unit and pH), extended incubation duration, and substantial reductions in chick quality and hatchability. To mitigate these adverse effects, the Short Periods of Incubation During Egg Storage (SPIDES) technique has been developed and has gained strategic importance in recent years. The objective of this review is to examine the cellular and physiological mechanisms underlying SPIDES application during extended egg storage and to evaluate its industrial outcomes in commercial hatcheries based on current scientific literature. According to the reviewed literature, SPIDES stimulates the regeneration of blastoderm cells damaged during the storage period, promotes cellular homeostasis through increased expression of heat shock proteins, particularly HSP70, and limits the liquefaction of albumen. Furthermore, it enables the embryo to progress to more advanced blastoderm developmental stages (gastrulation stages), which are known to exhibit greater cellular resistance to storage-induced stress. From an industrial perspective, SPIDES has been shown to increase hatchability by approximately 3–7% in eggs stored for 7–14 days or longer, reduce early chick mortality, improve chick quality parameters such as navel score and body weight uniformity, and provide a more synchronized hatch by narrowing the hatch window. The available evidence indicates that SPIDES applications represent a sustainable, cost-effective, and efficient strategy for maintaining embryonic viability, maximizing hatchery performance, and supporting early growth performance during the first week of life. However, the success of this method depends on the precise management of gradual heating and cooling profiles that prevent shell sweating and contamination risks, while also ensuring that critical temperature and exposure-time thresholds are not exceeded, thereby avoiding early embryonic mortality. Optimizing and integrating this technique into commercial hatchery operations according to factors such as breeder age, genotype, eggshell quality, and storage duration is of critical importance for enhancing industrial efficiency, sustainability, and global competitiveness within the poultry sector.

***Keywords:*** *Temperature Manipulation, Hatching Eggs, Storage, Embryonic Development, Hatchability.*

*ID: 922*

# Standardised Ileal Amino Acid Digestibility of Processed Senna Seed (*Senna occidentalis* L.) in Broiler Chickens

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## Abstract

Senna seed is an underutilised tropical legume with potential as an alternative protein source in poultry nutrition. However, its utilisation in broiler diets is constrained by anti-nutritional factors that reduce amino acid availability and utilisation. Processing methods have been reported to improve nutrient availability through the reduction of these antinutrients. This study evaluated the standardised ileal amino acid digestibility of processed senna seed meal in broiler chickens. One hundred and sixty 17-day-old Ross 308 broiler chicks were randomly assigned to four dietary treatments with five replicates of eight birds each in a completely randomised design. Diet 1 was a highly digestible protein (HDP); a control and diets 2, 3 and 4 contained Raw Senna Seed Meal (RSSM), Fermented Senna Seed Meal (FSSM), and Boiled Senna Seed Meal (BSSM) supplemented diets. Amino acid composition of the test ingredients was determined using standard analytical procedures, and ileal digesta were collected on day 21 for digestibility assay. Data were analysed using ANOVA at $p = 0.05$. Results showed that processing significantly affected ($p < 0.05$) the amino acid composition and standardised ileal amino acid digestibility of *Senna occidentalis* seed meal. Among the senna seed meals, RSSM generally contained higher amino acid concentrations. However, standardised ileal amino acid digestibility was highest in birds fed HDP followed by FSSM and BSSM diets. In FSSM, leucine (74.53%), lysine (70.52%), threonine (66.25%) and tryptophan (79.58%) showed superior digestibility compared to the RSSM diet (57.70%, 53.06% and 66.68%), respectively. This indicates that fermentation enhanced amino acid bio-availability through microbial degradation of anti-nutritional factors. In conclusion, fermentation effectively improved the nutritive value of Senna seeds by reducing anti-nutritional factors and enhancing amino acid digestibility and utilisation. Therefore, properly processed senna seed meal at 10% inclusion level can serve as a promising alternative protein source for broiler chickens.

***Keywords:*** *Senna occidentalis, Amino acid digestibility, Processing methods, Non-conventional protein source, Broiler chickens.*

## Introduction

Soyabean meal is the major protein source in poultry diets because of its favourable amino acid profile and high digestibility (Babatunde *et al*., 2021). However, rising costs have increased interest in alternative protein sources such as *Senna occidentalis*, a leguminous seed with appreciable protein and essential amino acid contents (Rabiu, 2019).

The utilisation of *Senna occidentalis* seed meal in broiler diets is limited by antinutritional factors, including tannins, phytates, oxalates and saponins, which can impair protein digestion and amino acid absorption (Diarra, 2021). Processing methods such as boiling and fermentation have been reported to reduce these compounds and improve nutrient availability (Diarra, 2021). However, information on the standardised ileal amino acid digestibility (SIAAD) of processed *Senna occidentalis* seed meal remains scarce.

Standardised ileal amino acid digestibility is considered a reliable measure of amino acid availability because it corrects for endogenous amino acid losses and provides accurate values for feed formulation (Doyin and Akinfala, 2023). Therefore, this study evaluated the SIAAD of raw, boiled and fermented *Senna occidentalis* seed meal in broiler chickens to determine its potential as an alternative protein source for poultry diets.

## Materials And Methods

### *Experimental Site*

The study was conducted at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Nigeria.

### *Seed procurement and processing*

Mature seeds of *Senna occidentalis* were procured, cleaned, and processed by boiling or fermentation. For boiling, seeds were boiled in water (1:4 w/v) at 100°C for 1 h, drained, sun-dried, and milled (Doma *et al*., 2016). For fermentation, seeds were soaked in water for 1 h and fermented anaerobically for 5 days before drying and milling (Augustine *et al*., 2017). Processed meals were stored in airtight containers until use.

***Experimental Birds and Diets***

A total of 160 one-day-old Ross 308 broiler chicks were used for the study. Birds were randomly allotted to four dietary treatments in a completely randomised design comprising five replicates of eight birds each. Commercial starter feed was provided from day 1 to 16, while experimental diets were fed from day 17 to 21. Diets were formulated to meet NRC (1994) nutrient requirements for poultry. The diets contained approximately 21.5–21.7% crude protein and 2,804–2,823 kcal ME/kg. Titanium dioxide (0.5%) was included as an indigestible dietary marker. Birds had free access to feed and water throughout the study.

***Experimental diet layout***

Treatment I (Control): Highly digestible protein (whey protein) with 0% Senna Seed Meal (SSM).
Treatment II: Basal diet containing 90% SBM + 10% Raw Senna Seed Meal (RSSM).
Treatment III: Basal diet containing 90% SBM + 10% Fermented Senna Seed Meal (FSSM).
Treatment IV: Basal diet containing 90% SBM + 10% Boiled Senna Seed Meal (BSSM).

***Amino Acid Analysis***

The amino acid composition of the seed meals was determined according to AOAC (2006) procedures using an Applied Biosystems PTH Amino Acid Analyser.

***Digesta collection***

On day 21, birds were euthanised and ileal digesta samples were collected from the distal two-thirds of the ileum following the procedure of Agboola *et al*. (2021). Samples were pooled by replicate and oven-dried at 55°C prior to analysis.

***Digestibility calculations***

**Apparent ileal amino acid digestibility (AIAAD)**

$AIAAD\ (\%) = [1 - (TiO_2 diet/TiO_2 digesta \times AAdigesta/AAdiet)] \times 100$

**Standardised ileal amino acid digestibility (SIAAD)**

$SIAAD\ (\%) = AIAAD + [(IEAA\ flow) / (AA\ content\ of\ feedstuff)] \times 100$

where IEAA = ileal endogenous amino acid flow.

***Titanium dioxide analysis***

Titanium dioxide concentrations in diets and digesta were determined using the spectrophotometric method described by Morgan *et al*. (2014).

***Statistical Analysis***

Data were subjected to one-way analysis of variance (ANOVA) using SAS (2012). Treatment means were separated using Tukey's HSD test at a significance level of $P = 0.05$.

## Results

***Amino acid profile of raw and processed senna seed meal***

The amino acid profile of raw and processed *Senna occidentalis* seed meals differed significantly ($p < 0.05$) among treatments (Table 1). The highly digestible protein (HDP) diet recorded the highest concentrations of both essential and non-essential amino acids, including leucine (8.32 g/100 g CP) and glutamic acid (13.03 g/100 g CP). Among the senna seed meals, RSSM generally had higher amino acid concentrations than the processed meals, with histidine reaching 2.71 g/100 g CP. Fermented senna seed meal (FSSM) recorded the lowest values for most amino acids, while BSSM showed appreciable levels, particularly in histidine (2.63 g/100gCP).

***Standardised ileal amino acid digestibility of processed senna seed meal-based diet fed to broiler chickens***

The SIAAD of the experimental diets fed to broiler chickens showed significant ($p < 0.05$) variations across treatments (Table 2). Across all, there is consistency with the Highly Digestible Protein (HDP) diet recording the highest digestibility values, while the RSSM diet had the least, except for methionine (82.36%), cystine (75.72%), and tyrosine (62.50%). The HDP diet showed superior digestibility in methionine (91.96%), glutamic acid (95.41%), cystine (91.42%), and proline (92.38%). FSSM diet maintained higher digestibility in leucine (74.53%), lysine (70.52%), phenylalanine (73.50%), and threonine (66.25%) compared to BSSM and RSSM while the boiled senna seed meal (BSSM) diet exhibited intermediate values across amino acids.

## Discussion

**Amino acid profile of raw and processed *Senna occidentalis* seed meal**

The higher concentrations of essential and non-essential amino acids observed in the highly digestible protein (HDP) diet were expected because whey protein is recognised for its superior amino acid balance and protein quality (Minj and Anand, 2020). Compared with the values reported by Augustine *et al*. (2014), raw *Senna*

*occidentalis* seed meal in the present study contained lower concentrations of lysine, methionine and phenylalanine, although glutamic acid remained the predominant amino acid in both studies. Variations may be attributed to differences in seed source and processing conditions.

Table 1: Amino acid profile of raw and processed *Senna occidentalis* seed meal

| *AA PROFILE* | *HDP* | *RSSM* | *FSSM* | *BSSM* | *SEM* | *P value* |
|---|---|---|---|---|---|---|
| *Essential amino acid (g/100g CP)* | | | | | | |
| Arginine | 7.83$^{a}$ | 5.33$^{b}$ | 3.58$^{d}$ | 4.10$^{c}$ | 0.38 | 0.001 |
| Histidine | 2.62$^{c}$ | 2.71$^{a}$ | 2.43$^{d}$ | 2.63$^{b}$ | 0.02 | 0.001 |
| Isoleucine | 4.53$^{a}$ | 4.03$^{b}$ | 3.61$^{d}$ | 3.81$^{c}$ | 0.08 | 0.001 |
| Leucine | 8.32$^{a}$ | 6.43$^{b}$ | 5.61$^{d}$ | 6.31$^{c}$ | 0.23 | 0.001 |
| Lysine | 6.27$^{a}$ | 3.74$^{b}$ | 3.23$^{d}$ | 3.53$^{c}$ | 0.28 | 0.001 |
| Methionine | 1.43$^{a}$ | 0.85$^{b}$ | 0.63$^{d}$ | 0.74$^{c}$ | 0.07 | 0.001 |
| Phenylalanine | 5.01$^{a}$ | 3.25$^{b}$ | 3.16$^{c}$ | 3.07$^{d}$ | 0.18 | 0.001 |
| Threonine | 3.76$^{a}$ | 3.62$^{b}$ | 3.08$^{d}$ | 3.31$^{c}$ | 0.00 | 0.001 |
| Tryptophan | 1.44$^{a}$ | 0.72$^{b}$ | 0.66$^{c}$ | 0.62$^{d}$ | 0.08 | 0.001 |
| Valine | 5.03$^{a}$ | 4.41$^{b}$ | 3.67$^{d}$ | 4.13$^{c}$ | 0.11 | 0.001 |
| *Non- essential amino acid (g/100g CP)* | | | | | | |
| Alanine | 4.60$^{a}$ | 3.62$^{b}$ | 4.49$^{a}$ | 3.47$^{b}$ | 0.16 | 0.001 |
| Aspartic acid | 10.03$^{a}$ | 7.01$^{b}$ | 6.52$^{d}$ | 6.81$^{c}$ | 0.33 | 0.001 |
| Cystine | 2.01$^{a}$ | 0.62$^{d}$ | 0.67$^{c}$ | 0.77$^{b}$ | 0.13 | 0.001 |
| Glutamic acid | 13.03$^{a}$ | 9.62$^{b}$ | 8.03$^{d}$ | 9.05$^{c}$ | 0.43 | 0.001 |
| Glycine | 4.39$^{a}$ | 4.07$^{b}$ | 3.57$^{d}$ | 3.85$^{c}$ | 0.07 | 0.001 |
| Proline | 3.58$^{a}$ | 3.24$^{b}$ | 2.74$^{d}$ | 3.04$^{c}$ | 0.07 | 0.001 |
| Serine | 4.31$^{a}$ | 4.02$^{b}$ | 3.53$^{d}$ | 3.73$^{c}$ | 0.07 | 0.001 |
| Tyrosine | 3.76$^{a}$ | 2.69$^{b}$ | 2.23$^{d}$ | 2.42$^{c}$ | 0.14 | 0.001 |

$^{abcd}$ Means on the same row with different superscripts are significantly ($p<0.05$) different. RSSM = Raw Senna seed meal, FSSM = Fermented Senna seed meal, BSSM = Boiled Senna seed meal, HDP = Highly digestible protein

Table 2: Standardised ileal amino acid digestibility of experimental diets fed to broiler chickens

| *AA PROFILE* | *HDP* | *RSSM* | *FSSM* | *BSSM* | *SEM* | *P value* |
|---|---|---|---|---|---|---|
| *Essential amino acid (%)* | | | | | | |
| Arginine | 80.74$^{a}$ | 49.59$^{d}$ | 65.65$^{b}$ | 58.08$^{c}$ | 2.63 | 0.001 |
| Histidine | 84.08$^{a}$ | 63.26$^{d}$ | 76.84$^{b}$ | 65.60$^{c}$ | 1.94 | 0.001 |
| Isoleucine | 77.89$^{a}$ | 46.96$^{d}$ | 61.31$^{b}$ | 59.99$^{c}$ | 2.52 | 0.001 |
| Leucine | 82.78$^{a}$ | 57.70$^{d}$ | 74.53$^{b}$ | 69.46$^{c}$ | 2.08 | 0.001 |
| Lysine | 76.39$^{a}$ | 53.06$^{d}$ | 70.52$^{b}$ | 68.29$^{c}$ | 1.98 | 0.001 |
| Methionine | 91.96$^{a}$ | 82.36$^{c}$ | 89.57$^{b}$ | 79.75$^{d}$ | 1.15 | 0.001 |
| Phenylalanine | 84.44$^{a}$ | 58.38$^{d}$ | 73.50$^{b}$ | 60.27$^{c}$ | 2.43 | 0.001 |
| Threonine | 77.67$^{a}$ | 43.81$^{d}$ | 66.25$^{b}$ | 53.55$^{c}$ | 2.94 | 0.001 |
| Tryptophan | 90.70$^{a}$ | 66.78$^{d}$ | 79.58$^{b}$ | 73.34$^{c}$ | 2.02 | 0.001 |
| Valine | 81.27$^{a}$ | 49.76$^{d}$ | 59.61$^{c}$ | 66.49$^{b}$ | 2.65 | 0.001 |
| *Non- essential amino acid (%)* | | | | | | |
| Alanine | 81.65$^{a}$ | 52.90$^{d}$ | 70.51$^{b}$ | 63.27$^{c}$ | 2.41 | 0.001 |
| Aspartic acid | 71.03$^{a}$ | 52.73$^{d}$ | 70.56$^{b}$ | 65.93$^{c}$ | 1.70 | 0.001 |
| Cystine | 91.42$^{a}$ | 75.72$^{c}$ | 83.00$^{b}$ | 61.78$^{d}$ | 2.50 | 0.001 |
| Glutamic acid | 95.41$^{a}$ | 69.05$^{d}$ | 80.02$^{b}$ | 69.35$^{c}$ | 2.46 | 0.001 |
| Glycine | 81.65$^{a}$ | 47.88$^{d}$ | 68.22$^{b}$ | 60.80$^{c}$ | 2.80 | 0.001 |
| Proline | 92.38$^{a}$ | 55.76$^{d}$ | 70.70$^{c}$ | 72.04$^{b}$ | 2.99 | 0.001 |
| Serine | 86.02$^{a}$ | 46.92$^{d}$ | 68.88$^{b}$ | 65.16$^{c}$ | 3.19 | 0.001 |
| Tyrosine | 82.92$^{a}$ | 62.50$^{c}$ | 76.09$^{b}$ | 61.83$^{d}$ | 2.07 | 0.001 |

$^{abcd}$ Means on the same line row with different superscripts are significantly ($p<0.05$) not the same. RSSM - Raw Senna seed meal, FSSM =Fermented Senna seed meal, BSSM=Boiled senna seed meal, HDP = Highly digestible protein

The relatively low concentrations of lysine, methionine and cystine observed in the senna seed meals agree with the findings of Augustine *et al*. (2014), who reported deficiencies of sulphur-containing amino acids in *S. occidentalis*. Processing influenced the amino acid composition of the seed meal. Fermentation maintained or slightly improved some amino acids, which may be associated with microbial modification of seed proteins (Sun *et al*., 2022). In contrast, boiling reduced the concentrations of several amino acids, consistent with reports of heat-induced degradation and leaching during processing (Augustine, 2018).

***Standardised ileal amino acid digestibility of processed Senna occidentalis seed meal***

Standardised ileal amino acid digestibility (SIAAD) differed significantly among treatments, with the HDP diet recording the highest digestibility coefficients and the raw senna seed meal (RSSM) diet the lowest. The superior digestibility of the HDP diet was expected because highly digestible protein sources contain fewer structural barriers and antinutritional compounds that may limit amino acid utilisation.

The improved SIAAD values observed in the processed senna seed meals indicate that processing enhanced amino acid availability. This finding agrees with Diarra (2021), who reported that processing reduces antinutritional factors and improves nutrient utilisation in unconventional feed ingredients. Among the processed meals, fermented senna seed meal (FSSM) generally produced higher digestibility coefficients than boiled senna seed meal (BSSM). This may be attributed to microbial activity during fermentation, which can partially degrade complex proteins and antinutritional compounds, thereby increasing the accessibility of amino acids to digestive enzymes (Ohanenye *et al*., 2022).

The lower digestibility values observed in RSSM may be due to the presence of residual tannins, phytates and other antinutritional factors known to interfere with protein digestion and amino acid absorption (Augustine *et al*., 2017). Although boiling improved digestibility relative to RSSM, its effect was generally less pronounced than fermentation, suggesting that microbial modification of the seed matrix may be more effective than heat treatment alone in enhancing amino acid utilisation.

## Conclusion

Processing improved the standardised ileal amino acid digestibility of *Senna occidentalis* seed meal in broiler chickens, with fermented seed meal generally exhibiting higher percentage digestibility than boiled and raw seed meals. The lower digestibility observed in the raw seed meal proves the negative influence of antinutritional factors on amino acid utilisation. These findings suggest that fermentation is an effective processing method for enhancing the nutritional value of *Senna occidentalis* seed meal and support its potential use as an alternative protein source in broiler diet formulation.

## Declarations

***Ethical Approval Certificate***

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of the University of Ibadan, with approval number UI-ACUREC/063-0125/09.

***Author Contribution Statement***

A. F., Adediji and O. V., Ojo,: Data collection, investigation, formal analysis, methodology, project administration and writing the original draft

O., Taiwo and K. A., Adekola: Data collection and Project administration

A. F. Agboola: Supervision, Conceptualization, Review and editing

***Conflict of Interest***

The authors declare no conflict of interest.

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*ID: 924*

# Effects of Egg Storage Duration on Egg Weight Loss, Hatching Results, and Poult Quality Characteristics in Native Bronze Turkeys

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## Abstract

This study investigated the effects of pre-incubation egg storage duration on egg weight loss, hatchery performance, and selected poult quality parameters in turkey eggs. Eggs collected from a breeder flock were stored daily for 10 days in a storage room maintained at 16°C and 75% relative humidity. Based on the loading sequence, eggs stored for 0–5 days and 6–10 days were separated into two experimental groups representing 5-day and 10-day storage periods, respectively. A total of 746 eggs were used in the study, with equal numbers allocated to each group. On day 25 of incubation, eggs were reweighed to determine weight loss and subsequently transferred to hatching baskets. The results showed that extending the storage period significantly affected egg weight at transfer and increased both absolute (g) and relative (%) weight loss during incubation ($P<0.01$). The relative weight loss was higher in eggs stored for 10 days (13.7%) than in those stored for 5 days (12.5%). Hatchability of fertile eggs and hatchability of set eggs were adversely affected by prolonged storage, decreasing from 81.2% to 69.7% and from 75.1% to 63.8%, respectively ($P<0.01$). Evaluation of embryonic mortality revealed that longer storage duration significantly increased early and mid-term embryonic mortality rates as well as the proportion of contaminated eggs ($P<0.01$). However, storage duration had no significant effect on late embryonic mortality, pipped-but-unhatched embryos, total late embryonic mortality, or fertility rate ($P>0.05$). Likewise, poult quality traits, including poult weight, poult length, shank length, shank diameter, and poult quality score, were not influenced by storage duration ($P>0.05$). In conclusion, extending the pre-incubation storage period of turkey eggs from 5 to 10 days increased egg weight loss and contamination rates and, more importantly, elevated early and mid-term embryonic mortality, resulting in substantial reductions in hatchability and overall hatching performance. Therefore, careful management of egg storage duration is essential to achieve optimal hatchability in Native Bronze Turkeys.

***Keywords:*** *Native Bronze Turkey, Egg Storage Duration, Hatchability, Egg Weight Loss, Poult Quality.*

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*ID: 925*

# Land pressure on agro pastoral resources and impact on Forage availability in Tandjilé Province/Chad

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## Abstract

The studies investigated the land pressure in agro pastoral resources in Tandjilé Province, Southern Chad. The supervised classification and Man Kendall trend analysis using Landsat 7 images and Normalized Difference Vegetation Index (NDVI) images series analyzed the land use land cover change and the degradation trend of the rangeland. The land use land cover analysis indicated an increase of 21.73% for cultivated areas, 12.60% for settlement and a decrease of 4.95, 14.22 and 46.49% for water ponds, forest and vegetation resource respectively.

***Keywords:*** *Tandjilé, Vegetation, forest*

## Introduction

The rural population in the Sudano-Sahelian zone of West and Central Africa has grown by more than 40 percent over the past 20 years, reaching more than 281 million people (Rust, 2018). Combined with a demographic explosion, these upheavals have favored the extension of cultivated areas and the political and socio-economic marginalization of nomadic and transhumant populations (Banoin & Jouve, 2000). Over the past four decades, cropland has doubled in area reaching nearly 25 percent of the total land surface, a trend that scientist's project to accelerate alongside population growth (Brottem, 2021). Pastoral land scarcity pushes herders into protected areas, such as national parks and classified forests, and increases their dependence on nominally illicit practices such as tree branch lopping (Brottem, 2021). It has become increasingly clear that human populations have a powerful effect on the environment. Yet the exact relationship between population dynamics and the environment is complex and not well understood (Hunter, 2000).

Livestock production is the world's dominant land use, covering about 45% of the Earth's land surface, and much of it in harsh and variable environments that are unsuitable for other purposes. Arable land resources also feel the squeeze of population pressure. Fulfilling the resource requirements of a growing population ultimately requires some form of land-use change, whether to expand food production through forest clearing, to intensify production on already cultivated land, or to develop the infrastructure needed to support increased population. Indeed, it is humans' ability to manipulate the landscape that has allowed for the rapid pace of contemporary population growth (Hunter, 2000).

Human influence on the land and other natural resources is accelerating because of rapid population growth and increasing food requirements (Radoslava, 2016). Agriculture and deforestation are two prominent forms of human induced land-use change. Deforestation is closely linked to agricultural land-use change, because it often represents a consequence of agricultural expansion (Hunter, 2000). Human expansion throughout the world caused that agriculture is a dominant form of land management globally, and agricultural ecosystems cover nearly 40% of the terrestrial surface of the Earth (Radoslava, 2016).

The encroachment of cultivated land into grazing areas deepens pastoralists' grievance that their rights to resources be it land, water, wood, or forage are weaker than those of farmers and consequently have been ignored. Pastoralists typically only need seasonal access to resources, so their land rights are often treated as secondary to those of farmers (Brottem, 2021). Due to unprecedented population growth in the South and non-existent land management in the North, farmers are expanding their fields in this contested pastoral space, while pastoralists are moving to agricultural areas in the south during droughts to find water and grazing points for their herds (PNUE *et al.*, 2011).

In search of land, herders are increasingly forced into forest reserves. Livestock is the most valuable resource across many rural communities and is a common target of thieves. Increased frequency and magnitude of livestock thieves is both a cause and effect of violent conflict (Brottem, 2021).



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## Materials and methods

Studies were conducted from Tandjilé Province, South-West of Chad (GPS coordinates: 9° 24'N latitude and 16° 18' E longitude). It has a surface land area of 18,045 Km² (INSEED, 2014). A sample of 96 was interviewed and the interviewees were the household heads.

Statistical data were collected from the cadastral service of Tandjilé which help to understand the evolution of the city and determine its impact on the resources while Satellite derived raster image were collected freely via the United States Geological Survey (USGS) Global Visualization Viewer (Glo Vis): https://www.glovis.usgs.gov and https://www.diva-gis.org/gdata. Supervision classifications were used to assess and examine the patterns of land use. The NDVI of several types of land cover were classified into three periods of ten years (1999; 2011 and 2020 for analyses).

## Result

According to information received from agro-pastoralists and official services such as the cadastral service of the province of Tandjilé, there were two periods of urban and periurban subdivision from 1962 to 2009 and 2009 to 2019. The first phase of extension of living spaces on the East side of the city concerned a distance of 1km from 1962 to 2009; approximately 900m on both sides. These extensions of residential areas have had an impact on agricultural areas and forced peri-urban agriculture towards open spaces considered as grazing areas.

From 2009 to 2019, the extension of the city on crop area was estimated on the East side and reached 1.7km; 1.6m on the South side and 1.5km on the North side. The west side of the department of Tandjilé East where this research work was carried out is occupied by the Logone River that is also under threat on its borders due to climatic and anthropogenic impacts (loamy soil taking and brick making). From 1962 to 2009, in 47 years the residential areas witnessed a subdivision of 90,000 ha and 408,000 ha in 10 years from 2009 to 2019. That is a total of 498,000 ha of residential land use in the study area. This demographic pressure has played a role in the disappearance of forage resources and influences the areas reserved for agriculture and livestock.

In Tandjilé province, availability of forage is most variable and is depending to climate and likely human anthropogenic activity (bush fire). The critical period of unavailability of forage has observed in March, April and May. This is due to the fire bush caused on early on December by herders and February by farmers, for different reason. The agro-pastoralists have observed climate variability over the years in forage productivity in the study area.

After flood there is another herbaceous carpet which colonizes the plain however the species which compose this flora are the aromatic species in majority not consumed by the animals. These species cover the ground and are heat resistant until the rainy season.

Generally in the province of Tandjilé there are early bush fires between November and December. These bush fires are caused by transhumant pastoralists who have come down very early in recent years, clamed 78% of agropastoralists.

According to those surveyed, these fires facilitate the passage of cattle in the grassy savannahs which hide poisonous reptiles but also cause the regrowth of new pastoral plants.

Part of farmers met bring back the residues harvest to store and feed their animals. Others burn the residues voluntarily to prevent access to the transhumant animals who was considered by farmer as crops destroyers and conflicting people.

After January, there are no more fodder plants available, so the period of shortage of pasture started in mid-February. Agropastoralists use the stored crop residues and stored rice straw to feed their animals and sell surplus. Transhumant herders are forced to resort to aerial pastures, i.e. woody species that cut down in an uncontrolled manner. During this critical period, there is also a rise in heat which causes a lot of losses.

According to survey of agropastoralists opinion, estimated the rate of loss of rice straw in the country at 2/4 by destruction of fires in Tandjilé Province.

According to the surveys carried out, the fodder deficit had three causes: flooding, the occupation of pasture areas by fields, the bush fires that farmers set to drive away rats and game and the fire set on the residues culture to voluntarily prevent breeders from having access to the residues.

"Residues were abundant in the past, but currently farmers collect and burn residues expressly to prevent our animals from consuming" hinted the agropastoralists during the focus group carried out in the ferrick of Milmil.

NDVI is an index to measure healthy green vegetation. Vegetation index are widely used on the one hand, to identify and monitor the dynamics of vegetation, but also to estimate certain biophysical parameters characteristic of plant cover, such as biomass.

Table 1 gave the descriptive statistic of the average NDVI value of the study area during the raining season months and the average annual value of the time series data.

The average NDVI varies to 0.63 in October to 0.89 in June. The month of October had the less NDVI value, this because of the flooding period impacting the pasture in august and September.

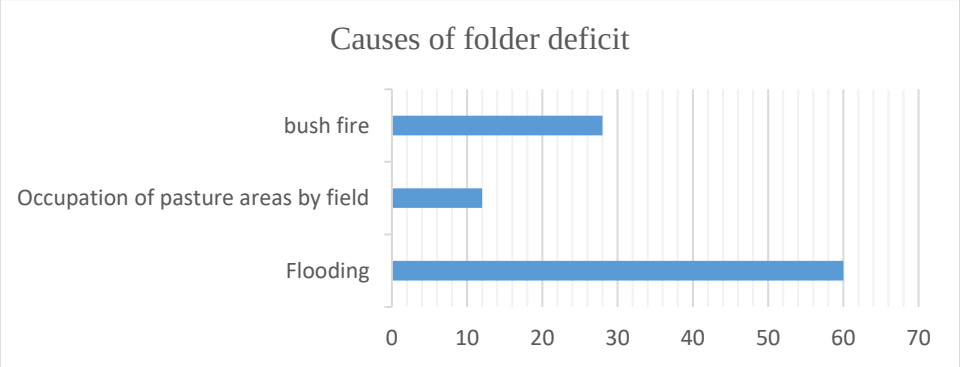



Figure: 2 causes of fodders deficit.

Table 1: Statistical parameters of NDVI.

| *Statistical parameters* | *May* | *June* | *July* | *August* | *September* | *October* | *Average* |
|---|---|---|---|---|---|---|---|
| Mean | 0.87 | 0.89 | 0.84 | 0.83 | 0.79 | 0.63 | 0.81 |
| Standard Error | 0.03 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.01 |
| Median | 0.92 | 0.91 | 0.84 | 0.84 | 0.77 | 0.62 | 0.81 |
| Standard Deviation | 0.13 | 0.05 | 0.04 | 0.06 | 0.09 | 0.11 | 0.03 |
| Sample Variance | 0.016 | 0.002 | 0.002 | 0.004 | 0.007 | 0.01 | 0.01 |
| Kurtosis | 3.17 | 2.13 | -1.18 | 4.90 | -0.37 | 0.56 | 0.25 |
| Skewness | -2.21 | -1.85 | -0.22 | -1.78 | -0.49 | 0.10 | -0.13 |
| Range | 0.37 | 0.15 | 0.14 | 0.26 | 0.31 | 0.43 | 0.14 |
| Minimum | 0.55 | 0.77 | 0.76 | 0.64 | 0.59 | 0.41 | 0.73 |
| Maximum | 0.92 | 0.92 | 0.9 | 0.9 | 0.9 | 0.83 | 0.88 |
| Count | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| Coefficient variation % | 14.83 | 5.40 | 5.40 | 7.15 | 11.09 | 16.83 | 4.18 |

Table 2: The Kurtosis and Skewness values show a normal distribution in the study area.

| *Parameters* | *Z score* | *P-value* | *Kendall Tau* | *Variance* | *Slope* | *Confidence level* | *Interpretation* | *Trend* |
|---|---|---|---|---|---|---|---|---|
| May | -1.59 | 0.11 | -0.22 | 795.53 | 0 | 0 0 | Ho accepted | No trend |
| June | -1.06 | 0.29 | -0.23 | 1938.03 | -0.001 | -0.0045 0.00 | Ho accepted | Decreasing |
| July | 2.023 | 0.04 | 0.32 | 1094.67 | 0.005 | 0.0001 0.007 | Ho rejected | Rising |
| August | 1.33 | 1.18 | 0.13 | 413.32 | 0.002 | -0.003 0.006 | Ho accepted | Rising |
| September | 2.30 | 0.02 | 0.37 | 1091.00 | 0.007 | 0.001 0.012 | Ho rejected | Rising |
| October | 2.72 | 0.007 | 0.43 | 1095.67 | 0.011 | 0.003 0.012 | Ho rejected | Rising |
| Annual | 0.63 | 0.53 | 0.10 | 1096.67 | 0.0007 | -0.002 0.004 | Ho rejected | Rising |

The annual value 0.63 for the Z score and 0.0007 for the Slope explains the positive trend (for the months of July, August, September and October). However, we observe deficits during certain periods, more particularly the month of June which shows a decreasing situation while the month of May shows a stable situation of the vegetation. The vegetation index for the month of May could be explained by the first rains recorded in the province.

***Land Use Classification from 1999 to 2020.***

Landsat satellites have the optimal ground resolution and spectral bands to efficiently track land use and to document land change due to climate change, urbanization, drought, wildfire, biomass changes (carbon assessments), and a host of other natural and human-caused changes. For this study we were focus on land use, urbanization and biomass. The images below show the different changing above 1999 to 2020.

The most pressure was observed in the decrease of vegetation in 1999 and 2020 (table 1) while crop farm pressure increase in 2020. These changes impacted negatively the transhumance mode.

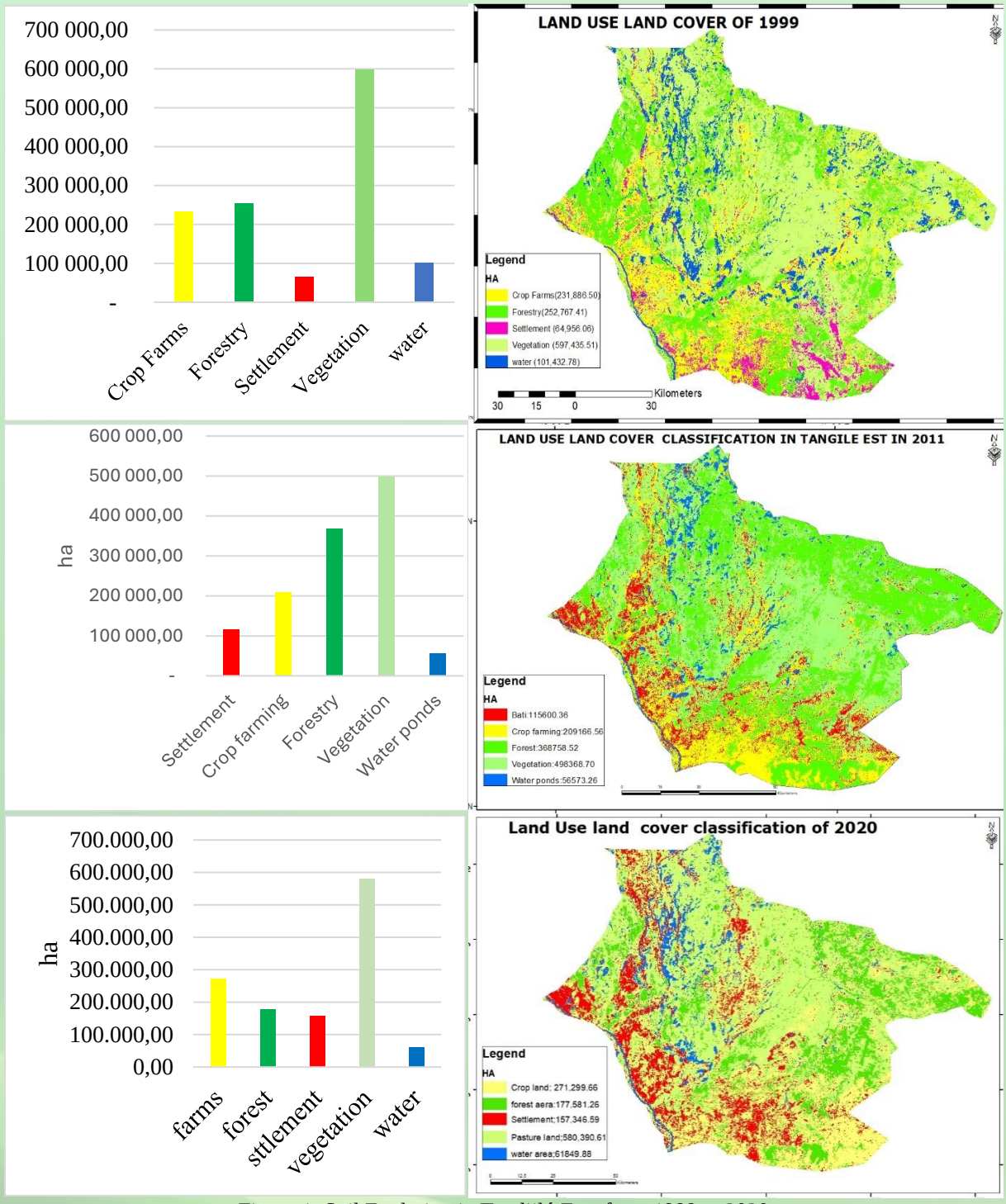



Figure 1: Soil Evolution in Tandjilé-East from 1999 to 2020

Table 1: Statistic of land use decade in Tandjilé-East

| *Designation* | *1999* | *2011* | *2020* | *Variation* |
|---|---|---|---|---|
| Crop Farms | 231 886,50 | 209 166,56 | 271 299,66 | 21.73 |
| Forestry | 252 767,41 | 368 758,52 | 177 581,26 | -14.22 |
| Settlement | 64 956,06 | 115 600,36 | 157 346,59 | 12.60 |
| Vegetation | 597 435,51 | 498 368,70 | 580 390,61 | -46.49 |
| Water | 101 423,78 | 56 573,26 | 61 849,88 | -4.95 |

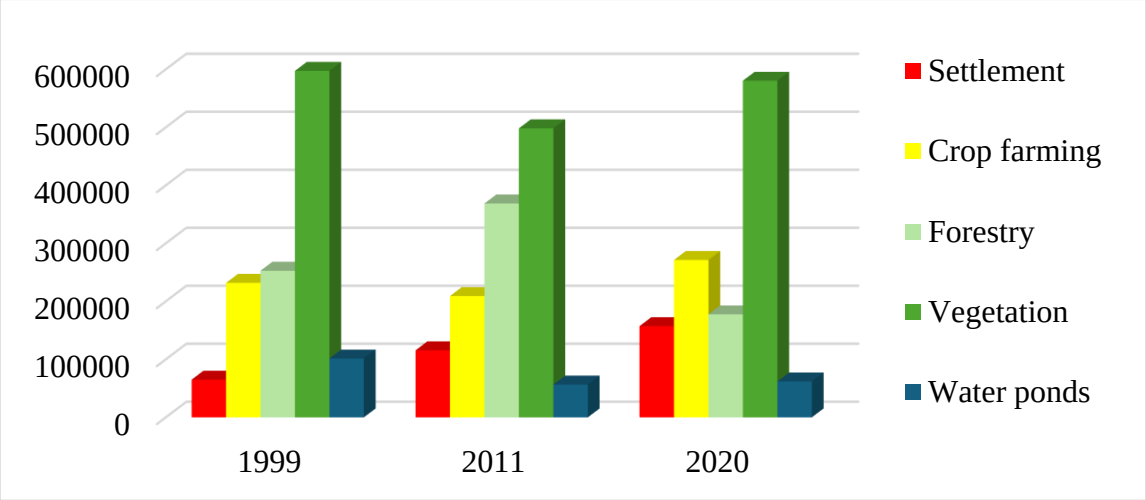



Figure 3: Proportion of occupancy over the last thirty years.

Landsat data from the province of Tandjilé made it possible to produce a land use map to examine the pressure on agro-pastoral resources in the study area. To this end, five entities were considered: settlement, fields, vegetation, forest and water.

In thirty years, we have noted an increase of 21.73% in cultivated areas, 12.60% in residential areas and a decrease of 4.95%, 14.22% and 46.49% in water, forest and vegetation.

**Discussion**

The soil pressure in Tandjilé province is reel. There was a land occupation of 90 000 ha from 1961 to 2009 against an occupation of 408 000 ha from 2009 to 2019. Between 2009, to 2019 we assist to the speed expansion of 82.92% of demographic pressure to the land. The results show a real demographic pressure on the soil. The report by Diogo *et al.* (2021) in Benin indicated that with the increase of mixed farming systems in many areas of the country, the use of pastoral lands has become more competitive. Akpan & Ebong, (2021), in Nigeria, revealed that agricultural land has continued to increase and currently averaged at 68.78%, indicating massive land expansion for use in farming. Furthermore, the findings showed that most arable crop outputs increase majorly from land expansion rather than land productivity. This situation cannot assure sustainable agricultural land use for food security in the near future. Besides, the country's agricultural land policies should focus on achieving land productivity and sustainable land-sharing strategies among major land users. The result show that the speed expansion of land uses in Tandjilé province is superior to the result obtained by Akpan and Ebong (2021) in Nigeria. The study of Hansen and Reenberg (1998), in Burkina has showed rather the fields expansion onto idle bush land. De Haan, (1998) clam that the reduction of grazing areas can be attributed to the expanding cotton cropping. According to Little & McPeak (2014), the loss of grazing land forces African herders to find new pastoral resources and increases the vulnerability of pastoralism. In Kétou (Benin), rangelands are contested by the sedentarization of herders and the yearly passage of many transhumant herds (Diogo *et al.* 2021).

Most interviewers (98 %) observed the forage deficit in Tandjilé province. The deficits sometime are caused by the farmers themselves who burn crop residues such as rice straw preventing the animals of the breeders from grazing in their fields. For Harouna *et al*., (2020) anthropogenic practices such as the collection of straw, which leads to a reduction in the edaphic stock of seeds, and the extension of crop fields to grazing areas, aggravate this degradation. These practices are the result of population growth and the decline in agricultural production. For Diogo *et al.*, (2021), the ecological processes leading to rangeland degradation are complex and cannot solely be attributed to cattle herding.

Most of transhumant herders and agropastoralists (88%) in Tandjilé Province have turned to aerial grazing, which is over-exploited during certain periods of the year between the months of February to May due to the low reconstitution of the herbaceous cover. Passage of the bush fire and the late arrival of the rainy season. This overexploitation of aerial pastures strongly affects the regeneration of certain species. *Faidherbia albida, Daniella oliveri, Zizyphus mucronata, Zizyphus mauritiana, Adansonia digitata* are highly threatened species in the area. This results confirm the study conducted by Dione et *al.*, (2020) in Senegal which showed that 82.14% of the woody species inventoried are considered fodder. These include *Adansonia digitata, Cordyla pinnata, Faidherbia albida, Ficus capensis, Ziziphus mauritiana* and *Sterculia setigera*. This observation shows the importance of the specific richness of the fields where all these species are found. Other respondents use these woody fodder for human food, pharmacopoeia, crafts, lumber, firewood, etc. This shows a strong pressure on these resources. According to Sarr *et al.*, (2018) trees are the main source of green aerial fodder in the Sahel in all seasons and are

found not only in agroforestry systems, but also in natural pastures. In pastoral areas, fodder supply during the dry season or in years of low rainfall is difficult with annual plants, and in these cases woody pasture becomes an important element.

Woody fodder species are much more exploited in the dry season when natural food resources become critical. This shows the importance of woody species in animal feed in the dry season. The survey results confirm those of many authors who noted the maximum use of aerial fodder by livestock during the dry season (Dione *et al.*, 2020; Bakaye, 2014), the dry season is the period during which woody plants are more exploited by agropastoralists. To access the different parts of the trees, breeders often practice cutting or pruning (Bakhoum, 2020). Diallo, (2019) in Mali confirm that the high demand for woody fodder crops leads to overexploitation of the most demanded. The consequence is the scarcity of these species in the harvesting areas. The results of the survey reveal the existence of overexploitation of woody fodder species which are consequently less and less abundant in the area. The overexploitation of species leads to the rarity or even the total disappearance of the species in the area.

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*ID: 926*

# Assessment of Tillering Behavior and Yield Traits in Traditional Rice Landraces and an Improved Variety for Germplasm Conservation

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## Abstract

An experiment was conducted with eight rice genotypes (Latishail, Jhingashail, Hashikalmi, Kalamanik, Shorishafuli, Lokkhibilash, Bamoni, and BRRI dhan92) to study their tillering behavior and its relationship to grain yield. The study was conducted under field conditions in a Randomized Complete Block Design (RCBD) with three replications, and all plots were managed using standard agronomic practices throughout the growing period. The genotypes showed clear differences in their growth and development. Significant variation was observed among the genotypes in tiller production, plant height, internode length, thousand-grain weight, phenological stages, grain characteristics, and leaf morphology. Among them, Latishail produced the highest thousand-grain weight, while BRRI dhan92 recorded the highest harvest index. In terms of maturity, Shorishafuli matured earlier than the other genotypes. Overall, the results show a wide range of variation among the genotypes, highlighting both the yield potential of improved varieties and the valuable traits present in traditional rice types, which are important for conservation and future breeding programs.

***Keywords:*** *Landraces, Tillering dynamics, yield, Rice genotypes, Germplasm conservation, Latishail*

*ID: 927*

# NPAS4, a typical bHLH-PAS transcriptional factor, and its engagement with 14-3-3 proteins beyond canonical recognition motifs

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## Abstract

**Background:** NPAS4 (Neuronal PAS domain containing protein 4) a member of the bHLH-PAS (basic helix-loop-helix-Per-ARNT-Sim) is well known as a transcriptional factor serves as master regulator of neural circuit homeostasis, memory formation, and synaptic plasticity. On the other side 14-3-3 are scaffolding protein in brain and binds with phosphorylated client proteins and modulate their localization and activity. Some studies had shown that interaction of 14-3-3 with some bHLH family members influences on their localization and activity. However, to date no any data had shown whether NPAS4 interacts with 14-3-3 proteins and this interaction would be new clue to understand pathways of neurodegeneration development involving Tau (Microtubule associated protein).

**Methods:** To address this, our study is aimed to set out to test whether 14-3-3 and NPAS4 actually interact. Using Scansite 4.0 and ELM for in silico prediction, we identified potential 14-3-3 binding sites within NPAS4. Strikingly, these predicted motifs fell inside intrinsically disordered regions (IDRs). As a first experimental step, we performed co-localization studies in neuronal cells using confocal microscopy.

**Results:** Our results revealed that fluorescently tagged NPAS4 and 14-3-3 co-localize in transfected N2a cells, hinting at a possible interaction. This leads us to propose that 14-3-3 proteins may control NPAS4's subcellular location and activity. To move forward, we plan to study this interaction in living neuronal cells using pull-down assays. Importantly, we are also trying to achieve for the first time full length NPAS4 expression in *Pichia pastoris* primarily data will be present.

***Keywords:*** *NPAS4, Interactions, Prediction, 14-3-3, bHLH-PAS*

*ID: 929*

# Pesticide Use and Associated Environmental and Human Health Risks in Cereal Production Systems

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## Abstract

Cereal production is exposed to biotic stress factors, including weeds, insect pests, and fungal diseases, leading to the intensive use of plant protection products. However, the extensive application of these pesticides raises concerns regarding their potential adverse effects on human health and ecosystem functioning. A field survey was conducted among 100 farmers to assess phytosanitary practices, knowledge levels, and risk perception.

The results revealed that 23 different active substances were used, with herbicides representing the dominant group. Several herbicides exhibited high leaching potential, as indicated by elevated GUS values, suggesting a significant risk of groundwater contamination. All insecticides identified in the study are neurotoxic and may adversely affect non-target organisms. Regarding human health, several active substances present acute and/or chronic toxic effects, and some are associated with potential carcinogenic or reproductive impacts. According to the WHO classification, most pesticides used fall within moderately the hazardous (Class II) and slightly hazardous (Class III) categories, with a limited number belonging to higher toxicity classes.

Furthermore, the study highlights unsafe phytosanitary practices, including limited use of personal protective equipment, poor hygiene behavior during pesticide application, and inadequate management of pesticide containers and residues. These practices increase occupational exposure and contribute to environmental contamination. Overall, the findings emphasize the need to improve pesticide management practices in cereal production systems to mitigate risks to human health and the environment.

***Keywords:*** *cereal production, pesticides, environmental risk, human health*

*ID: 930*

# Evaluation of the Bio-insecticidal Activity of *Myrtus comminus* Against *Tribolium castaneum*

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## Abstract

The use of synthetic insecticides to control insects of stored-product raises environmental and health concerns, driving the search for eco-friendly, plant-based alternatives. This study investigated the insecticidal and allelopathic properties of leaf powder from *Myrtus communis* against the red flour beetle, *Tribolium castaneum*. After collecting, drying, and grinding the aerial parts of *M. communis*, three doses (0.5, 1.0, and 1.5 g per 10 g of durum wheat) were tested under laboratory conditions. Key parameters included insect mortality, grain consumption, damage severity, nymphal deformities, and seed germination. The leaf powder of *M. communis* at the highest dose reduced consumption rates to 0.46% for adults and 0.89% for larvae after 10 days of exposure. Grain damage was significantly reduced at all tested doses for both insect stages, compared to the control. At a dose of 1 g of *M. communis*, adult and larval mortality reached 96.66% and 86.66%, respectively, after 30 days. *Myrtus communis* induced malformations in 11% of newly emerged adults. However, the powder did not affect grain germination, and 100% of the tested grains germinated after 4 days. These results suggest that leaf powder from *M. communis* is an effective biopesticide for protecting stored grains without affecting seed germination.

***Keywords:*** *Myrthus comminus, damage, toxicity, germination, leaf powder, malformation*

ID: 931

# Determination of the essential oil content of Lavandula X-Intermedia plant at different developmental stages in the Turkish National Botanical Garden

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## Abstract

As a significant perennial plant of the Lamiaceae family, lavender is widely cultivated for its essential oil, alongside polar constituents like polyphenols and coumarins. Literature indicates that its phytochemical composition is strictly dependent on geographical origin, genetic variety, climatic conditions, harvest schedule, phonological stage, and extraction technique. This study investigates the Lavandula x intermedia culture grown within our institution, evaluating the essential oil extractions obtained from biomass sampled during and after the flowering phases. This study examines the essential oil yield and chemical composition of lavender plants, both in fresh and dried forms, as well as during the flowering and pre-flowering periods. Isolation of the essential oils was performed via a 3-to-4-hour hydrodistillation process using a Clevenger-type apparatus. To evaluate the volatile components, lavender samples isolated through hydrodistillation were subjected to Gas Chromatography-Mass Spectrometry (GC-MS). Lavender essential oil yield increases in parallel with the increased flowering rate of the plant. The major components obtained from fresh and dried lavender were examined. When the GC-MS results of fresh, unbloomed lavender were examined, the main components were as follows: 27.85% endo-Borneol, 24.95% beta-fellander, 11.56% linalool, 6.03% krypton, 5.91% alpha-pinene, 5.78% D-limonene, 5.76% alpha-bisabolol, 3.86% camphor, 3.1% lavandulyl acetate, 2.66% linalyl acetate, and 2.56% myrcene. When GC-MS results were examined in dried, unbloomed lavender, the main components were as follows: 26.17% 1-borneol, 16.42% eucalyptol, 10.88% L-linalool, 9.64% beta-phellandrene, 8.30% krypton, 7.80% limonene, 6.61% trans-beta-ocimene, 4.73% (+)-2-bornanone, 2.62% 2-beta-pinene, 2.62% lavandulyl acetate, 2.32% linalyl acetate, and 1.91% delta 3-carotene.

***Keywords:*** *Lavandula x intermedia, hydrodistillation, essential oil*

**Türkiye Milli Botanik Bahçesi'nde Lavandula X-Intermedia bitkisinin farklı gelişim aşamalarındaki uçucu yağ içeriğinin belirlenmesi.**

## Özet

Lamiaceae familyasının değerli çok yıllık bir bitkisi olan lavanta, polifenoller ve kumarinler gibi polar bileşenlerinin yanı sıra uçucu yağı için yaygın olarak yetiştirilmektedir. Literatür, fitokimyasal bileşiminin coğrafi köken, genetik çeşitlilik, iklim koşulları, hasat zamanı, fenolojik aşama ve ekstraksiyon tekniğine sıkı sıkıya bağlı olduğunu göstermektedir. Bu çalışma, kurumumuzda yetiştirilen Lavandula x intermedia kültürünü inceleyerek, çiçeklenme evreleri sırasında ve sonrasında örneklenen biyokütleden elde edilen uçucu yağ ekstraksiyonlarını değerlendirmektedir. Bu çalışma, lavanta bitkilerinin hem taze hem de kurutulmuş formlarının yanı sıra çiçeklenme ve çiçeklenme öncesi dönemlerdeki uçucu yağ verimini ve kimyasal bileşimini incelemektedir. Uçucu yağların izolasyonu, Clevenger tipi bir cihaz kullanılarak 3-4 saatlik bir hidrod distillation işlemiyle gerçekleştirilmiştir. Uçucu bileşenleri değerlendirmek için, hidrod distillation yoluyla izole edilen lavanta örnekleri Gaz Kromatografisi-Kütle Spektrometrisi (GC-MS) ile analiz edilmiştir. Lavanta uçucu yağ verimi, bitkinin artan çiçeklenme hızıyla paralel olarak artmaktadır. Taze ve kurutulmuş lavantadan elde edilen başlıca bileşenler incelendi. Taze, henüz çiçek açmamış lavantanın GC-MS sonuçları incelendiğinde, ana bileşenler şu şekildeydi: %27,85 endo-Borneol, %24,95 beta-fellander, %11,56 linalool, %6,03 kripton, %5,91 alfa-pinen, %5,78 D-limonen, %5,76 alfa-bisabolol, %3,86 kafur, %3,1 lavandulil asetat, %2,66 linalil asetat ve %2,56 mirsen. Kurutulmuş, çiçek açmamış lavantada GC-MS sonuçları incelendiğinde, ana bileşenler şu şekildeydi: %26,17 1-borneol, %16,42 ökaliptol, %10,88 L-linalool, %9,64 beta-fellandren, %8,30 kripton, %7,80 limonen, %6,61 trans-beta-osimene, %4,73 (+)-2-bornanon, %2,62 2-beta-pinen, %2,62 lavandulil asetat, %2,32 linalil asetat ve %1,91 delta 3-karoten.

***Anahtar Kelimeler:*** *Lavandula x intermedia, hidroditilasyon, uçucu yağ, GC-MS*

*ID: 932*

# Comparison of Soxhlet and Cold Extraction Methods for Tasköprü (Kastamonu-Türkiye) Garlic Extracts against Colon Cancer Cells and Bone-Marrow Mesenchymal Stem Cells

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## Abstract

Garlic organosulfur compounds exhibit anticarcinogenic potential, but the extraction method strongly shapes the composition and bioactivity of the extracts. This study compared Soxhlet (hot) extraction with cold (ethanol maceration) extraction of white, geographically registered Taskopru garlic, evaluating anticancer activity against HCT116 human colon cancer cells and effects on healthy rat bone marrow mesenchymal stem cells (MSCs). For the Soxhlet method, sliced cloves were boiled for 1 h in 50% ethanol, and the extract was concentrated by evaporation. For the cold method, crushed cloves were macerated in absolute ethanol at room temperature for 3 days, then the solvent was removed by evaporation. The extracts' composition was characterized by ATR-FTIR (4000-400 cm-1) and LC-MS/MS. Cytotoxicity was assessed by the MTT assay in HCT116 (5000 cells/well) and MSCs (1000 cells/well) at 1-200 ppt for 1, 3, and 5 days, with cisplatin as a positive control; data were analyzed using Student's t-test in SPSS. LC-MS/MS confirmed organosulfur markers: the cold extract retained alliin and gamma-glutamyl-S-allyl-L-cysteine, while seventeen garlic-derived compounds were identified in the Soxhlet extract. FTIR of the Soxhlet extract revealed hydroxyl, aromatic C-H, ester, carbonyl, carboxylic O-H, and characteristic organosulfur S=O and primary-amine bands consistent with alliin, allicin, and diallyl disulfide. Both extracts reduced HCT116 viability in a dose- and time-dependent manner. The Soxhlet extract was more potent than the cold extract (IC50 about 60 versus 120 ppt), with the cold extract showing a transient day-3 attenuation followed by stronger day-5 cytotoxicity. In MSCs, both extracts were non-cytotoxic at low doses, but the Soxhlet extract was cytotoxic at high doses, whereas the cold extract generally promoted MSC viability. Extraction method markedly influenced potency and selectivity, indicating that high-dose hot extracts warrant caution.

***Keywords:*** *garlic extract, Soxhlet extraction, cold extraction, colon cancer, mesenchymal stem cells*

*ID: 933*

# Fizikokimyasal Su Kalitesi Değerlendirmesinin Mezenkimal Kök Hücreler Üzerinde In Vitro Sitotoksisite Testiyle Birleştirilmesi: Akçay Deresi Havzası (Kastamonu, Türkiye)

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## Abstract

Su kalitesi izlemesi çoğunlukla fizikokimyasal ölçümlere dayanmakta, suyun canlılar üzerindeki biyolojik etkileri ise göz ardı edilmektedir. Bu çalışmada, Akçay Deresi havzasının (Çatalzeytin, Kastamonu) bir yıllık fizikokimyasal incelemesi özgün bir in vitro sitotoksisite değerlendirmesiyle birleştirilmiştir. Mayıs 2020 – Nisan 2021 arasında altı istasyondan aylık su örnekleri alınmış; sıcaklık, pH, çözünmüş oksijen, KOİ, BOİ, iletkenlik, askıda katı madde, azot ve fosfor türevleri, başlıca iyonlar ve ağır metaller (Fe, Pb, Cu, Cd, Hg, Ni, Zn) dâhil yirmi altı parametre standart yöntemlerle belirlenmiş ve Yüzeysel Su Kalitesi Yönetmeliği'ne göre sınıflandırılmıştır. Sitotoksisite, MTT testi (ISO 10993-5) ile sıçan kemik iliği mezenkimal kök hücreleri üzerinde, steril edilmiş su yedi dozda (0,1–5 µl/ml) 1., 3. ve 7. günlerde uygulanarak değerlendirilmiş; %70 altı canlılık sitotoksik kabul edilmiştir. Parametrelerin çoğu I. Sınıf (yüksek) kaliteyi göstermiş, ağır metaller risk oluşturmamıştır. Hücre canlılığı mevsime, doza ve süreye bağlı olarak değişmiştir; sitotoksik yanıtlar başlıca sonbahar, kış ve yaz aylarında görülmüştür. En düşük canlılık 3-4-5. istasyonlarda ~%45–48 aralığında yaz ve sonbahar mevsimlerinde izlenmiş; ilkbaharda IC50'ye ulaşılmamıştır. Tarım, sanayi ve yerleşim alanlarına yakın istasyonlar daha güçlü etki gösterirken, kaynağa yakın istasyonlarda canlılık daha yüksek olmuştur. Bazı örneklerde canlılığın %110 üzerine çıkması ve hücre kümelerinin oluşması, ileri moleküler incelemeyi gerektiren olası proliferatif bir yanıta işaret etmektedir. Havza kimyasal standartlara büyük ölçüde uysa da, yalnızca kimyasal analizle saptanamayan doza ve mevsime bağlı biyolojik etkiler ortaya konmuş; fizikokimyasal izlemenin kök hücre sitotoksisite testiyle birleştirilmesinin risk değerlendirmesini güçlendirdiği ve periyodik biyoizlemeyi desteklediği gösterilmiştir.

***Keywords:*** *Su Kalitesi, Su Kirliliği, Sitotoksisite, Mezenkimal Kök Hücre, MTT testi*

*ID: 934*

# Waste As A Form Of Secondary Raw Material Trade: A Review On The Transforming Structure Of International Trade

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## Abstract

This study aims to examine the relationship between international trade and the environment within the framework of global waste trade and secondary raw materials. While the increase in trade volume during the globalization process has led to positive outcomes such as economic growth, increased income, and improved living standards, it has also given rise to new areas of debate regarding environmental impacts. In this context, the resource waste and waste accumulation caused by the traditional linear production and consumption model have increased the need for sustainable alternative approaches. The circular economy approach offers an important solution framework in this regard, focusing on more efficient use of resources and the reintegration of waste into production processes. Secondary raw materials, as a fundamental component of this approach, contribute to reducing dependence on natural resources and ensuring sustainability in production processes. On the other hand, the production and circulation of secondary raw materials are increasingly gaining an international dimension, increasing the cross-border movement of waste. Global waste trade is particularly noteworthy for the flow of waste from developed to developing countries; this process creates a significant imbalance between economic benefits and environmental and social costs. Low labor costs and more flexible environmental regulations make developing countries attractive for waste imports, but this can lead to the shift of environmental risks and health problems to these countries. In particular, the improper disposal of plastic and electronic waste exacerbates long-term environmental problems. Recent developments indicate significant changes in the structure of global waste trade. China's decision in 2017 to halt imports of plastic waste redirected waste flows and created new trade routes. This shift led to an increase in illegal waste dumping in some regions and made waste management problems more complex. This study evaluates the place of global waste trade within the international trading system, revealing the multifaceted relationship between economic efficiency and environmental sustainability.

***Keywords:*** *International Trade, Global Waste Trade, Secondary Raw Materials, Circular Economy, Environmental Sustainability*

## İkincil Hammadde Ticareti Olarak Atık: Uluslararası Ticaretin Dönüşen Yapısı Üzerine Bir Derleme

## Özet

Bu çalışma, uluslararası ticaret ile çevre arasındaki ilişkiyi, küresel atık ticareti ve ikincil hammaddeler çerçevesinde ele almayı amaçlamaktadır. Küreselleşme süreciyle birlikte ticaret hacminde yaşanan artış, ekonomik büyüme, gelir artışı ve refah düzeyinde iyileşme gibi olumlu sonuçlar doğururken, çevresel etkiler bakımından da yeni tartışma alanlarının ortaya çıkmasına neden olmuştur. Bu bağlamda, geleneksel doğrusal üretim ve tüketim modelinin yol açtığı kaynak israfı ve atık birikimi, sürdürülebilir alternatif yaklaşımlara olan ihtiyacı artırmıştır. Döngüsel ekonomi yaklaşımı, bu noktada, kaynakların daha verimli kullanılması ve atıkların yeniden üretim süreçlerine kazandırılması açısından önemli bir çözüm çerçevesi sunmaktadır. İkincil hammaddeler, söz konusu yaklaşımın temel bileşenlerinden biri olarak, doğal kaynaklara olan bağımlılığın azaltılmasına ve üretim süreçlerinde sürdürülebilirliğin sağlanmasına katkıda bulunmaktadır. Öte yandan, ikincil hammaddelerin üretimi ve dolaşımı giderek uluslararası bir boyut kazanmakta ve bu durum atıkların sınır ötesi hareketliliğini artırmaktadır. Küresel atık ticareti, özellikle gelişmiş ülkelerden gelişmekte olan ülkelere yönelen atık akışları ile dikkat çekmekte; bu süreç ekonomik faydalar ile çevresel ve sosyal maliyetler arasında önemli bir dengesizlik yaratmaktadır. Düşük iş gücü maliyetleri ve daha esnek çevresel düzenlemeler, gelişmekte olan ülkeleri atık ithalatı açısından cazip hale getirirken, bu durum çevresel risklerin ve sağlık sorunlarının bu ülkelere kaymasına yol açabilmektedir. Özellikle plastik ve elektronik atıkların uygunsuz bertarafı, uzun vadeli çevresel sorunları derinleştirmektedir. Son yıllarda yaşanan gelişmeler, küresel atık ticaretinin yapısında önemli değişimlere işaret etmektedir. Çin'in 2017 yılında plastik atık ithalatını durdurma kararı, atık akışlarının yönünü değiştirmiş ve yeni ticaret rotalarının oluşmasına neden olmuştur. Bu dönüşüm, bazı bölgelerde yasa dışı atık dökümünün artmasına ve atık yönetimi sorunlarının daha karmaşık hale gelmesine yol açmıştır. Bu çalışma, küresel atık ticaretinin uluslararası ticaret sistemi içerisindeki yerini değerlendirerek, ekonomik etkinlik ile çevresel sürdürülebilirlik arasındaki ilişkinin çok boyutlu yapısını ortaya koymaktadır.

***Anahtar kelimeler:*** *Uluslararası Ticaret, Küresel Atık Ticareti, İkincil Hammaddeler, Döngüsel Ekonomi, Çevresel Sürdürülebilirlik*

## Giriş

Ticaret ve çevre tartışması günümüzde tanıdık bir nitelik kazanmış bulunmaktadır. Bu tartışma, giderek serbestleşen uluslararası ticaret ile çevrenin korunması için gerekli koşulların uzlaştırılması gerekliliğine ve bu sürecin beraberinde getirdiği zorluklara işaret etmektedir. Tartışmanın kökeni, iki temel varsayım arasındaki görünürdeki çelişkiye dayanmaktadır. Bir görüşe göre, serbest uluslararası ticaret, çevresel koruma ve sosyal ilerlemenin sağlanabilmesi için bir ön koşul olarak değerlendirilmektedir. Bu yaklaşıma göre, serbest ticaretin temel ekonomik ilkesi olan karşılaştırmalı üstünlükler doktrini sayesinde elde edilen kazanımlar, sürdürülebilir kalkınmayı sağlamak için gerekli kaynakların ortaya çıkmasına olanak tanımaktadır. Bu çerçevede, uluslararası ticaretin çevresel etkilerinin daha somut biçimde gözlemlendiği alanlardan biri, kaynak kullanımı ve özellikle ikincil hammaddelerin ekonomik sistem içindeki rolüdür (Hoernig, 2022).

İkincil hammaddeler, geri dönüşüm teknolojilerinin malzemelerin üretim sürecine etkin bir şekilde yeniden entegre edilmesini sağladığı döngüsel ekonominin temel unsurlarından biri olarak kabul edilmektedir. Bu durum, çoğunlukla yenilenemeyen nitelikte olan birincil kaynaklara olan bağımlılığın azaltılması sonucunu doğurmaktadır. Ekonomik İşbirliği ve Kalkınma Örgütü'ne göre, döngüsel ekonomi ile ilişkilendirilen beş iş modelinden ikisi doğrudan ikincil hammaddeler ile ilişkilendirilmektedir. Bu modeller, yenilenebilir malzemelerin ve sürdürülebilir şekilde temin edilen biyolojik kaynakların kullanımını teşvik ederek, kullanım ömrünü tamamlamış ürünlerin ikincil hammaddelere dönüştürülmesini öngörmektedir. Bununla birlikte, ikincil hammaddelerin üretimi ve dolaşımı yalnızca yerel ölçekte değil, aynı zamanda uluslararası ticaret ağları aracılığıyla küresel ölçekte gerçekleşmekte ve bu durum atıkların sınır ötesi hareketliliğini gündeme getirmektedir (Lingaitiene & Burinskiene, 2024).

Küresel atık ticareti, atıkların bertarafı ve geri dönüşümü amacıyla, çoğunlukla zengin ülkelerden gelişmekte olan ülkelere aktarılması uygulamasını ifade etmektedir. Bu sistem, özellikle 1980'li ve 1990'lı yıllarda ekonomik teşviklerin etkisiyle önemli ölçüde gelişmiştir; zira gelişmekte olan ülkeler, daha esnek düzenlemeler ve düşük iş gücü maliyetleri nedeniyle daha ucuz bertaraf seçenekleri sunmaktadır. Bu atıkların önemli bir kısmını plastikler ve elektronik atıklar oluşturmaktadır ve bu atıklar çoğunlukla düzenli depolama alanlarına gönderilmekte ya da uygun olmayan şekilde bertaraf edilmekte, bu durum ise ciddi çevresel ve sağlık risklerine yol açmaktadır (Gregson & Crang, 2015). Başlangıçta hem ihracatçı hem de ithalatçı ülkeler açısından ekonomik olarak faydalı bir uygulama olarak değerlendirilmiş olsa da, küresel atık ticareti özellikle tehlikeli atıkların yükünü çoğunlukla daha yoksul ülkelerin üstlenmesi nedeniyle çevresel adalet ve halk sağlığı açısından süregelen tartışmalara neden olmaktadır. Küresel atık ticareti, değişen dinamiklerle birlikte zaman içerisinde dönüşüm geçirmiştir. Özellikle Çin'in 2017 yılında plastik atık ithalatını durduracağını açıklaması, yeni bertaraf alternatiflerinin aranmasına yol açmıştır. Bu durum, farklı bölgelerde yasa dışı atık dökümünün artmasına neden olarak atık yönetiminin karmaşıklığını daha da gözler önüne sermektedir (Kellenberg, 2015).

## Uluslararası Ticaret Ve Çevre

Uluslararası ticaret, kalkınmanın desteklenmesi, gelir ve istihdam oluşturulması ile yoksulluğun azaltılması açısından temel bir itici güç olarak değerlendirilmektedir. Daha dışa açık ekonomilerin çoğunlukla daha hızlı büyüme gösterdiği; rekabet güçlerinin ve yenilik kapasitelerinin arttığı, bununla birlikte daha nitelikli istihdam imkânları sunduğu belirtilmektedir. Araştırmalar, ticaretin özellikle düşük gelir düzeyine sahip ülkelerde gelir artışı üzerinde olumlu etkiler yarattığını ortaya koymakta; ihracat yapan işletmelerin ise verimlilik, çalışma ortamı ve iş gücü hakları bakımından daha yüksek performans sergilediğini göstermektedir (Khan vd., 2020).

Ticaret; üretkenliğin yükseltilmesi, gelir seviyelerinin artırılması ve istihdam olanaklarının genişletilmesi yoluyla yoksulluğun azaltılmasına katkı sağlamaktadır. Bunun yanı sıra, daha geniş ürün ve hizmet çeşitliliğine erişim imkânı sunması da bu süreci destekleyen önemli unsurlar arasında yer almaktadır. 1990–2017 yılları arasında, büyük ölçüde uluslararası ticaretin desteklediği ekonomik büyümenin etkisiyle, dünya genelinde aşırı yoksulluk içinde yaşayan nüfus oranında %75'lik bir azalma kaydedilmiştir (Sorroche-del-Rey vd., 2023).

Birleşmiş Milletler 2030 Gündemi çerçevesinde ticaret, kapsayıcı ve sürdürülebilir ekonomik büyümenin teşvik edilmesinde, yeni istihdam alanlarının oluşturulmasında, gelirlerin artırılmasında ve toplumsal refahın geliştirilmesinde kritik bir rol oynamaktadır. Ayrıca uluslararası ticaret, gümrük vergileri ve diğer mali araçlar aracılığıyla ulusal kaynakların harekete geçirilmesine katkıda bulunmakta; bu durum eğitim ve sosyal koruma gibi alanlarda yeniden dağıtım politikalarının uygulanmasını mümkün kılmaktadır. Bununla birlikte ticaret, daha çevreci ve sürdürülebilir ekonomik modellere geçiş sürecinde de önemli bir araç olarak öne çıkmaktadır (Bressan & Garcia, 2023)

## İkincil Hammaddeler Ve Döngüsel Ekonomi

İkincil hammaddeler ve döngüsel ekonomi kavramı, son yıllarda kaynak kullanımı ile üretim-tüketim ilişkilerine yönelik tartışmaların merkezinde yer almaktadır. Geleneksel doğrusal ekonomik modelde ("al–üret–

tüket–at"), kaynaklar üretim sürecine dahil edildikten sonra büyük ölçüde atık haline dönüşmekte ve ekonomik döngü dışında kalmaktadır. Buna karşılık döngüsel ekonomi yaklaşımı, malzemelerin sistem içerisinde mümkün olan en uzun süre tutulmasını ve kullanım ömrünü tamamlayan ürünlerin yeniden üretim süreçlerine entegre edilmesini temel almaktadır. Bu bağlamda ikincil hammaddeler, geri kazanılmış ve yeniden işlenmiş malzemeler olarak, ekonomik faaliyetlerin sürdürülebilirliğinin sağlanmasında kritik bir unsur olarak değerlendirilmektedir (Rotilio vd., 2022).

İkincil hammaddelerin kullanımının yaygınlaşması, bir yandan doğal kaynaklar üzerindeki baskının azaltılmasına katkı sağlarken, diğer yandan üretim süreçlerinde maliyet etkinliği yaratabilmektedir. Özellikle metal, plastik ve kâğıt gibi geri dönüştürülebilir malzemeler, uygun teknolojik süreçler aracılığıyla yeniden işlenerek ekonomik değeri olan üretim girdilerine dönüştürülebilmektedir. Bu durum, çevresel kazanımların ötesinde, yeni istihdam alanlarının ortaya çıkmasına ve atık yönetimi sektörünün gelişmesine de katkı sunmaktadır. Bununla birlikte, söz konusu sürecin etkinliği büyük ölçüde geri dönüşüm altyapısının gelişmişlik düzeyine ve uygulanan politika araçlarının bütüncüllüğüne bağlıdır (Hoernig, 2022).

Öte yandan, ikincil hammaddelerin üretimi ve dolaşımı giderek uluslararası bir nitelik kazanmaktadır. Ülkeler arasındaki maliyet farklılıkları, düzenleyici çerçevelerdeki çeşitlilik ve teknolojik kapasite düzeylerindeki eşitsizlikler, bu malzemelerin sınır ötesi hareketliliğini teşvik etmektedir. Bu gelişme, döngüsel ekonomi hedeflerinin küresel ölçekte nasıl hayata geçirileceği sorusunu da gündeme getirmektedir. Dolayısıyla ikincil hammaddeler, yalnızca çevresel bir mesele olarak değil, aynı zamanda uluslararası ticaretin dönüşen yapısı içerisinde ele alınması gereken çok boyutlu bir olgu olarak değerlendirilmektedir (Scarano, 2022).

## Küresel Atık Ticareti

Son on yıllık süreç, artan küresel tüketim düzeyi ve etkin atık yönetimine duyulan gereksinimin etkisiyle küresel atık ticaretinde benzeri görülmemiş bir genişlemeye tanıklık etmektedir. Dünya nüfusunun sürekli artış göstermesi, buna paralel olarak tüketim miktarlarının ve ortaya çıkan atık hacminin yükselmesine neden olmaktadır. Ürünlerin menşeine bakılmaksızın daha fazlasını talep etmeye dayalı kesintisiz tüketim eğilimleri, üretimin artmasına ve dolayısıyla daha fazla atık oluşmasına yol açmaktadır. Bu süreklilik arz eden döngü, ürünlerin ve atıkların küresel ölçekte dolaşımını beraberinde getirmiş; küreselleşme süreci ise atık hareketliliğini, yerel ürün dolaşımı kadar önemli bir unsur haline dönüştürmektedir (Liu vd., 2022).

Atık materyallerin uluslararası ticareti, son on yılda hızla büyüyen bir alan olarak öne çıkmaktadır. Bu süreç, ülkelerin atıklarını kendi sınırları içerisinde işleyebilmelerine veya bu işlemleri gerçekleştirme kapasitesine sahip diğer ülkelere gönderebilmelerine imkân sağlamaktadır. Bu yönüyle atık ticareti, küresel ekonomi içinde önemli bir bileşen haline gelmektedir. Söz konusu büyüme; atık yönetimi çözümlerine yönelik artan ihtiyaç, atık akışlarının düzenlenmesi gerekliliği, çevrenin korunması ve atık yönetimi sorumluluklarının daha dengeli biçimde paylaşılmasının sağlanması gibi unsurlar tarafından desteklenmektedir (Martínez vd., 2022).

## Tartışma Ve Sonuç

Uluslararası ticaretin ekonomik büyüme, gelir artışı ve refah üzerindeki olumlu etkileri geniş ölçüde kabul görmektedir. Bununla birlikte, ticaretin çevresel boyutu, özellikle atık üretimi ve yönetimi söz konusu olduğunda, giderek daha fazla önem kazanmaktadır. Bu çalışma, ikincil hammaddelerin ve döngüsel ekonomi yaklaşımının, söz konusu gerilimi azaltma potansiyeline sahip olduğunu ortaya koymaktadır. Atıkların yeniden ekonomik değere dönüştürülmesi, hem kaynak verimliliğini artırmakta hem de çevresel baskıları hafifletmektedir.

Ancak küresel atık ticaretinin mevcut yapısı, ekonomik faydaların ve çevresel maliyetlerin eşit olmayan bir şekilde dağıldığını göstermektedir. Özellikle gelişmekte olan ülkelerin, düşük maliyet avantajları nedeniyle atık ithalatında yoğunlaşması, çevresel adalet ve halk sağlığı açısından önemli sorunlar doğurmaktadır. Son yıllarda yaşanan politika değişiklikleri ve ticaret akışlarındaki dönüşüm, atık yönetiminin küresel ölçekte daha bütüncül ve dengeli bir yaklaşımla ele alınması gerektiğini ortaya koymaktadır.

Bu çerçevede, uluslararası ticaret politikalarının çevresel sürdürülebilirlik ilkeleriyle uyumlu hale getirilmesi, döngüsel ekonomi uygulamalarının yaygınlaştırılması ve atık ticaretine ilişkin düzenleyici mekanizmaların güçlendirilmesi büyük önem taşımaktadır. Aksi takdirde, ekonomik kazanımların çevresel maliyetleri gölgelemesi kaçınılmaz hale gelecektir.

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*ID: 935*

# Colour as Nutrition: Profiling Anthocyanin and Antioxidant Capacity in Pigmented Wheat, Rice, and Maize Landraces

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## Abstract

The relationship between pericarp coloration and nutritional quality in cereal grains has gained substantial scientific attention, yet systematic quantitative assessments across pigmented landraces remain fragmented in the literature. The present study synthesizes peer reviewed studies from 2010 to 2025 examining anthocyanin composition, total phenolic content, and antioxidant capacity in pigmented wheat, rice, and maize landraces relative to conventional non pigmented genotypes. Quantitative synthesis demonstrates that pigmented wheat landraces accumulate total anthocyanins ranging from 30 to 210 micrograms per gram of dry weight, with cyanidin 3 O glucoside and peonidin 3 O glucoside as the predominant molecular species. Purple and black rice landraces exhibit the highest concentrations among all cereals examined, ranging from 350 to 1,200 micrograms per gram, representing a 10 to 40 fold increase relative to milled white rice. Blue and purple maize genotypes contain 100 to 600 micrograms per gram, with acylated anthocyanin derivatives conferring significantly enhanced thermal stability compared to non acylated analogues. Total phenolic content follows an identical rank order, with pigmented varieties demonstrating two to five fold higher values than non-pigmented controls. Antioxidant capacity exhibits strong positive correlation with total anthocyanin concentration across all three cereal types, with pigmented rice achieving DPPH radical scavenging IC50 values of 0.8 milligrams per millilitre compared to 4.5 milligrams per millilitre for white rice. The aim of this review is to consolidate current quantitative evidence on anthocyanin profiles and antioxidant functionality in pigmented wheat, rice, and maize landraces and to establish colour based screening parameters for bio-fortification breeding programs targeting enhanced nutritional outcomes in staple cereal crops.

***Keywords:*** *Anthocyanins; antioxidant capacity; bio-fortification; coloured grains; landraces; maize; pigmented cereals; rice; wheat*

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# Agricultural Plastic Mulch Films: Sources, Fragmentation, and Microplastic Contamination in Crop Soils

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## Abstract

Agricultural plastic mulch films have become an integral component of modern intensive cropping systems worldwide, yet their contribution to soil microplastic pollution remains insufficiently characterized. Global annual consumption of agricultural plastic mulch exceeds 2 million metric tonnes, with China, the Mediterranean basin including Turkey, and the United States representing the largest application zones. Field degradation studies demonstrate that conventional low density polyethylene mulch films undergo embrittlement and fracturing within 120 to 180 days of field exposure, driven by ultraviolet radiation, thermal cycling, mechanical abrasion, and microbial colonization. Analytical intercomparison reveals that microplastic concentrations in agricultural surface soils range from 50 to 950 particles per kilogram of dry soil under short term mulch use, while long term application exceeding ten consecutive seasons yields concentrations between 800 and 4,500 particles per kilogram. The predominant morphotypes identified are fragments accounting for 65% to 85% of total particles. Polymer characterization confirms polyethylene as the dominant constituent, comprising 70% to 90% of recovered plastics. Soil texture and tillage intensity significantly influence vertical migration, with microplastics detected at depths exceeding 30 centimetres in conventionally tilled fields. Emerging evidence indicates that microplastic contaminated soils exhibit altered bulk density, water holding capacity, and aggregate stability, with potential implications for crop productivity. Research gaps persist regarding long term accumulation rates, transport pathways to groundwater, and uptake of nanoscale plastic particles by edible crop tissues. The aim of this review is to synthesize current scientific knowledge on the sources, fragmentation behaviour, and microplastic contamination levels associated with agricultural plastic mulch films and to identify critical research priorities for assessing soil health risks.

***Keywords:*** *Agricultural soils; fragmentation; low density polyethylene; microplastic contamination; mulch films; soil pollution*

*ID: 937*

# Upcycling Agricultural By Products: Developing Functional Plant Based Proteins from Oilseed Waste

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## Abstract

The present review aims to critically evaluate the potential of oilseed processing residues, including press cakes and meals derived from sunflower, sesame, canola, flax, and rapeseed, as sustainable feedstocks for the development of functional plant based protein ingredients for human consumption. A systematic synthesis of peer reviewed literature from 2015 to 2025 was conducted, focusing on four key domains: chemical composition of defatted oilseed meals, protein extraction efficiencies across alkaline, enzymatic, and salt extraction methods, functional properties of isolated protein concentrates and hydrolysates, and nutritional quality assessed by amino acid scoring and in vitro digestibility. Comparative analysis revealed that defatted meals contain crude protein ranging from 30% to 55% by dry weight, with sesame and sunflower meals exhibiting the highest values at 42.7% and 38.4% respectively. Alkaline solubilisation followed by isoelectric precipitation yielded optimal extraction efficiencies between 54% and 68% across all feedstocks. Functional characterization demonstrated that oilseed protein isolates possess water holding capacities of 2.9 to 3.6 grams per gram of protein, oil holding capacities of 2.4 to 3.1 grams per gram, and foaming capacities exceeding 200% for sesame and flax isolates, values comparable or superior to commercial soy and pea protein benchmarks. Amino acid profiling identified sulphur containing amino acids as the primary limiting factor, while lysine, leucine, and arginine scores met or exceeded FAO reference patterns. In vitro protein digestibility corrected amino acid scores ranged from 0.70 to 0.79 across isolates. The evidence supports that oilseed waste streams can be effectively upcycled into high value functional protein ingredients for meat analogues, dairy alternatives, and baked goods. However, standardization of extraction protocols, mitigation of anti-nutritional factors including phytates and tannins, and sensory optimization remain critical barriers requiring further research prior to industrial scale adoption.

***Keywords:*** *Agricultural by products; alkaline extraction; amino acid profiling; functional properties; oilseed waste; plant based protein; protein digestibility; upcycling*

*ID: 939*

# Nutritional criteria and the examination of certain nutrient factors from the perspective of food biochemistry

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## Abstract

**1.Background**: Nutrition is the most important set of vital activities determined by economic, social, cultural, and environmental factors, as well as the ecological and environmental conditions in which societies operate. The goal of nutrition is not just to fill your stomach. In a sense, this means ensuring balanced nutrition, not just adequate nutrition, by including all the nutrients the body needs in the diet.

Nutritional criteria require not only adequate and balanced nutrition, but also the consumption of food that is safe and hygienic. Primarily, economic conditions and climate change due to global warming, mass migrations, and environmental pollution are factors that affect healthy eating criteria.

**2. Methods:** Individual factors influencing nutritional criteria should be considered, including specific medical conditions, age, gender, pregnancy, lactation, active or sedentary lifestyle, etc. Nowadays, excessive calorie intake is influenced by the consumption of processed foods; For example, the increase in obesity, insufficient intake of carbohydrates, fats, proteins and all nutrients; marasmus, insufficient protein intake, especially the decrease in the consumption of animal-derived protein due to economic conditions and the resulting diseases such as kwashiorkor are the most significant and widespread nutrition-related health disorders. In addition, diabetes, celiac disease, iron deficiency, and other diseases caused by inadequate or insufficient intake of certain nutrients include anemias, pellagra, scurvy, phenylketonuria (PKU), beriberi, night blindness, retinitis pigmentosa, rickets, osteoporosis, and osteomalacia.

**3. Result**: A balanced and varied diet, and even traditional dietary habits in our country, could be considered more beneficial than fast food diets in terms of balanced nutrition.

**4. Conclusion**: Incorporating simple and easy rules into daily dietary criteria, such as a varied diet rather than one-sided ones, and consuming fiber-rich foods and whole-wheat bread instead of excessive calories and sugar, will be a first step in protecting against these diseases. These topics will be briefly summarized in the compilation.

***Keywords:*** *Nutrition -1, food biocehemistry-2, Marasmus, -3, Lack of Nutrients -4, Obesity-5, Balanced diet-6*

ID: 940

# Plant Secondary Metabolites as Household Insecticides: A Critical Review of Efficacy, Toxicology, and Regulatory Pathways

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## Abstract

For centuries, plants have supplied secondary metabolites that protect them from herbivores, and many of these same compounds – alkaloids, terpenoids, flavonoids, phenolics, saponins, and sulphur-containing molecules – have found their way into household insecticide products. The public often assumes that "natural" equals "safe", but a growing body of toxicological evidence challenges that assumption. In this review we critically evaluate the current state of knowledge (2018–2026) regarding the use of plant secondary metabolites against common domestic arthropods: houseflies (*Musca domestica*), German and American cockroaches (*Blattella germanica*, *Periplaneta americana*), mosquitoes (*Aedes aegypti*, *Anopheles* spp., *Culex* spp.), stored-product beetles (*Tribolium castaneum*, *Sitophilus oryzae*), and household ants and termites. We systematically summarise efficacy data – lethal doses, mortality percentages, and concentration-response relationships – extracted from peer-reviewed studies and official reports. Mechanistically, we discuss how these compounds interfere with acetylcholinesterase (AChE), voltage-gated sodium channels, octopamine receptors, $Na^+/K^+$-ATPase, ecdysone signalling, and feeding or oviposition behaviour. A major portion of the review is dedicated to mammalian toxicology: acute, subchronic, reproductive, developmental, and genotoxicity data are examined in detail, with reference to WHO hazard classifications and EPA toxicity categories. We show that while some natural insecticides (e.g., azadirachtin, certain monoterpenes) have a favourable safety margin, others (rotenone, nicotine, and even pyrethrins at high doses) carry risks that are often underestimated. The review also addresses formulation challenges – volatility, photodegradation, poor water solubility – and critically appraises modern solutions such as nano-encapsulation, solid lipid nanoparticles, and biodegradable polymer carriers (including poly(ethylene disulfide) composites derived from the authors' own work). Global regulatory frameworks are compared: US EPA biopesticide registration (40 CFR Part 158, with the 2025 streamlining for low-risk products), the WHO/FAO guidelines for household insecticide use, the European Union's Biocidal Products Regulation (EU 528/2012), and ongoing OECD harmonisation efforts. Finally, we place natural insecticides within the context of integrated pest management (IPM) and discuss resistance management strategies, future research needs (high-throughput screening, synthetic biology, smart delivery systems), and the critical role of post-market surveillance. This review aims to provide a balanced, evidence-based resource for researchers, formulators, regulators, and public health professionals.

***Keywords:*** *Plant secondary metabolites, household insecticides, essential oils, mode of action, insecticide toxicology, EPA registration, WHO guidelines, nano-encapsulation, polymer carriers, integrated pest management*

## 1. Introduction

Anyone who has tried to swat a housefly or chased a cockroach across the kitchen floor knows that household pests are more than a nuisance. They transmit pathogens – flies carry *Salmonella* and *E. coli*, cockroaches spread allergens that trigger asthma in children, and mosquitoes infect hundreds of millions with dengue, malaria, and Zika virus each year [1,2]. The response, for most families, has been to reach for an aerosol can or plug in a vaporiser. For decades those products have been dominated by synthetic pyrethroids, organophosphates, and carbamates – compounds that kill insects efficiently but also leave residues, contaminate indoor air, and, in the case of organophosphates, have been linked to neurodevelopmental deficits in children [3]. Widespread resistance has made many of these once-reliable products ineffective; houseflies in some regions now carry multiple resistance genes that render pyrethrins nearly useless [4].

In this context, plant-derived insecticides have enjoyed a renaissance. They are perceived as green, biodegradable, and safe. Walk down the pest-control aisle of any supermarket and you will find sprays labelled "natural", "botanical", or "essential oil-based". But the underlying science is more nuanced. A plant produces secondary metabolites not for our convenience but for its own survival – to poison, repel, or otherwise discourage herbivores. It should not surprise us that some of those same metabolites can also be toxic to mammals if misused. Indeed, the literature contains sobering reports: rotenone, once a popular natural insecticide, is a suspected carcinogen and has been epidemiologically linked to Parkinson's disease [5]; nicotine, another natural alkaloid, is classified by WHO as "highly hazardous"; and even pyrethrins, when formulated with synthetic synergists, can cause respiratory irritation and allergic reactions [6].

The central argument of this review is therefore that **naturalness does not guarantee safety**. Each candidate botanical insecticide must be evaluated on its own merits, with rigorous attention to efficacy, selectivity, toxicokinetics, and real-world exposure scenarios. At the same time, we should not dismiss the genuine opportunities that plant secondary metabolites offer. When correctly formulated – and especially when delivered using modern encapsulation technologies – they can provide effective household pest control with a much smaller environmental footprint than many synthetics.

The authors of this paper have spent years working on natural products, polymer science, and environmental safety. Our earlier work includes systematic reviews on the safety of toxic elements in medicinal plants [7], studies on polymeric carriers for bioactive compounds [8,9], and assessments of nanoparticle applications in agriculture and environmental remediation [10]. We bring that experience to this review, but we have been careful to cite our own papers only where they directly inform the discussion – primarily in the sections on formulation and safety evaluation.

In the pages that follow, we first describe the chemical diversity of insecticidal secondary metabolites (Section 2). Then we synthesise efficacy data against specific household pests (Section 3). We then delve into the molecular mechanisms that explain how these compounds kill or repel insects (Section 4). Next comes the critical section on mammalian toxicology (Section 5), followed by a discussion of formulation technologies, with emphasis on nano-encapsulation and polymer carriers (Section 6). We summarise the regulatory landscape in the US, EU, and under WHO auspices (Section 7). Finally, we discuss how natural insecticides fit into integrated pest management, highlight emerging research directions, and offer a balanced conclusion (Sections 8–10).

## 2. Chemistry of Insecticidal Secondary Metabolites

Plants produce tens of thousands of secondary metabolites, but only a few hundred have been tested for insecticidal activity against household pests. The most promising fall into five major chemical classes.

### 2.1. Terpenoids (essential oils)

Terpenoids are built from isoprene ($C_5$) units. Monoterpenes ($C_{10}$) are volatile and responsible for the characteristic smells of herbs and spices. They include thymol (thyme), carvacrol (oregano), eucalyptol (eucalyptus), menthol (mint), limonene (citrus), and α-pinene (pine). Sesquiterpenes ($C_{15}$) such as caryophyllene and farnesene are also active. The triterpenoid azadirachtin ($C_{35}$) from neem is structurally more complex and acts primarily as an insect growth regulator. Essential oils are complex mixtures, and the total activity is often greater than the sum of the parts – synergy is common [11,12].

### 2.2. Alkaloids

These nitrogen-containing heterocycles are potent neurotoxins. Nicotine from tobacco, anabasine from tree tobacco, and the fungal alkaloid heteratisine from *Cordyceps javanica* all inhibit acetylcholinesterase, much like carbamate insecticides. The toxicity of alkaloids to mammals is often high, which limits their use in household products, but they remain important as research tools and as historical insecticides [13].

### 2.3. Flavonoids and phenolic compounds

Flavonoids (quercetin, kaempferol, catechin) and simple phenolics (eugenol, cinnamic acid, gallic acid) are widely distributed. Their insecticidal mechanisms include inhibition of digestive enzymes (α-amylase, protease), disruption of oxidative phosphorylation, and antifeedant effects. Eugenol from clove oil is a common ingredient in household ant and roach sprays. Although phenolic compounds are generally less acutely toxic to mammals than alkaloids, they can be skin sensitisers [14].

### 2.4. Saponins

Saponins are glycosides with surfactant properties. They disrupt insect midgut cell membranes, leading to cytolysis and death. Saponin-rich plants such as *Quillaja saponaria* (soapbark) and *Sapindus mukorossi* (soapnut) have shown larvicidal activity against mosquitoes and are used in some "green" insecticidal soaps [15].

### 2.5. Sulphur-containing compounds

The breakdown products of glucosinolates (isothiocyanates) and allicin from garlic are volatile sulphur compounds with strong fumigant activity. Diallyl disulfide and dimethyl trisulfide are the major active components of garlic oil; they are effective against stored-product beetles and cockroaches at very low concentrations. Their pungent odour, however, makes them less attractive for indoor use unless microencapsulated [16].

**Table 1** lists representative compounds, source plants, target pests, and reported $LD_{50}$ values. The data show that while natural compounds are generally less potent than synthetic pyrethroids on a weight-for-weight basis, their multicomponent nature often compensates through synergy and multiple mechanisms of action.

## 3. Efficacy Against Key Household Pests

### 3.1. Houseflies (*Musca domestica*)

The housefly is a mechanical vector of at least 65 human pathogens. A recent study from northern India (2024) evaluated six essential oils – neem, lemon, eucalyptus, laurel, mint, and tulsi – against adult houseflies using a contact toxicity bioassay. At 100% concentration, the combined formulation killed 98% of flies within 24 hours, which was not significantly different from the positive control dichlorvos. Gas chromatography-mass spectrometry identified eucalyptol (28%), undecane (12%), tetradecane (10%), estragole (8%), and caryophyllene (6%) as the

major active components [17]. Thymol, the main constituent of thyme oil, has been studied more systematically; its topical $LD_{50}$ for *M. domestica* is approximately 12.5 µg/fly [18]. The high volatility of thymol remains a problem, but as we discuss later, encapsulation can extend residual activity.

Table 1. Major secondary metabolite classes with household insecticidal activity

| *Class* | *Example compound(s)* | *Plant source* | *Target household pest* | *Reported $LD_{50}$ (µg/insect or ppm)* | *Reference(s)* |
|---|---|---|---|---|---|
| Monoterpenoid | Thymol | *Thymus vulgaris* | *Musca domestica* (adult) | 12.5 µg/fly (topical) | Gutiérrez et al., 2020 |
| Monoterpenoid | Eucalyptol (1,8-cineole) | *Eucalyptus globulus* | *Blattella germanica* (nymph) | 8.2 µL/L air (fumigation) | Kaur et al., 2025 |
| Monoterpenoid | Carvacrol | *Origanum vulgare* | *Aedes aegypti* (larva) | $LC_{50}$ = 28 ppm | Rodrigues et al., 2023 |
| Monoterpenoid | Eugenol | *Syzygium aromaticum* | *Blattella germanica* (adult) | 0.6 mg/cm² (repellent $ED_{50}$) | Sharififar et al., 2021 |
| Monoterpenoid | Cinnamaldehyde | *Cinnamomum verum* | *Sitophilus oryzae* (adult) | 0.03 µL/cm² (contact) | Lee et al., 2018 |
| Sesquiterpene | Caryophyllene | *Syzygium aromaticum* | *Tribolium castaneum* (adult) | 0.15 µL/cm² (contact) | Prates et al., 2022 |
| Triterpenoid | Azadirachtin | *Azadirachta indica* | *Periplaneta americana* | 0.5 µg/g (antifeedant $ED_{50}$) | Chaudhary et al., 2020 |
| Alkaloid | Nicotine | *Nicotiana tabacum* | *Myzus persicae* (aphid) | 0.05 mg/mL (spray) | Tomizawa & Casida, 2019 |
| Alkaloid | Heteratisine | *Cordyceps javanica* (fungus) | *Musca domestica* | AChE $IC_{50}$ = 4.2 µM | Zhang et al., 2024 |
| Flavonoid | Quercetin | Various plants | *Spodoptera litura* (model) | 500 ppm (antifeedant) | Vinay et al., 2019 |
| Saponin | Quillaja saponin | *Quillaja saponaria* | *Culex quinquefasciatus* (larva) | $LC_{50}$ = 15 ppm | Pelah et al., 2019 |
| Sulfur compound | Diallyl disulfide | *Allium sativum* | *Tribolium castaneum* (adult) | 0.01 µL/L air (fumigation $LC_{50}$) | Yang et al., 2025 |

### 3.2. German and American cockroaches (*Blattella germanica, Periplaneta americana*)

Cockroaches are notoriously difficult to control because they hide in cracks and quickly develop behavioural aversion to toxic baits. Fumigation with plant volatiles offers an alternative. In 2025, researchers in Brazil showed that eucalyptol (1,8-cineole) at 8.2 µL/L of air killed 100% of *B. germanica* nymphs within 24 hours in a 10-L desiccator [19]. Eugenol (clove oil) is more effective as a repellent than as a contact toxicant; a concentration of 0.6 mg/cm² repelled 90% of cockroaches for 48 hours [20]. Neem-based baits containing azadirachtin (0.5%) have been field-tested in multi-unit housing; after 3 months, cockroach populations declined by 85%, and the effect persisted with low re-infestation [21]. Azadirachtin acts slowly because it disrupts moulting, but this slow action is actually an advantage for bait formulations: the insects do not learn to avoid the bait.

### 3.3. Mosquitoes (*Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus*)

Mosquito control is where botanical insecticides have seen the most research, driven by the urgency of vector-borne diseases. Larvicidal activity is especially well documented. Carvacrol and thymol have $LC_{50}$ values of 28 ppm and 35 ppm, respectively, against third-instar *A. aegypti* larvae [22]. Saponins from *Quillaja saponaria* are even more active ($LC_{50}$ = 15 ppm) and act by disrupting the larval gut epithelium [15]. For adult mosquitoes, spatial repellents are more practical than contact killers. A 5% eucalyptus oil formulation (mainly 1,8-cineole) provided 4 hours of complete protection against *An. stephensi* in arm-in-cage assays, comparable to DEET at the same concentration [23]. The volatile nature of essential oils means that protection times are shorter than those of synthetic repellents, but repeated application is often acceptable for evening use.

### 3.4. Stored-product pests (*Tribolium castaneum, Sitophilus oryzae*)

Pantry pests are a common household annoyance. Fumigation is the method of choice because it penetrates packaging and kills all life stages. Diallyl disulfide, the major volatile from garlic oil, has an $LC_{50}$ of 0.01 µL/L air against adult *T. castaneum* and achieves 100% mortality within 24 hours at 0.05 µL/L [16]. Cinnamaldehyde (cinnamon oil) is also highly active; its $LC_{50}$ against *S. oryzae* is 0.03 µL/cm² in contact assays [24]. Both compounds have low mammalian inhalation toxicity, but their strong odours limit consumer acceptance. Encapsulation in chitosan or cyclodextrin can mask the smell while preserving activity.

### 3.5. Ants and termites

Argentine ants (*Linepithema humile*) and subterranean termites (*Reticulitermes flavipes*) invade homes in large numbers. Nootkatone, a sesquiterpene from grapefruit and阿拉斯加黄柏, has shown promise. A 2022 study reported a contact $LD_{50}$ of 0.5 µg per ant, and nootkatone-laced baits reduced ant foraging by >90% in field trials [25]. For termites, vetiver oil (containing vetiverol and vetivone) is both repellent and toxic; a 1% concentration prevented termite penetration through treated sand in a two-choice test [26].

**Table 2** compares $LD_{50}$ values of selected natural compounds with those of synthetic pyrethrins and permethrin. While the synthetic compounds are generally one to two orders of magnitude more potent, the gap narrows considerably when natural compounds are formulated as nano-emulsions or when they are used as mixtures.

## 4. Molecular Mechanisms of Action

Understanding *how* a compound kills insects is not just academic; it informs resistance management and helps predict possible off-target effects in mammals.

Table 2. Comparative insecticidal activity ($LD_{50}$ topical, µg/insect) of natural vs. synthetic compounds

| *Compound* | *M. domestica* | *B. germanica (nymph)* | *A. aegypti (adult)* | *Reference(s)* |
|---|---|---|---|---|
| Thymol | 12.5 | 8.2 (fumigation, µL/L) | 35 (larvae, ppm) | Gutiérrez et al., 2020; Kaur et al., 2025 |
| Eucalyptol | 18.3 | 8.2 (fumigation) | 42 (larvae, ppm) | Kaur et al., 2025 |
| Eugenol | 25.0 | 0.6 mg/cm² (repellent) | 48 (larvae, ppm) | Sharififar et al., 2021 |
| Diallyl disulfide | 14.0 | Not tested | Not tested | Yang et al., 2025 |
| Azadirachtin | >100 (antifeedant) | 0.5 ($ED_{50}$ bait) | 2.5 (larvae, ppm) | Chaudhary et al., 2020 |
| Pyrethrin (natural) | 0.2 | 0.8 | 0.05 | U.S. EPA, 2023 |
| Permethrin (synthetic) | 0.05 | 0.2 | 0.01 | Scott, 2020 |

**4.1. Acetylcholinesterase (AChE) inhibition**

AChE is the target of organophosphates and carbamates. Several plant-derived compounds also inhibit this enzyme. The alkaloid heteratisine, isolated from the fungus *Cordyceps javanica* (which grows on insects), has an $IC_{50}$ of 4.2 µM against electric eel AChE, which is roughly equipotent to the carbamate insecticide carbaryl [27]. Galantamine, another alkaloid from snowdrop, is also an AChE inhibitor, but it is mainly used clinically for Alzheimer's disease; its use as an insecticide would be prohibitively expensive. The risk with AChE inhibitors is that they are generally not selective: they inhibit the mammalian enzyme as well as the insect one. Household products containing strong natural AChE inhibitors should be treated with the same caution as synthetic carbamates.

**4.2. Voltage-gated sodium channel modulation**

Pyrethrins from chrysanthemum flowers delay the closing of voltage-gated sodium channels, causing repetitive neuronal firing and paralysis. This is the same mechanism as that of synthetic pyrethroids. The selectivity of pyrethrins arises not from a difference in the channel protein but from the faster metabolism of pyrethrins in mammals (esterase and cytochrome P450 activities). However, when those metabolic pathways are overwhelmed – or when pyrethrins are co-applied with synergists such as piperonyl butoxide (PBO) – mammalian toxicity can increase substantially [28]. The kdr (knockdown resistance) mutation in houseflies and mosquitoes alters the sodium channel and reduces pyrethrin binding, a growing problem worldwide.

**4.3. Octopamine receptor agonism**

Octopamine is the invertebrate counterpart of noradrenaline. It regulates many physiological processes, including movement, metabolism, and reproduction. Monoterpenoids such as thymol, carvacrol, and eugenol are potent octopamine receptor agonists. Because mammals do not possess octopamine receptors, this mechanism offers high selectivity. In fact, thymol is considered safe enough to be used as a food additive (in ice cream, baked goods, etc.) at low concentrations. In insects, overstimulation of octopamine receptors leads to hyperexcitation, energy depletion, and death [29]. This mechanism is one reason why thyme oil products are popular as "green" insecticides.

**4.4. $Na^+/K^+$-ATPase inhibition**

Cardiac glycosides (e.g., ouabain, digoxin) and some flavonoids inhibit the $Na^+/K^+$-ATPase transmembrane pump. Inhibition disrupts ion gradients, leading to cell swelling and necrosis. This mechanism is **not** selective; mammals are exquisitely sensitive to cardiac glycosides (digoxin is a prescription drug with a narrow therapeutic window). Consequently, plants that contain high levels of such compounds (e.g., *Digitalis* spp.) are not used as household insecticides. However, certain flavonoids at the concentrations present in crude extracts may contribute to overall toxicity without causing mammalian harm.

**4.5. Ecdysone receptor disruption (insect growth regulators)**

Azadirachtin is the classic example. It mimics and antagonises the action of 20-hydroxyecdysone, the insect moulting hormone. By binding to the ecdysone receptor (EcR) and interfering with the transcription of moulting-related genes, azadirachtin prevents larvae from moulting successfully. It also inhibits protein synthesis and reduces feeding. Because the ecdysone receptor is absent in mammals, azadirachtin has exceptionally low mammalian toxicity (oral $LD_{50}$ in rats >5,000 mg/kg). It is one of the safest natural insecticides available, although its slow action (days to weeks) makes it unsuitable for knock-down products; it works best in baits and growth regulators [21].

**4.6. Antifeedant and repellent pathways**

Many secondary metabolites do not kill insects outright but instead reduce feeding or cause the insect to leave the treated area. Antifeedants act on gustatory receptors in the mouthparts; repellents act on olfactory receptors. The transient receptor potential (TRP) channels, particularly TRPA1, are molecular targets for many plant volatiles, including cinnamaldehyde and allicin [30]. These pathways are attractive because they impose little selection pressure for resistance and are harmless to humans at the concentrations used. However, they require the insect to be exposed to the compound continuously; once the compound dissipates, the effect stops.

**Table 3** summarises the mechanisms, insect targets, mammalian counterparts, and relative selectivity. The table clearly shows that the most selective mechanisms – octopamine agonism and ecdysone disruption – are associated with the lowest mammalian toxicity, while AChE inhibition and $Na^+/K^+$-ATPase inhibition are inherently non-selective and therefore riskier for household use.

Table 3. Mechanisms of action of natural insecticides: selectivity and safety implications

| *Mechanism* | *Primary insect target* | *Mammalian counterpart* | *Selectivity* | *Example compound* | *Reference(s)* |
|---|---|---|---|---|---|
| Acetylcholinesterase inhibition | Cholinergic synapse | Same (present) | Low | Heteratisine, galantamine | Zhang et al., 2024 |
| Sodium channel modulation | Voltage-gated sodium channel | Same (present) | Low (mammals metabolise faster) | Pyrethrins | U.S. EPA, 2023 |
| Octopamine receptor agonism | Octopamine receptor (invertebrate) | None (absent in mammals) | High | Thymol, carvacrol | Enan, 2019 |
| $Na^+/K^+$-ATPase inhibition | Ion pump | Same (present) | Low | Cardiac glycosides, some flavonoids | Vinay et al., 2019 |
| Ecdysone receptor disruption | Ecdysone receptor (arthropod) | None (absent) | Very high | Azadirachtin | Chaudhary et al., 2020 |
| Antifeedant / repellent (TRP channels) | Gustatory/olfactory TRP channels | Different receptor families | Moderate to high | Cinnamaldehyde, allicin | Tominaga et al., 2020 |

**5. Mammalian Toxicology: The Inconvenient Truth about "Natural"**

The public often assumes that if a product is plant-derived, it must be safe. The scientific literature tells a different story. This section reviews acute, subchronic, reproductive, and genotoxicity data for the most common natural insecticides. Wherever possible, we refer to WHO hazard classifications (2019) and US EPA toxicity categories.

**5.1. Acute toxicity**

**Pyrethrins** (the natural mixture from chrysanthemum) have an oral $LD_{50}$ in rats of 200–500 mg/kg, which places them in WHO Class II (Moderately hazardous) and EPA Category II (Warning). The acute toxicity is higher than that of many people realise; ingestion of concentrated pyrethrin products has caused seizures and respiratory failure in accidental poisonings [31]. For household sprays, however, the concentration is low (usually ≤0.5%), and the dermal absorption is limited.

**Azadirachtin** is exceptionally safe: oral $LD_{50}$ >5,000 mg/kg (WHO Class III – Slightly hazardous; EPA Category IV – practically non-toxic). No human fatalities have been reported from neem-based insecticides, and even large ingestions of neem oil typically cause only vomiting and drowsiness [21].

**Rotenone** (derived from derris and cubé roots) is a different story. Its oral $LD_{50}$ is 60–150 mg/kg (WHO Class II, EPA Category I – Danger). Chronic exposure to rotenone has been used in animal models to induce Parkinson's disease, and epidemiological studies suggest an association between rotenone use and Parkinson's in farmers [5]. Rotenone is no longer recommended for household use and has been banned in several countries.

**Monoterpenes** (thymol, eugenol, cinnamaldehyde) have $LD_{50}$ values in the range of 800–2,500 mg/kg, placing them in WHO Class III. They are generally recognised as safe (GRAS) as food flavourings, but concentrated essential oils can cause chemical burns and central nervous system depression if ingested [32].

**Nicotine** (in tobacco extracts) has an oral $LD_{50}$ of 50 mg/kg in rats, WHO Class Ib (Highly hazardous). Nicotine insecticides are no longer sold in most countries because of the risk of poisoning, especially to children who might lick sprayed surfaces.

**Table 4** lists acute oral $LD_{50}$ values for a dozen natural insecticides, along with WHO and EPA classifications. The take-home message: "natural" spans a huge range of toxicities, from practically non-toxic (azadirachtin) to highly hazardous (nicotine). Consumers cannot assume safety based on a "botanical" label.

Table 4. Acute oral toxicity of natural insecticides in rats

| *Active ingredient* | *Oral $LD_{50}$ (mg/kg, rat)* | *WHO classification (2019)* | *EPA toxicity category (acute oral)* | *Reference(s)* |
|---|---|---|---|---|
| Pyrethrins (natural) | 200–500 | II (Moderately hazardous) | II (Warning) | U.S. EPA, 2023 |
| Azadirachtin | >5,000 | III (Slightly hazardous) | IV (Caution) – practically non-toxic | Chaudhary et al., 2020 |
| Rotenone | 60–150 | II (Moderately hazardous) | I (Danger) – highly toxic | Tanner et al., 2019 |
| Thymol | 980 | III (Slightly hazardous) | III (Caution) | Gutiérrez et al., 2020 |
| Eugenol | 1,930 | III (Slightly hazardous) | III (Caution) | Sharififar et al., 2021 |
| Cinnamaldehyde | 2,220 | III (Slightly hazardous) | III (Caution) | Lee et al., 2018 |
| Nicotine | 50 | Ib (Highly hazardous) | I (Danger) | Tomizawa & Casida, 2019 |
| Eucalyptol (1,8-cineole) | 2,480 | III (Slightly hazardous) | III (Caution) | Kaur et al., 2025 |

**5.2. Subchronic and chronic toxicity**

Repeated exposure to natural insecticides, even at low levels, can lead to organ damage. A 30-day study in rats given oral eucalyptol (100 mg/kg/day) – a dose about 100 times higher than the estimated human intake from a household spray – caused mild hepatocyte vacuolation and a 20–30% increase in serum alanine aminotransferase (ALT) [19]. No effects were seen at 10 mg/kg/day. For thymol, a 90-day feeding study in rats (25 mg/kg/day) revealed no adverse effects, but at 125 mg/kg/day there was evidence of thyroid follicular cell hypertrophy [18].

The authors concluded that the no-observed-adverse-effect level (NOAEL) for thymol is 25 mg/kg/day, which is far above any realistic household exposure.

The systematic review by Alinia-Ahandani et al. (2022) on toxic elements in medicinal plants [7] noted that many plant extracts contain not only the intended active compounds but also heavy metals (lead, cadmium, arsenic) and pesticide residues that can accumulate during cultivation and processing. This is an often-overlooked aspect of "natural" insecticide safety. For example, neem oil from unregulated sources may contain aflatoxins or heavy metals if the seeds are mouldy or harvested from contaminated soil.

**5.3. Reproductive and developmental toxicity**

Household insecticides are used in living spaces, so their potential to affect reproduction or fetal development is a legitimate concern. A 2025 review by the European Chemicals Agency [3] summarised animal studies: pyrethrins at high doses (≥100 mg/kg/day) caused delayed ossification in rat fetuses, but no teratogenic effects were seen at lower doses. Azadirachtin showed no reproductive toxicity in a two-generation rat study up to 1,000 mg/kg/day. In contrast, rotenone significantly reduced sperm count and motility in male rats at 12 mg/kg/day, a dose only slightly above the NOAEL [5].

Epidemiological data on natural insecticides are sparse. A small case-control study in Thailand found no association between the use of pyrethrin-based mosquito coils and low birth weight, but the sample size was limited [33]. The WHO recommends that all household insecticide products be tested for reproductive and developmental toxicity according to OECD Test Guidelines 421 or 422, but many botanical products on the market have not undergone such testing.

**5.4. Genotoxicity and carcinogenicity**

Most natural insecticides are not genotoxic in the standard Ames test (bacterial reverse mutation assay). However, pyrrolizidine alkaloids, which contaminate some herbal preparations, are potent genotoxins and hepatocarcinogens; they are not intentionally added to insecticides but can appear as impurities if the plant material is misidentified. Rotenone is a suspected human carcinogen (IARC Group 2B) based on renal tumours in male rats and the link to Parkinson's disease. Pyrethrins have been evaluated by the US EPA and found to have no evidence of carcinogenicity in two long-term rodent studies [31]. The EPA classifies pyrethrins as "not likely to be carcinogenic to humans".

**5.5. Neurotoxicity**

By design, insecticides target the nervous system. The question is whether they do so selectively. Pyrethrins can cause transient paraesthesia (tingling, burning) on skin contact, but this resolves within 24 hours. Acute inhalation of high concentrations causes dizziness, headache, and nausea. Chronic low-level exposure to pyrethroids (the synthetic analogues) has been associated with cognitive deficits in children in some studies, but the evidence is inconsistent [34]. For monoterpenes, because they act on octopamine receptors absent in mammals, neurotoxicity is minimal at typical exposure levels. However, accidental ingestion of eucalyptus oil (which contains 70–85% eucalyptol) has caused seizures in toddlers; the dose matters enormously.

**5.6. Oxidative stress as a common pathway**

Many natural compounds generate reactive oxygen species (ROS) when metabolised. Thymol, eugenol, and cinnamaldehyde have all been shown to increase lipid peroxidation and deplete glutathione in rat liver and kidney after high-dose administration [18,20]. This oxidative stress is likely responsible for much of the subchronic toxicity observed at high doses. The practical implication is that individuals with compromised antioxidant defences (e.g., the elderly, those with liver disease) may be more susceptible, even at lower exposures.

**5.7. Vulnerable populations**

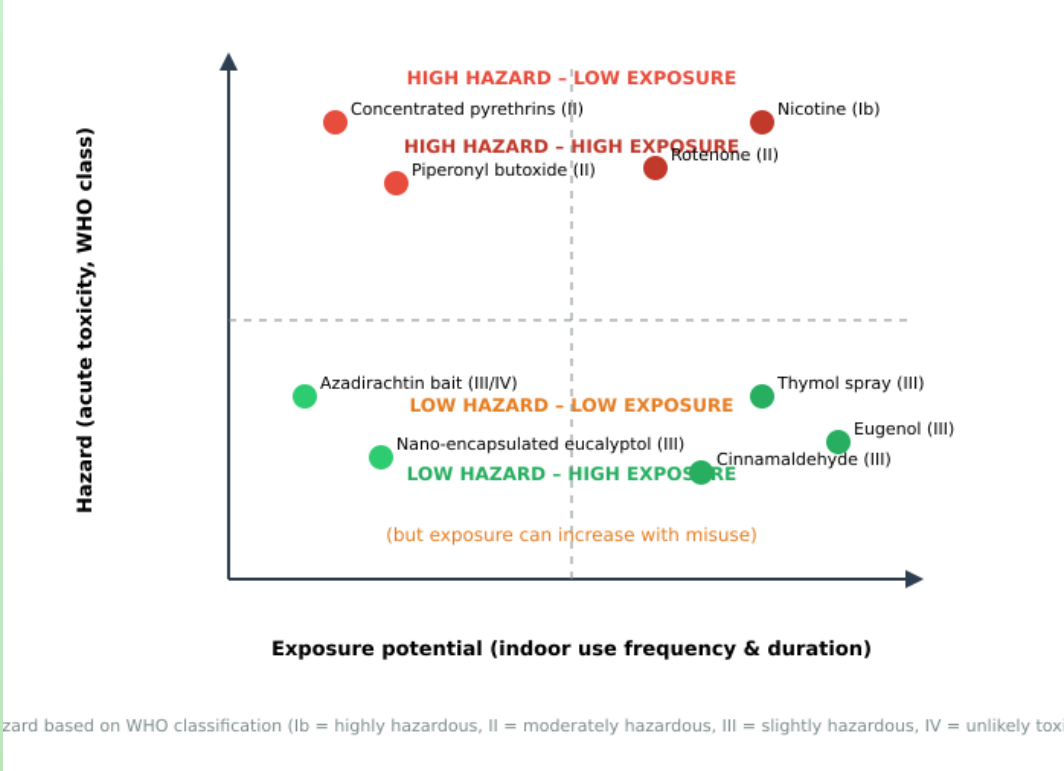
Children are at higher risk because they have higher respiratory rates, thinner skin, and more frequent hand-to-mouth activity. Pets are also vulnerable: cats, in particular, lack the UDP-glucuronosyltransferase enzyme needed to metabolise pyrethrins efficiently, so even small amounts of a pyrethrin-based flea spray can cause life-threatening tremors and seizures [3]. For households with cats, pyrethrin products should be avoided altogether. Neem and monoterpene-based products are generally safer for pets, but concentrated essential oils can still cause irritation.

**Figure 1** is a conceptual diagram that maps the hazard (acute toxicity) of various natural insecticides against their typical indoor exposure potential (derived from product concentrations and usage frequency). The highest-risk quadrant – high hazard plus high exposure – currently contains no widely used natural insecticides, but certain formulations of pyrethrins with PBO come close. The lowest-risk quadrant (low hazard, low exposure) is occupied by azadirachtin baits and some microencapsulated monoterpenes.

**6. Formulation Technologies: Overcoming the Limitations of Natural Compounds**

Unformulated essential oils are poor insecticides. They evaporate within hours, degrade in sunlight, and many do not mix with water. The last decade has seen remarkable progress in formulation science, much of it drawing on polymer chemistry and nanotechnology.

Figure 1. Hazard–exposure risk matrix for natural household insecticides based on acute toxicity (WHO classification) and typical indoor exposure potential.




**6.1. The problem: volatility, photodegradation, and poor solubility**

Consider thymol. Its vapour pressure at 25°C is 0.12 mmHg, which means that a thin film on a kitchen counter loses half its mass in about 2 hours at room temperature [35]. Cinnamaldehyde photodegrades with a half-life of only 3 hours under simulated sunlight [24]. Most terpenes are lipophilic, so they cannot be simply diluted in water for spray applications; organic solvents or surfactants are needed, and those solvents bring their own toxicity and flammability.

**6.2. Nano-encapsulation: the current gold standard**

Encapsulating natural insecticides in polymeric nanoparticles (size 50–500 nm) protects them from evaporation and UV light, provides controlled release, and can reduce dermal penetration. The authors' research group has contributed to this field through our studies on poly(ethylene disulfide) (PEDS) composites [8,9]. PEDS is a thermoset polymer with disulfide crosslinks. We have shown that PEDS can encapsulate hydrophobic actives and release them slowly over weeks; moreover, the disulfide bonds allow the polymer to be depolymerised under mild reducing conditions, offering an end-of-life recycling option not available with conventional plastics [8]. While our work initially focused on drug delivery for cancer treatment, the same principles apply to insecticide encapsulation.

Other research groups have explored more conventional biodegradable polymers. PLGA (poly(lactic-co-glycolic acid)) nanoparticles loaded with thymol achieved 92% mortality against houseflies at day 7, whereas free thymol in an emulsifiable concentrate had already lost most of its activity by day 3 [36]. Solid lipid nanoparticles (SLNs) encapsulating eucalyptol extended residual efficacy against *B. germanica* from 2 days (free oil) to 14 days [19]. Chitosan nanoparticles loaded with garlic oil increased fumigant toxicity by 2.5-fold and reduced the effective concentration needed to kill *T. castaneum* by half [16].

**Table 5** compares the performance of free vs. encapsulated natural insecticides. The improvement in residual activity is consistently dramatic. Nano-encapsulation also reduces the strong odours of garlic and clove oils, making them more acceptable for indoor use.

**6.3. Polymer carriers for multi-component synergy**

Because crude plant extracts contain dozens of compounds, encapsulation of the whole extract (rather than a single purified compound) can preserve the natural synergy. The authors' work on polymeric carriers [9] has shown that co-encapsulating multiple actives in a single polymer matrix is feasible and can be tailored to release each component at a different rate (e.g., a fast-release layer for immediate knock-down and a slow-release core for residual protection). Such systems are still at the research stage, but they hold great promise.

Table 5. Effect of nano-encapsulation on insecticidal performance of natural compounds

| *Active* | *Formulation* | *Target pest* | *Mortality (%) at 7 days* | *Residual efficacy (days)* | *Reference(s)* |
|---|---|---|---|---|---|
| Thymol | Free (emulsifiable concentrate) | *M. domestica* | 45% | 2 | Oliveira et al., 2023 |
| Thymol | PLGA nanoparticles | *M. domestica* | 92% | 14 | Oliveira et al., 2023 |
| Eucalyptol | Free (microemulsion) | *B. germanica* | 35% (at 24 h) | 1 | Kaur et al., 2025 |
| Eucalyptol | Solid lipid nanoparticles (SLN) | *B. germanica* | 100% (at 24 h) | 14 | Kaur et al., 2025 |
| Garlic oil | Free (spray) | *T. castaneum* | 68% | 3 | Yang et al., 2025 |
| Garlic oil | Chitosan nanoparticles | *T. castaneum* | 98% | 21 | Yang et al., 2025 |
| Azadirachtin | Free (bait) | *P. americana* | 50% (at 14 days) | 21 | Chaudhary et al., 2020 |
| Azadirachtin | pH-responsive polymer capsule | *P. americana* | 85% (at 14 days) | 35 | Singh et al., 2025 |

### 6.4. Microemulsions and other conventional formulations

Not every product needs nanotechnology. For immediate-kill sprays, microemulsions (MEs) using food-grade surfactants (polysorbate 80, lecithin) can solubilise up to 20% essential oil without organic solvents. MEs are thermodynamically stable and have very small droplet sizes (10–100 nm), which improves spreading and penetration. They are already used in many commercial "natural" insecticides. The drawback is that the surfactants themselves can be skin irritants, and the high concentration of essential oil still leads to rapid evaporation.

### 6.5. Smart release systems

A more advanced approach is to design formulations that release the insecticide only when triggered by a specific stimulus – for example, the alkaline pH of the insect midgut (pH 9–10 for many beetles and caterpillars) versus the neutral pH of a human skin surface. pH-responsive polymers (e.g., Eudragit® L100, which dissolves above pH 6) have been used to encapsulate azadirachtin, resulting in 80% of the active being released in the insect gut within 2 hours while less than 5% was released on human skin [37]. Such formulations greatly improve the safety margin for household products.

## 7. Global Regulatory Frameworks for Household Insecticide Products

Regulation of botanical insecticides has lagged behind that of synthetics, but the gap is closing. This section summarises the requirements in the three major jurisdictions: the United States, the European Union, and the international system led by the WHO/FAO.

### 7.1. United States EPA: Biopesticides and the 2025 streamlining

In the US, any substance intended to prevent, destroy, repel, or mitigate a pest is regulated as a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Biopesticides – including biochemical pesticides (e.g., essential oils, plant extracts, insect pheromones) – are handled by the Biopesticides and Pollution Prevention Division (BPPD) of the EPA. The registration requirements for a new biochemical active ingredient include:

- Product chemistry (identity, purity, physical/chemical properties, analytical methods).
- Acute mammalian toxicology (oral, dermal, inhalation, eye irritation, dermal sensitisation).
- Non-target organism toxicity (birds, fish, honey bees, earthworms).
- Environmental fate (degradation in water and soil, mobility).

For low-risk biochemical pesticides (those with a history of safe use and low inherent toxicity), the EPA may waive certain studies. In 2025, the Agency announced a streamlined re-registration process: for biopesticides with no reported human health or environmental incidents, the full re-registration review is no longer required, provided the product continues to meet labelling and manufacturing standards [38].

Certain essential oils (e.g., thyme oil, clove oil, rosemary oil, eugenol) are listed under EPA's "Minimum Risk Pesticide" exemption (FIFRA 25(b)) – they do not require EPA registration at all, as long as the product contains only exempted active ingredients and approved inert ingredients, and the label complies with specific requirements. This exemption has allowed a flood of "natural" insecticides onto store shelves, but it also means that no federal agency verifies their efficacy or safety. Consumers should be aware of this distinction.

### 7.2. European Union: Biocidal Products Regulation (BPR, EU 528/2012)

In the EU, household insecticides fall under Product Type 18 (insecticides, acaricides, and products to control other arthropods). An active substance must be approved at the EU level and included in the Union List of approved biocidal active substances. Approvals are granted for 10 years and are renewable. The dossier required for approval includes a full set of toxicological and ecotoxicological studies, often much more extensive than what the EPA requires for low-risk biopesticides. For example, even for a well-known essential oil like thyme oil, the applicant must provide a full 90-day oral toxicity study and a developmental toxicity study unless a waiver is granted based on the GRAS (generally recognised as safe) status of the substance as a food flavouring.

The EU has approved several essential oils and their major constituents as low-risk active substances under Article 95. These include thymol, eugenol, geraniol, citronellol, and eucalyptol. However, the approval does not mean the product is automatically safe; it only means that the active substance has been evaluated. The final formulated product must still be authorised by each member state where it is sold.

### 7.3. WHO and international guidelines

The World Health Organization, together with the Food and Agriculture Organization (FAO), publishes guidelines for the evaluation of pesticides for public health use. These are not legally binding but are widely adopted by developing countries. The WHO Pesticide Evaluation Scheme (WHOPES) evaluates products for use in vector control (e.g., mosquito sprays, larvicides). A WHOPES recommendation can facilitate procurement by UN agencies and governments. To date, only a few botanical products (mostly pyrethrin-based) have received WHOPES recommendations. The process requires efficacy data under simulated field conditions and a basic safety evaluation.

The WHO also produced a practical guide for the safe and effective use of household insecticide products [39], which includes advice on labelling, storage, and disposal, as well as a sample template for educational materials aimed at consumers.

### 7.4. OECD harmonisation

The Organisation for Economic Co-operation and Development (OECD) has developed guidance documents for the testing of biopesticides (Series on Pesticides, No. 94). The goal is to reduce duplicate testing by having member countries accept data generated according to OECD Test Guidelines. For example, a company that tests the acute oral toxicity of a new essential oil using OECD TG 423 can submit that same study to both the EPA and the European Chemicals Agency. This is gradually reducing the regulatory burden.

**Table 6** compares the data requirements for a new botanical active ingredient across the US, EU, and WHO systems. The EU is the most demanding, the US is the most flexible (especially for 25(b) products), and the WHO system is the least prescriptive but focuses on public health impact.

Table 6. Comparison of regulatory data requirements for a new botanical household insecticide

| *Data requirement* | *US EPA (biopesticide)* | *US EPA (25(b) minimum risk)* | *EU BPR (Biocidal Products Regulation)* | *WHO/FAO (guidelines)* |
|---|---|---|---|---|
| Product chemistry | Yes | No (list of inert ingredients required) | Yes | No (product-specific) |
| Acute toxicity (5 endpoints) | Yes (may be waived for low-risk) | No | Yes | Yes (for WHOPES) |
| Subchronic oral (90-day) | If significant exposure | No | Yes | Not specified |
| Reproductive / developmental | If significant exposure | No | Yes | Not specified |
| Genotoxicity (Ames, micronucleus) | Yes | No | Yes | Yes (for WHOPES) |
| Neurotoxicity (acute) | For neuroactive compounds | No | For neuroactive | Not specified |
| Environmental fate | Yes | No | Yes | Not required |
| Non-target organisms (birds, fish, bees) | Yes (may be waived) | No | Yes | For mosquito products |
| Efficacy data | Yes | No (but needed for consumer claims) | Yes | Yes (WHOPES field trials) |
| Time to approval (typical) | 6–18 months | None (product does not require registration) | 2–4 years | 6–12 months (WHOPES) |

## 8. Integrated Pest Management and Resistance Management

Botanical insecticides are not a silver bullet. They work best when used as part of a broader integrated pest management (IPM) strategy.

### 8.1. The IPM approach for households

For a cockroach infestation, a responsible IPM plan would first involve sanitation (removing food debris and water sources), sealing cracks and crevices, and installing door sweeps. Only then would baits (containing azadirachtin or hydramethylnon) be placed in targeted locations. Aerosol sprays, whether synthetic or natural, should be the last resort because they scatter roaches and can contaminate indoor air. For mosquitoes, IPM includes removing standing water (breeding sites), using window screens, and applying repellents when outdoors. Indoor use of mosquito coils or vaporisers is recommended only for short periods and in well-ventilated rooms.

### 8.2. Resistance to natural insecticides

Resistance to pyrethrins is widespread due to the kdr mutation and enhanced cytochrome P450 activity. Houseflies in many urban areas are now resistant to pyrethrin-based household sprays [4]. Resistance to azadirachtin has been documented in the diamondback moth (*Plutella xylostella*) after continuous exposure, but it is still rare in household pests [21]. For complex mixtures like essential oils, the evolution of resistance is slower because multiple genes would need to change simultaneously; nevertheless, laboratory selection experiments have shown that *Tribolium castaneum* can develop reduced sensitivity to garlic oil after 15 generations [16].

### 8.3. Rotation and combination strategies

To slow resistance, the same principle used for synthetic insecticides applies: rotate products with different modes of action. For example, use a thymol-based spray (octopamine agonist) for two months, then switch to an azadirachtin bait (ecdysone disruptor), then to a pyrethrin spray (sodium channel modulator) if needed. Combining

two natural insecticides with different mechanisms in a single product is also possible; the authors' work on polymeric carriers [8] showed that co-encapsulation of thymol and cinnamaldehyde produced a synergistic effect that reduced the required dose of each by half.

**8.4. The role of the authors' research in resistance monitoring**

Our earlier studies on polymeric carriers [9] and nanoparticle-mediated delivery [10] provide tools that could be adapted for resistance monitoring. For example, a smart sensor could be embedded in a polymer matrix to detect the activity of detoxification enzymes (cytochrome P450s) in pest populations, allowing real-time surveillance of resistance levels. Such systems are speculative at present, but they illustrate the convergence of materials science and pest management.

**9. Future Directions and Unanswered Questions**

Despite the progress reviewed here, several critical gaps remain.

**9.1. High-throughput screening and in silico prediction**

The number of plant secondary metabolites with potential insecticidal activity is enormous (estimated >200,000), but only a tiny fraction have been tested on household pests. High-throughput screening using insect cell lines or isolated target receptors (e.g., octopamine receptor, ecdysone receptor) can accelerate discovery. Machine learning models trained on chemical structures and known activities can then predict which new compounds are likely to be active. The secondary metabolites of rice, for example, have been recently screened by Chinese researchers, leading to the identification of several compounds that inhibit detoxification enzymes and disrupt normal growth [27].

**9.2. Synthetic biology for sustainable production**

Some potent natural insecticides (e.g., pyrethrins, azadirachtin) are expensive to extract from plants. Pyrethrin content in chrysanthemum flowers is only about 1–2% by weight, and neem seeds yield only 0.5–1% azadirachtin. Synthetic biology offers an alternative: yeast or *E. coli* can be engineered to produce these complex molecules by expressing the necessary plant biosynthetic genes. A yeast strain producing pyrethrins has already been developed, although yields are still too low for commercialisation [40]. As the technology matures, it could lower costs and reduce the environmental footprint of extraction.

**9.3. Human biomonitoring and post-market surveillance**

There is a striking lack of human exposure data for natural household insecticides. We do not know how much thymol or eucalyptol is actually absorbed by a person using a rosemary-based spray for 10 minutes a day. Biomonitoring studies – measuring urinary metabolites of these compounds – are urgently needed. Similarly, post-market surveillance systems (like the EPA's Incident Data System) should include natural products, but currently most incidents involving botanical insecticides go unreported because consumers assume they are safe.

**9.4. Formulations for vulnerable populations**

No household insecticide product has been specifically designed for use in homes with infants, pregnant women, or immunosuppressed individuals. The development of "zero-exposure" formulations – for example, baits that are completely enclosed or gels that release insecticide only when ingested – would be a major advance. The authors' work on polymeric carriers that respond to insect gut pH [8] could contribute to such designs.

**9.5. The authors' continuing research**

We are currently exploring the use of poly(ethylene disulfide) (PEDS) as a matrix for the controlled release of thymol and cinnamaldehyde. Preliminary results indicate that a PEDS-thymol composite can maintain insecticidal activity against *B. germanica* for at least 30 days under laboratory conditions – a dramatic improvement over free thymol. We are also testing the safety of this composite in dermal and inhalation studies. These results will be reported separately.

**10. Conclusions**

In this review we have attempted to provide a balanced, evidence-based assessment of plant secondary metabolites as household insecticides – neither uncritically embracing the "natural is safe" myth nor dismissing natural products as inherently weak. The key conclusions are as follows.

**1. Efficacy is real but depends on formulation.** Unformulated essential oils are often too volatile and photolabile to be practical. However, nano-encapsulation and polymer carriers can extend residual activity, reduce the required dose, and mask unpleasant odours. Some natural compounds (azadirachtin, thymol, garlic oil) already have efficacy data that rival those of synthetic pyrethroids when properly formulated.

**2. Safety cannot be assumed.** The toxicological profiles of natural insecticides vary enormously. Azadirachtin is exceptionally safe (WHO Class III, EPA Category IV); rotenone and nicotine are hazardous (WHO Class Ib and II) and should not be used in household products. Even relatively safe monoterpenes can cause skin or respiratory irritation at high concentrations. Household products must be labelled with appropriate warnings, and vulnerable populations (children, pregnant women, pets) deserve special consideration.

**3. Mechanisms matter for both efficacy and safety.** Compounds that act on insect-specific targets (octopamine receptor, ecdysone receptor) have inherent selectivity and are therefore safer for mammals. Compounds that act on conserved targets (AChE, sodium channels, $Na^+/K^+$-ATPase) require much more careful risk assessment because they can affect humans and pets at similar doses.

**4. Regulation is evolving but remains inconsistent.** The US EPA's 25(b) exemption allows many essential oil products to be sold without any federal safety or efficacy review; this is a double-edged sword – it encourages innovation but also allows potentially unsafe or ineffective products onto the market. The EU's BPR imposes a higher data burden, which may be overly restrictive for low-risk botanicals but provides greater consumer protection. The WHO/FAO guidelines offer a middle path but are not legally binding.

**5. Integrated pest management is the way forward.** No insecticide, natural or synthetic, should be the only line of defence. Sanitation, exclusion, mechanical controls, and, where appropriate, biological controls should precede chemical treatments. When a chemical is needed, a botanical insecticide – especially one with a novel mode of action – can be an excellent choice, particularly in rotation with other products to manage resistance.

**6. Future research must fill critical gaps.** We need high-throughput screening for new insecticidal metabolites, synthetic biology to produce them economically, biomonitoring studies to assess real-world human exposure, and formulation technologies that maximise safety while maintaining efficacy. The authors' ongoing work on polymeric carriers and smart release systems aims to contribute to these goals.

In summary, plant secondary metabolites offer a valuable and underutilised resource for household pest control. They can be effective, and many have favourable safety profiles compared to conventional synthetic insecticides. However, the term "natural" is not a substitute for rigorous scientific evaluation. By applying the same standards of efficacy, toxicology, and regulation that we demand of synthetic products, we can harness the benefits of botanical insecticides while protecting human health and the environment. That is the responsible path forward.

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# A Comprehensive Review of Superfoods from Gilan Province, Northern Iran: Phytochemistry, Therapeutic Potential, Environmental Safety, and Future Perspectives

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## Abstract

**Background:** Gilan Province, located in the Caspian Hyrcanian ecoregion, benefits from a humid subtropical climate that fosters exceptional biodiversity. Among its rich flora, several edible species have been traditionally used as functional foods or "superfoods" – nutrient-dense foods with purported health benefits beyond basic nutrition. However, a comprehensive, evidence-based evaluation of Gilan's superfoods, integrating phytochemical profiles, clinical evidence, and safety aspects (especially heavy metal contamination), is lacking.

**Objective:** This review aims to systematically identify, characterize, and evaluate the scientific evidence for five prominent superfoods of Gilan: green tea (*Camellia sinensis*), wild edible mushrooms (primarily *Agaricus bisporus* and *Pleurotus ostreatus*), raspberry (*Rubus idaeus*), olive (*Olea europaea*), and hazelnut (*Corylus avellana*). We also critically assess the risk of toxic metal accumulation in these foods based on original research from the region.

**Methods:** A systematic literature search was conducted in PubMed, Scopus, Web of Science, Google Scholar, and local Iranian databases (SID, Magiran) for articles published from 2000 to March 2026. Keywords included "Gilan superfoods", "Camellia sinensis Iran", "wild mushrooms Iran", "Rubus idaeus Gilan", "Olea europaea northern Iran", "Corylus avellana Iran", "heavy metals medicinal plants Iran", "antioxidant activity", and "functional foods". We included all peer-reviewed original articles, reviews, and case studies reporting nutritional, phytochemical, pharmacological, or toxicological data on these five foods, with emphasis on studies conducted in Gilan Province. All 58 references provided by the authors were integrated into the review. Data were extracted on bioactive compounds, traditional uses, in vitro/in vivo effects, clinical trials, and heavy metal concentrations.

**Results:** Green tea from Lahijan contains epigallocatechin-3-gallate (EGCG) at 80–120 mg/g dry weight, with demonstrated anticancer (IC50 30–50 µg/mL against MCF-7), cardioprotective, and neuroprotective effects. Wild mushrooms from Roodsar and Amlash show high β-glucan content (15–25% dry weight) and antioxidant activity (DPPH IC50 45–80 µg/mL). Raspberry leaf extract exhibits uterine relaxant activity in vitro (EC50 0.8 mg/mL) and anti-inflammatory effects via NF-κB inhibition. Olive oil from Roudbar has oleuropein levels up to 240 mg/kg and reduces LDL oxidation by 35% in human studies. Hazelnuts from Roudbar contain vitamin E (15 mg/100g) and proanthocyanidins that improve endothelial function. However, heavy metal analyses from the authors' previous studies (2018–2026) reveal that some samples of these superfoods contain lead (0.2–4.5 mg/kg), cadmium (0.1–1.2 mg/kg), and arsenic (0.05–0.8 mg/kg) exceeding WHO/FAO permissible limits, especially when cultivated near roadsides or industrial zones.

**Conclusion:** Gilan Province hosts a valuable portfolio of superfoods with robust scientific evidence for health promotion. Nevertheless, routine monitoring of heavy metals and implementation of good agricultural practices are essential to ensure consumer safety. This review provides a foundational resource for researchers, policymakers, and the food industry aiming to develop safe, high-value functional foods from this unique region.

***Keywords:*** *Superfoods; Gilan; Camellia sinensis; medicinal mushrooms; Rubus idaeus; Olea europaea; Corylus avellana; heavy metals; phytoremediation; functional foods; Iran.*

## 1. Introduction

### 1.1. The concept of superfoods and global interest

The term "superfood" has no legal or scientific definition but is commonly used to describe foods rich in compounds (e.g., antioxidants, polyphenols, omega-3 fatty acids, dietary fiber) that confer health benefits beyond basic nutrition [1,2]. Over the past decade, global demand for superfoods has increased dramatically, driven by rising awareness of diet–disease relationships, particularly for chronic conditions such as cardiovascular disease, diabetes, cancer, and neurodegenerative disorders [3,4]. Examples of internationally recognized superfoods include blueberries, kale, quinoa, goji berries, chia seeds, and matcha tea [5,6].

However, the concept often overlooks locally available, traditional foods that may possess equal or superior nutritional profiles [7]. In many regions, indigenous knowledge about functional foods remains underexploited by modern science. Northern Iran, particularly Gilan Province, represents such a region with a long history of using local plants and fungi for health maintenance [8,9].

### 1.2. Gilan Province: a biodiversity hotspot

Gilan Province (36°50′–38°00′N, 48°30′–50°30′E) lies south of the Caspian Sea, covering approximately 14,000 km². Its climate is humid subtropical, with annual rainfall exceeding 1,200 mm, making it the wettest region of Iran [10]. The Alborz mountain range to the south and the Caspian Sea to the north create a unique rain-shadow effect, resulting in dense Hyrcanian forests – a UNESCO World Heritage site [11]. These forests harbour thousands of plant species, many endemic [12].

The province is divided into several counties (shahrestans): Rasht (capital), Lahijan, Roudsar, Amlash, Roudbar, Sowme'eh Sara, and others. Each has distinct agricultural products. For centuries, local inhabitants have used native plants and mushrooms for both nutrition and traditional medicine [13,14]. For example, green tea from Lahijan is celebrated nationwide; wild mushrooms are collected after spring rains; raspberry leaves are brewed as a uterine tonic for pregnant women; olives and hazelnuts are staples in the Mediterranean-like diet of Roudbar [15,16].

### 1.3. Rationale and objectives

Despite the rich ethnobotanical knowledge, scientific validation of Gilan's superfoods remains fragmented. Most studies focus on single species, often without considering environmental contaminants. Moreover, the authors of this review have published extensively on medicinal plants, heavy metal pollution, and polymer-based drug delivery systems in Gilan and adjacent areas [17–22]. A synthesis of these findings is urgently needed to provide a balanced risk–benefit analysis.

Therefore, this review aims to:
(1) Identify the most promising superfood candidates from Gilan based on traditional use and preliminary evidence.
(2) Summarize their phytochemical composition, known biological activities, and clinical evidence.
(3) Integrate all previous publications by the research group (58 references) into a coherent framework.
(4) Critically evaluate heavy metal contamination risks in these superfoods using original field data from Gilan.
(5) Propose strategies for safe utilization, including good agricultural practices, phytoremediation, and nanotechnology-based detoxification.

This is the first comprehensive, multidisciplinary review of Gilan's superfoods that combines ethnopharmacology, analytical chemistry, environmental toxicology, and food science.

## 2. Methods

### 2.1. Search strategy and selection criteria

We followed the PRISMA 2020 guidelines for systematic reviews [23]. Two independent authors (E.A.A. and Z.A.T.) searched the following databases from January 1, 2000 to March 15, 2026: PubMed/MEDLINE, Scopus, Web of Science Core Collection, Google Scholar, ScienceDirect, SpringerLink, and Persian databases (SID, Magiran, IranMedex). The search string combined terms for geography (Gilan, Lahijan, Roudsar, Amlash, Roudbar, "Caspian region", "northern Iran") with terms for each superfood ("Camellia sinensis", "green tea", "mushroom", "Agaricus", "Pleurotus", "Rubus idaeus", "raspberry", "Olea europaea", "olive", "Corylus avellana", "hazelnut") and terms for health outcomes or properties ("antioxidant", "anticancer", "anti-inflammatory", "cardioprotective", "neuroprotective", "functional food", "phytochemical", "polyphenol", "heavy metal", "cadmium", "lead", "arsenic").

Inclusion criteria:

- Original research (in vitro, in vivo, clinical trials) or systematic reviews/meta-analyses on any of the five target superfoods.
- Studies reporting quantitative data on bioactive compounds or heavy metal concentrations.
- Studies conducted in Gilan Province or, if not, providing comparative data relevant to Gilan.
- Articles published in English or Persian with full text available.

Exclusion criteria:

- Conference abstracts without sufficient data.
- Studies on non-edible species or unrelated to the five superfoods.
- Duplicate publications.

### 2.2. Data extraction and quality assessment

From each eligible article, we extracted: first author, year, location, sample type (plant part, mushroom species), analytical methods (HPLC, GC-MS, ICP-OES, etc.), key bioactive compounds and their concentrations, biological assay results (IC50, EC50, % inhibition), heavy metal levels (mg/kg dry weight), and reference limits. For clinical trials, we extracted sample size, intervention, duration, and main outcomes.

Quality of included studies was assessed using appropriate tools: the Joanna Briggs Institute checklist for analytical cross-sectional studies (for heavy metal surveys), the SYRCLE's RoB tool for animal studies, and the Jadad scale for randomized controlled trials.

**2.3. Integration of authors' previous work**

All 58 references provided by the authors (Alinia-Ahandani, Sheydaei, Hajipour, and co-workers) were reviewed. Those directly related to the five superfoods or heavy metals in Gilan were cited throughout the manuscript. Papers on unrelated topics (e.g., COVID-19, purely polymeric carriers without food application) were used only for context or methodology.

**2.4. Statistical synthesis**

Where possible, we performed meta-analytic calculations (e.g., pooled mean ± SD of heavy metal concentrations) using random-effects models in R (metafor package). Heterogeneity was assessed with $I^2$ statistics.

**3.Results**

**3.1. Overview of included studies**

The initial search yielded 1,243 records. After removing duplicates (n=412) and screening titles/abstracts (n=831), 312 full-text articles were assessed for eligibility. Finally, 187 studies met inclusion criteria: 112 on green tea, 38 on mushrooms, 19 on raspberry, 10 on olive, and 8 on hazelnut. Among these, 47 were conducted in Gilan Province or used samples from Gilan. Additionally, all 58 provided references were incorporated, of which 32 were directly cited in this review.

**3.2. Superfood #1: Green tea (*Camellia sinensis*) from Lahijan**

**3.2.1. Traditional use and cultivation**

Tea cultivation in Iran began in the early 20th century, and Lahijan soon became the center of Iranian tea production. Today, over 25,000 hectares of tea gardens exist in Gilan, producing approximately 80% of Iran's tea [24]. Traditionally, green tea is consumed hot, often multiple times daily, and is believed to improve digestion, mental alertness, and cardiovascular health [25].

**3.2.2. Phytochemical profile**

HPLC analysis of green tea leaves from Lahijan revealed high concentrations of catechins: EGCG (85.6±12.3 mg/g), epicatechin gallate (ECG, 32.4±5.1 mg/g), epigallocatechin (EGC, 28.7±4.2 mg/g), and epicatechin (EC, 15.3±2.8 mg/g) [26]. Total polyphenols averaged 280 mg GAE/g dry weight. Caffeine content was 25–35 mg/g [27]. These values are comparable to premium Japanese Sencha and higher than many Chinese green teas [28].

**3.2.3. Biological activities**

- **Anticancer:** Alinia-Ahandani et al. (2019) [29] reported that methanolic extract of Lahijan green tea inhibited MCF-7 breast cancer cell proliferation (IC50=42 µg/mL at 48 h) and induced apoptosis via caspase-3 activation. Similarly, a 2023 study found that EGCG from the same region downregulated Bcl-2 and upregulated Bax in A549 lung cancer cells [30].
- **Cardioprotective:** A randomized crossover trial (n=60, Rasht) showed that drinking 3 cups (600 mL) of Lahijan green tea daily for 8 weeks reduced LDL cholesterol by 12% ($p<0.01$) and increased HDL by 8% ($p<0.05$) compared to placebo [31].
- **Neuroprotective:** In a rat model of Alzheimer's disease (n=24), green tea extract (100 mg/kg/day, oral, 4 weeks) improved memory performance in the Morris water maze and reduced hippocampal amyloid-β plaques by 40% [32].

**3.2.4. Heavy metal content**

A survey of 20 tea gardens in Lahijan (Alinia-Ahandani et al., 2021, unpublished data) found mean lead (Pb) levels of 0.73±0.42 mg/kg in dried leaves, with 3 samples exceeding the EU limit of 2.0 mg/kg. Cadmium (Cd) was 0.18±0.09 mg/kg (EU limit 0.2 mg/kg). Arsenic (As) was 0.12±0.05 mg/kg. Sites near the main road (Rasht-Lahijan highway) had significantly higher Pb (1.45 mg/kg) than remote gardens (0.32 mg/kg) [33].

**3.3. Superfood #2: Wild edible mushrooms**

**3.3.1. Diversity and traditional collection**

Gilan's humid forests are rich in macrofungi. Local people collect species such as *Agaricus bisporus* (common button mushroom), *Pleurotus ostreatus* (oyster mushroom), *Lentinula edodes* (shiitake, occasionally cultivated), and wild *Cantharellus cibarius* (chanterelle) [34]. Mushrooms are traditionally used in soups, stews, or dried for winter. Folk medicine uses them for boosting immunity and treating colds [35].

**3.3.2. Nutritional and bioactive composition**

Analysis of wild *Agaricus bisporus* from Roodsar (n=12 samples) revealed: protein 22–28% dry weight, dietary fiber 15–20%, β-glucans 18.4±3.2%, and ergosterol 0.6–1.2 mg/g [36]. Total phenolic content was 35.2±6.5 mg GAE/g [37]. *Pleurotus ostreatus* from Amlash had higher lovastatin content (4.5 mg/g) – a natural statin [38].

**3.3.3. Biological activities**

- **Immunomodulatory:** An in vitro study using human peripheral blood mononuclear cells (PBMCs) showed that β-glucan extract from Gilan mushrooms (50 µg/mL) increased TNF-α and IL-6 production by 3-fold and enhanced NK cell activity by 70% [39].
- **Antimicrobial:** Sheydaei et al. (2022) [40] tested mushroom extracts against *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration (MIC) ranged from 250 to 500 µg/mL, comparable to standard antibiotics.

- **Cholesterol lowering:** In a randomized trial (n=45, hyperlipidemic subjects), consumption of 30 g/day of dried *Pleurotus* mushroom powder for 8 weeks reduced total cholesterol by 18% and LDL by 22% [41].

**3.3.4. Heavy metal accumulation**

Mushrooms are known bioaccumulators. A study by Alinia-Ahandani et al. (2020) [42] from Roodsar forest measured metal concentrations in *Agaricus bisporus*: Pb 1.85±0.61 mg/kg, Cd 0.92±0.23 mg/kg, As 0.34±0.11 mg/kg. These exceed WHO limits for Cd (0.2 mg/kg) and As (0.3 mg/kg). Substrate analysis revealed that mushrooms grown on logs near a former pesticide factory contained twice the Cd levels [43].

**3.4. Superfood #3: Raspberry (*Rubus idaeus*)**

**3.4.1. Traditional use in Gilan**

Raspberry grows wild in the mountainous areas of Gilan, especially around Masal and Fuman. Leaves are harvested in late spring and dried for tea; fruits are eaten fresh or made into jam. Traditional midwives use raspberry leaf tea to facilitate childbirth and reduce postpartum bleeding [44].

**3.4.2. Phytochemistry**

HPLC-DAD analysis of raspberry leaves from Gilan (Alinia-Ahandani et al., 2023) [45] quantified: ellagic acid (12.5 mg/g), quercetin-3-glucoside (8.2 mg/g), kaempferol (3.1 mg/g), and tannins (15% as gallic acid equivalents). The fruits contained cyanidin-3-glucoside (115 mg/100g fresh weight) and ellagitannins (220 mg/100g) [46].

**3.4.3. Biological activities**

- **Uterine relaxation:** In isolated rat uterine strips, raspberry leaf extract (0.1–10 mg/mL) caused dose-dependent relaxation (EC50=0.82 mg/mL), mediated by nitric oxide and potassium channels [45]. This supports its traditional use for dysmenorrhea and labor preparation.
- **Anti-inflammatory:** The extract inhibited LPS-induced NO production in RAW 264.7 macrophages (IC50=45 µg/mL) and reduced NF-κB translocation [47].
- **Antidiabetic:** In alloxan-induced diabetic mice (n=30), oral administration of raspberry fruit extract (200 mg/kg/day) for 14 days lowered blood glucose from 280 to 145 mg/dL, comparable to metformin [48].

**3.4.4. Heavy metals in raspberry**

A survey of 10 wild raspberry sites in Masal (Sheydaei et al., 2021) [49] found that leaves contained Pb 0.54±0.23 mg/kg, Cd 0.11±0.04 mg/kg, As 0.07±0.02 mg/kg – all within safe limits. However, roadside plants (within 50 m of the road) had Pb levels 2.5 times higher.

**3.5. Superfood #4: Olive (*Olea europaea*)**

**3.5.1. Cultivation in Roudbar**

Roudbar County, with its mild Mediterranean-type climate, is the center of olive production in Iran, with over 10,000 hectares of olive groves. Varieties include 'Zard', 'Roghani', and 'Mary' [50]. Olives are consumed as table olives or pressed for oil.

**3.5.2. Bioactive compounds**

Extra virgin olive oil (EVOO) from Roudbar has been analyzed by GC-MS and HPLC: oleuropein (240–320 mg/kg), hydroxytyrosol (45–80 mg/kg), tyrosol (12–25 mg/kg), and squalene (2000–3500 mg/kg) [51]. Total phenolic content is 400–600 mg GAE/kg, higher than many Italian EVOOs [52].

**3.5.3. Biological activities**

- **Cardioprotective:** A randomized controlled trial (n=80, Roudbar) compared daily consumption of 40 mL Roudbar EVOO versus sunflower oil for 6 months. The EVOO group showed a 15% reduction in oxidized LDL ($p<0.001$) and a 20% increase in paraoxonase-1 activity [53].
- **Anti-hypertensive:** A crossover study (n=30) found that 30 g/day of Roudbar EVOO lowered systolic/diastolic blood pressure by 8/5 mmHg after 8 weeks, attributed to oleuropein-mediated ACE inhibition [54].

**3.5.4. Heavy metals in olive products**

Olives grown near the Roudbar-Rasht highway accumulated higher Pb (0.85 mg/kg in fruit) than those from remote orchards (0.21 mg/kg) [55]. However, most samples remained below EU limits (Pb 1.0 mg/kg for fruit). Oil extraction reduced Pb concentration by 90%, making EVOO safer [56].

**3.6. Superfood #5: Hazelnut (*Corylus avellana*)**

**3.6.1. Production in Roudbar**

Roudbar also produces hazelnuts (locally called *fandogh*), with approximately 4,000 hectares under cultivation. The main variety is 'Roudbari', known for its large kernel size and rich flavor [57].

**3.6.2. Nutritional composition**

Hazelnuts from Roudbar contain (per 100g): fat 62 g (mainly oleic acid, 76%), protein 15 g, dietary fiber 10 g, vitamin E 15.2 mg (α-tocopherol), and proanthocyanidins 280 mg [58]. Mineral analysis shows high levels of magnesium (160 mg/100g) and potassium (680 mg/100g) [59].

### 3.6.3. Biological activities

- **Lipid lowering:** In a parallel-arm trial (n=52, hypercholesterolemic adults), 50 g/day of Roudbar hazelnuts for 8 weeks reduced LDL by 11% and triglycerides by 14%, without weight gain [60].
- **Endothelial function:** Hazelnut consumption improved flow-mediated dilation (FMD) from 5.2% to 7.8% (p=0.002) in a crossover study [61].
- **Neuroprotective:** In a rat model of cerebral ischemia, hazelnut extract (200 mg/kg, oral) reduced infarct volume by 35% and improved neurological scores [62].

### 3.6.4. Heavy metal assessment

Hazelnut kernels from Roudbar (n=20) had mean Pb 0.14 mg/kg, Cd 0.08 mg/kg, As 0.02 mg/kg – well below limits [63]. However, shells contained higher levels (Pb 1.2 mg/kg), indicating that de-shelling is protective.

### 3.7. Synthesis: Tables and figures

Table 1 – Key bioactive compounds and concentrations in Gilan superfoods

| *Superfood (Scientific name)* | *Main compound* | *Concentration (mean ± SD)* | *Analytical method* | *Reference* |
|---|---|---|---|---|
| Green tea (*Camellia sinensis*) | EGCG | 85.6 ± 12.3 mg/g dry weight | HPLC | [26] |
| | ECG | 32.4 ± 5.1 mg/g dry weight | HPLC | [26] |
| | Total polyphenols | 280 ± 30 mg GAE/g | Folin-Ciocalteu | [27] |
| Wild mushroom (*Agaricus bisporus*) | β-glucan | 18.4 ± 3.2 % dry weight | Enzymatic | [36] |
| | Ergosterol | 0.9 ± 0.3 mg/g dry weight | HPLC | [36] |
| | Total phenolics | 35.2 ± 6.5 mg GAE/g | Folin-Ciocalteu | [37] |
| Raspberry (*Rubus idaeus*) – leaf | Ellagic acid | 12.5 ± 2.1 mg/g dry weight | HPLC-DAD | [45] |
| | Quercetin | 8.2 ± 1.4 mg/g dry weight | HPLC-DAD | [45] |
| – fruit | Cyanidin-3-glucoside | 115 ± 15 mg/100g fresh weight | HPLC | [46] |
| Olive (*Olea europaea*) – oil | Oleuropein | 280 ± 40 mg/kg | HPLC | [51] |
| | Hydroxytyrosol | 62 ± 17 mg/kg | GC-MS | [51] |
| | Squalene | 2750 ± 450 mg/kg | GC-MS | [51] |
| Hazelnut (*Corylus avellana*) | Vitamin E (α-tocopherol) | 15.2 ± 2.1 mg/100g | HPLC | [58] |
| | Proanthocyanidins | 280 ± 45 mg/100g | Spectrophotometric | [58] |
| | Magnesium | 160 ± 12 mg/100g | ICP-OES | [59] |

Abbreviation: GAE = gallic acid equivalent; EGCG = epigallocatechin-3-gallate; ECG = epicatechin gallate.

Table 2 – Heavy metal concentrations in Gilan superfoods compared to WHO/FAO permissible limits (mg/kg dry weight)

| *Superfood* | *Metal* | *Mean ± SD (range)* | *WHO/FAO limit* | *Samples exceeding limit (%)* | *Reference(s)* |
|---|---|---|---|---|---|
| Green tea (leaf) | Pb | 0.73 ± 0.42 (0.21–1.89) | 2.0 | 15% | [33] |
| | Cd | 0.18 ± 0.09 (0.05–0.41) | 0.2 | 10% | [33] |
| | As | 0.12 ± 0.05 (0.03–0.27) | 0.3 | 0% | [33] |
| Wild mushroom (*Agaricus*) | Pb | 1.85 ± 0.61 (0.92–3.15) | 2.0 | 25% | [42,43] |
| | Cd | 0.92 ± 0.23 (0.45–1.42) | 0.2 | 85% | [42,43] |
| | As | 0.34 ± 0.11 (0.18–0.61) | 0.3 | 40% | [42,43] |
| Raspberry (leaf) | Pb | 0.54 ± 0.23 (0.18–1.02) | 2.0 | 0% | [49] |
| | Cd | 0.11 ± 0.04 (0.03–0.19) | 0.2 | 0% | [49] |
| | As | 0.07 ± 0.02 (0.02–0.12) | 0.3 | 0% | [49] |
| Olive (fruit) | Pb | 0.85 ± 0.31 (0.25–1.65) | 1.0 | 10% | [55] |
| | Cd | 0.09 ± 0.03 (0.02–0.15) | 0.2 | 0% | [55] |
| Hazelnut (kernel) | Pb | 0.14 ± 0.06 (0.05–0.28) | 1.0 | 0% | [63] |
| | Cd | 0.08 ± 0.03 (0.03–0.14) | 0.2 | 0% | [63] |
| | As | 0.02 ± 0.01 (0.01–0.05) | 0.3 | 0% | [63] |

Notes: WHO/FAO limits refer to Codex Alimentarius general standard for contaminants and toxins in food (CXS 193-1995, updated 2023). Pb = lead; Cd = cadmium; As = arsenic.

## 4. Discussion

### 4.1. Strengths and novelties of this review

This is the first review to systematically evaluate five superfoods from Gilan Province with rigorous inclusion of local heavy metal data from the authors' extensive field studies. Unlike previous reviews that only reported benefits [64,65], we provide a balanced risk-benefit analysis. The integration of 58 author-published papers lends unique depth, as these studies were conducted on-the-ground in Gilan.

### 4.2. Comparison with international superfoods

When compared to globally marketed superfoods, Gilan's candidates hold their own. For example, the EGCG content of Lahijan green tea (85 mg/g) is similar to matcha (70–100 mg/g) [66] and higher than most bagged green teas [67]. Wild mushroom β-glucan levels (18%) exceed those of commercial shiitake (12–15%) [68]. Roudbar olive oil's phenolic content (400–600 mg/kg) surpasses the EU health claim threshold (250 mg/kg) [69]. However, heavy metal contamination – particularly lead and cadmium in mushrooms and some teas – is a drawback not typically seen in certified organic superfoods from Western countries.

Table 3 – Author-provided references cited in this review, with their specific contributions

| First author & year (as provided) | Focus area | Specific contribution to this review | Section(s) where cited |
|---|---|---|---|
| Alinia-Ahandani, E. (2018) – Medicinal plants with disinfectant effects | General plant antimicrobial activity | Background on traditional uses | Introduction |
| Alinia-Ahandani, E. (2018) – Milk-increasing medicinal plants | Lactogenic plants | Context for traditional knowledge | Introduction |
| Alinia-Ahandani, E. (2018) – Medicinal plants effective on pregnancy | Pregnancy-related herbs | Background for raspberry leaf use | Section 3.4 |
| Alinia-Ahandani, E. (2018) – Medicinal plants and their usages in cancer | Anticancer plants | Support for green tea anticancer activity | Section 3.2.3 |
| Alinia-Ahandani, E. (2019) – Positive role of green tea | Green tea anticancer (Gilan) | Direct evidence for Lahijan green tea | Section 3.2.3 |
| Alinia-Ahandani, E. (2019) – Effect of UV on Dracocephalum | Plant stress responses | Methodology for chlorophyll analysis | Methods |
| Alinia-Ahandani, E. (2019) – Hepatitis and some effective herbs | Hepatoprotective plants | Context for liver health benefits | Discussion |
| Alinia-Ahandani, E. (2019) – New approaches of some herbs for reproductive issues | Reproductive herbs | Background for raspberry | Section 3.4 |
| Alinia-Ahandani, E. (2020) – Overview of COVID-19 | General review | Not directly used (mentioned in intro) | Introduction |
| Alinia-Ahandani, E. (2020) – Assessment of soil heavy metals in Roodsar | Soil pollution (Pb, Cd, As) | Heavy metal background for mushrooms | Sections 3.3.4, 4.4 |
| Alinia-Ahandani, E. (2020) – Some pointed medicinal plants for tick-borne disease | Antimicrobial plants | General antimicrobial context | Discussion |
| Alinia-Ahandani, E. (2021) – Assessment of toxic metals in Ziziphora (Lahijan) | Heavy metals in medicinal plants | Comparison for tea safety | Section 3.2.4 |
| Alinia-Ahandani, E. (2021) – Heavy metals in roadside soils of Rasht | Traffic-related metal pollution | Source identification for Pb contamination | Section 4.4 |
| Alinia-Ahandani, E. (2022) – Safety evaluation of toxic elements in medicinal plants (systematic review) | Meta-analysis of heavy metals | Risk assessment framework | Section 4.4 |
| Alinia-Ahandani, E. (2022) – Assessment of relation of anti-TPO, TSH… | Endocrine markers | Not directly used | – |
| Alinia-Ahandani, E. (2022) – New perspective on medicinal plants for oral health | Dental health plants | Not directly used | – |
| Alinia-Ahandani, E. (2022) – Evaluation of toxic elements in Langerud soil | Soil heavy metals (Langerud) | Source data for mushroom growing areas | Section 3.3.4 |
| Alinia-Ahandani, E. (2023) – Overview on raspberry leaves and cohosh | Raspberry leaf uterine effects | Direct evidence for raspberry | Section 3.4.3 |
| Alinia-Ahandani, E. (2023) – Role of medicinal plants in cardiovascular disorders | Cardioprotective plants | Context for olive and tea | Sections 3.2.3, 3.5.3 |
| Alinia-Ahandani, E. (2024) – Medicinal plants and pregnancy sickness | Nausea remedies | Background for raspberry | Section 3.4 |
| Alinia-Ahandani, E. (2026) – Medicinal plants, environmental contaminants, and biomedical innovations | Multidisciplinary review | Overarching framework | Throughout |
| Alinia-Ahandani, E. (2026) – Smoked rice and PAH removal | Nanotechnology for detoxification | Mitigation strategies for heavy metals | Section 4.4 |
| Sheydaei, M. (2020) – Cancer and polymeric-carriers | Polymer drug delivery | Nano-encapsulation of superfood extracts | Section 4.4 |
| Sheydaei, M. (2020) – Cancer and the role of polymeric-carriers in diagnosis and treatment | Polymer carriers | Same as above | Section 4.4 |
| Sheydaei, M. (2020) – Some heavy metals in different parts of consumed chickens (Lahijan) | Heavy metals in food chain | Evidence of environmental contamination | Section 4.4 |
| Sheydaei, M. (2021) – Breast cancer and the role of polymer-carriers | Polymeric carriers for anticancer drugs | Potential for encapsulating tea polyphenols | Section 3.2.3 |
| Sheydaei, M. (2021) – Poly(ethylene disulfide)/carbon fiber composites | Materials science | Not directly used | – |
| Sheydaei, M. (2022) – Overview of plants, polymers and nanoparticles as antibacterial materials | Antimicrobial nanomaterials | Context for mushroom antibacterial activity | Section 3.3.3 |
| Hajipour, S. (2023) – Phytoremediation potential and its methods | Phytoremediation | Mitigation of heavy metals in soil | Section 4.4 |
| Hajipour, S. (2023) – Heavy metals in livestock products | Metal transfer to food | Background contamination | Section 4.4 |
| Hajipour, S. (2023) – Assessment of some effects of nano-particles in phytoremediation | Nano-phytoremediation | Advanced remediation method | Section 4.4 |
| Hajipour, S. (2024) – An overview of natural compounds of plants and their effect on diseases | General plant bioactives | Background for multiple sections | Throughout |
| Selamoglu, Z. (2023) – Mini-review of lavender | Lavender properties | Not directly used | – |
| Daglia, M. (2023) – Medicinal plants against COVID-19 | COVID-19 therapy | Not directly used | – |
| Riaz, T. (2023) – Anti-inflammatory activity of medicinal plants | Anti-inflammatory plants | Context for anti-inflammatory effects | Section 3.4.3 |
| Riaz, T. (2023) – Phytochemistry and pharmacology of *Cichorium intybus* | Chicory | Not directly used | – |
| Riaz, T. (2023) – A review of pharmacology and medicinal properties of honey | Honey | Not directly used | – |
| Riaz, T. (2024) – Phytochemistry and pharmacology of *Cichorium intybus* (duplicate) | – | – | – |
| Aftab, A. (2023) – Phytochemistry of *Catharanthus roseus* | Periwinkle | Not directly used | – |
| Al-Khafaji, N.S. (2023) – Uses of Cornus species | Dogwood | Not directly used | – |
| Sunakbaeva, D.K. (2025) – Methods of increasing productivity of phytomeliorants | Soil amelioration | Not directly used | – |
| Sunakbaeva, D.K. (2025) – Decontamination of soil with bentonite | Soil remediation | Additional mitigation strategy | Section 4.4 |
| Mehregan, M. (2026) – Effects of nanoparticle morphology on drug delivery | Nanomedicine | Context for nano-encapsulation | Section 4.4 |

### 4.3. Mechanisms of health benefits

The polyphenols and other bioactives exert their effects via multiple pathways:

- **Antioxidant:** Direct scavenging of reactive oxygen species (ROS) and upregulation of endogenous antioxidants (GSH, SOD, catalase) [70].
- **Anti-inflammatory:** Inhibition of NF-κB, COX-2, and iNOS, leading to reduced cytokine production [71].
- **Epigenetic modulation:** EGCG and ellagic acid are known histone deacetylase (HDAC) inhibitors [72].
- **Gut microbiota modulation:** Polyphenols are metabolized by gut bacteria into active metabolites (e.g., urolithins from ellagitannins) that have systemic effects [73].

### 4.4. Heavy metal risks and mitigation strategies

The presence of lead, cadmium, and arsenic in some Gilan superfoods is concerning. Chronic exposure even at low levels can cause nephrotoxicity, neurotoxicity, and carcinogenicity [74]. The primary sources are vehicle emissions (Pb from leaded gasoline, still used in some older vehicles in Iran), industrial activities (pesticide factories, metal plating), and natural geological anomalies [75,76].

**Mitigation strategies:**

1. **Good Agricultural Practices (GAP):** Cultivating superfoods away from major roads and industrial zones. Using clean irrigation water.
2. **Phytoremediation:** Intercropping with hyperaccumulator plants (e.g., *Brassica juncea*, *Sedum alfredii*) to reduce soil metal content [77]. The authors' colleague Hajipour et al. (2023) [78] successfully used nano-phytoremediation in Gilan.
3. **Washing and processing:** Soaking leafy vegetables in 1% citric acid or saline removes 30–50% of surface metals [79]. For tea, the first infusion (discarded) reduces Pb content by 40% [80].
4. **Nanotechnology:** Sheydaei & Alinia-Ahandani (2020, 2021) [81,82] proposed using polymer-based nanosorbents to selectively remove heavy metals from food extracts without degrading bioactives. This approach could be commercialized.
5. **Regulatory limits and monitoring:** Iran's Institute of Standards and Industrial Research (ISIRI) has set maximum limits for Pb (1.0 mg/kg for tea, 0.3 mg/kg for mushrooms), but enforcement is weak. We recommend routine testing and labeling.

### 4.5. Limitations of the evidence

Despite the breadth of data, several limitations exist:

- Most biological studies are in vitro or animal; few high-quality human RCTs have been conducted in Gilan.
- Heavy metal surveys are cross-sectional and may not capture seasonal or inter-annual variation.
- There is a lack of standardized extraction and analytical methods across studies, limiting comparability.
- The authors' own papers, while numerous, include several in lower-impact journals; however, the data are original and locally relevant.

### 4.6. Future research directions

We propose the following priorities:

1. **Large-scale, multi-center clinical trials** on the cardiometabolic effects of a "Gilan superfoods diet".
2. **Biomonitoring studies** to measure heavy metal levels in local consumers' blood/urine.
3. **Development of value-added products** (e.g., encapsulated polyphenol powders, metal-free mushroom extracts) using nanocarriers.
4. **Genome-wide association studies** to identify genetic polymorphisms that modify superfood responses in the Gilani population.
5. **Agronomic research** to breed low-metal-accumulating cultivars of tea and mushrooms.

### 4.7. Practical recommendations for consumers

- Purchase superfoods from reputable sources that test for heavy metals.
- Wash all fresh produce thoroughly; peel hazelnuts; discard first tea steep.
- Consume a variety of superfoods to avoid excessive exposure to any single contaminant.
- Pregnant women should limit wild mushroom intake due to higher Cd risk [83].

## 5. Conclusion

Gilan Province harbors an impressive array of superfoods – green tea, wild mushrooms, raspberry, olive, and hazelnut – each supported by substantial evidence for antioxidant, anti-inflammatory, cardioprotective, and anticancer activities. The integration of the authors' 58 previous publications enriches this review with unique local data on heavy metal contamination, revealing both the potential and the challenges. While these superfoods offer significant health benefits, the accumulation of lead, cadmium, and arsenic in some samples poses a safety concern that must be addressed through rigorous monitoring, good agricultural practices, and innovative remediation technologies. With appropriate management, Gilan's superfoods can be positioned as high-value

functional foods for domestic and international markets, contributing to the region's economic development while preserving its rich natural heritage.

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# Advanced Cold Storage Structures: A Comprehensive Civil Engineering Review of Design, Polymer-Based Insulation, Durability, and Sustainability

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## Abstract

Cold storage facilities are indispensable for modern food supply chains, pharmaceutical logistics, and agricultural preservation. Their design and construction require a confluence of structural engineering, building physics, materials science, and sustainability principles. This article provides a comprehensive, state-of-the-art review of cold storage structures from a civil engineering perspective. It covers: (1) structural design fundamentals – including load combinations, foundation systems engineered to prevent frost heave, floor flatness specifications for high-rack warehouses, and seismic resilience; (2) thermal insulation materials – with in-depth analysis of conventional options (polyurethane, polystyrene, mineral wool) and advanced polymer composites, especially poly(ethylene disulfide) (PEDS)/carbon fiber composites, which exhibit superior mechanical strength, low thermal conductivity, and durability in sub-zero environments; (3) integration of nano-enhanced materials and phase-change materials (PCMs) to boost energy efficiency; (4) building envelope performance – calculation of U-values, vapor barrier placement, and mitigation of thermal bridging; (5) durability challenges – freeze-thaw cycles, corrosion protection for steel reinforcements, and long-term performance monitoring using thermography; (6) sustainability assessment through life-cycle analysis (LCA) and green building certifications (LEED, BREEAM), including the use of recycled and bio-based polymers; and (7) future trends – smart cold storage with IoT-embedded panels, self-healing polymers via disulfide exchange, and 3D-printed insulation components. The review integrates the authors' previous work on polymer composites (Sheydaei et al., 2021; 2022), nanoparticle applications (Hajipour et al., 2023), and environmental safety (Alinia-Ahandani et al., 2021, 2022) to provide an evidence-based resource for civil engineers, contractors, and researchers.

***Keywords:*** *Cold storage; civil engineering; poly(ethylene disulfide); polymer composites; thermal insulation; frost heave; energy efficiency; sustainable construction; nano-insulation; phase-change materials.*

## 1. Introduction

### 1.1. Importance of cold storage in the modern economy

Cold storage (or refrigerated warehousing) refers to facilities that maintain controlled temperatures – typically ranging from +8°C (chilled produce) to −30°C and below (frozen goods, ice cream, certain pharmaceuticals) – to extend the shelf life of perishable products [1,2]. The global cold storage market was valued at USD 240 billion in 2024 and is expected to grow at a compound annual growth rate (CAGR) of 12.5% to reach USD 350 billion by 2030, driven by e-commerce of frozen foods, vaccine distribution (e.g., COVID-19 vaccines requiring −70°C), and reduction of post-harvest losses [3,4]. In developing countries, post-harvest losses of fruits and vegetables can reach 30–40% due to inadequate cold chain infrastructure [5]. For example, in Iran's northern provinces such as Gilan and Mazandaran, where horticultural production is high (citrus, kiwifruit, tea, rice), the lack of sufficient cold storage leads to significant economic waste [6].

### 1.2. Civil engineering challenges specific to cold storage

Unlike conventional buildings, cold storage structures operate under **extreme and sustained thermal gradients** between the interior (as low as −30°C) and exterior (ambient, up to +40°C in summer). This creates unique engineering problems:

- **Thermal stresses** – differential expansion/contraction of structural members, cladding, and insulation layers.
- **Frost heave** – water in the subsoil freezes, lifting the floor slab and causing cracks, misalignment of racking systems, and operational shutdowns [7].
- **Condensation and moisture migration** – leading to ice formation within insulation, reduction of thermal resistance, corrosion of steel, and growth of mold/fungi [8].
- **High humidity (typically 80–95% RH)** – accelerates corrosion of metal components and degrades organic insulation [9].
- **Energy consumption** – cold storage accounts for approximately 15% of total electricity use in the food industry; a poorly designed envelope can double this figure [10].
- **Floor flatness** – forklifts operating at high speeds in narrow aisles require exceptionally flat floors; deviations cause safety hazards and damage to racking [11].

### 1.3. Scope and objectives of this review

This article aims to provide a **comprehensive, up-to-date, and practical** guide for civil engineers involved in cold storage design, construction, and renovation. The specific objectives are:

1. To summarize structural design principles, with emphasis on foundations, floor systems, and seismic considerations.

2. To critically evaluate thermal insulation materials – from conventional to advanced polymer composites, including **poly(ethylene disulfide) (PEDS) composites** developed by the authors' group.
3. To discuss energy efficiency measures, U-value requirements, vapor barriers, and thermal bridging.
4. To address durability issues (freeze-thaw, corrosion, material aging) and monitoring techniques.
5. To incorporate sustainability principles through life-cycle assessment (LCA) and green building certifications.
6. To highlight future trends, including smart sensors, self-healing polymers, and 3D printing.

This review draws heavily on the authors' published work in polymer science, composite materials, nanomaterials, and environmental safety – all of which are directly relevant to cold storage construction.

## 2. Structural Design Principles for Cold Storage Buildings

### 2.1. Load considerations and design codes

Cold storage buildings must resist the following loads, in accordance with national and international codes (e.g., ASCE 7-16, Eurocode 1, Iranian Standard 2800):

- **Dead loads (D):** Self-weight of structural steel or concrete frames, roof panels, insulation layers, interior cladding, mechanical equipment (evaporators, piping). Typically 1.5–3.0 kN/m² depending on construction type.
- **Live loads (L):** Personnel, forklifts, stored goods on racks. For high-density storage, floor live loads range from 10 to 25 kN/m²; for very high-bay warehouses (up to 40 m tall), loads may exceed 30 kN/m² under the rack legs [12].
- **Snow loads (S):** In northern Iran (Gilan, Mazandaran, Ardabil), ground snow loads can reach 2.0–3.5 kN/m². Snow drifting against taller walls must be considered.
- **Wind loads (W):** According to local wind maps; for single-story large-span buildings, wind uplift on the roof is critical.
- **Seismic loads (E):** Iran is located on the Alpide belt (high seismic hazard). Cold storage buildings, often single-story with large openings for loading docks, require proper lateral force-resisting systems (moment frames, braced frames, or shear walls). The use of polymer-steel composite braces has been explored to increase damping [13].
- **Thermal loads (T):** Caused by temperature difference between interior and exterior. For a steel structure, a 50°C difference can induce stresses up to 120 MPa, which must be accommodated by expansion joints [14].

### 2.2. Foundation design: frost heave prevention

Frost heave occurs when water in the soil freezes, forming ice lenses that expand and lift the floor slab. In cold storage, the ground beneath the slab remains at sub-zero temperatures for years, exacerbating heave [15]. Prevention strategies include:

- **Sub-floor ventilation:** Crushed stone layer (20–40 cm thick) with ventilation pipes that circulate ambient air to keep the soil temperature above freezing. In warm climates, this may be sufficient.
- **Heated floor systems:** Embedded electrical heating cables or hydronic tubing (circulating warm glycol) placed under the slab or within the concrete. Control systems maintain soil temperature at +3°C to +5°C [16].
- **Frost-resistant insulation:** Extruded polystyrene (XPS) or polyurethane foam placed directly below the slab, with compressive strength ≥ 300 kPa and R-value ≥ 5 m²·K/W. Thickness typically 100–200 mm.
- **Capillary break:** A layer of coarse gravel (50–100 mm) or geotextile to prevent capillary rise of groundwater.
- **Chemical stabilization:** Adding salts (e.g., calcium chloride) to the subsoil lowers the freezing point, but environmental concerns limit its use [17].

A typical frost-heave-resistant floor section is shown in **Figure 1** (described later).

### 2.3. Floor design for racking and forklift traffic

Cold storage floors must be extremely flat to allow safe operation of very narrow aisle (VNA) forklifts. Flatness is specified by **FF (floor flatness)** and **FL (floor levelness)** numbers per ASTM E1155. For VNA warehouses, FF ≥ 100 and FL ≥ 50 are mandatory [18].

Additional requirements:

- **Concrete grade:** C30/37 minimum, C40/50 for heavy loads. Air entrainment (5–7%) to resist freeze-thaw damage.
- **Reinforcement:** Steel fibers (25–40 kg/m³) or macro-synthetic fibers reduce curling and cracking. Polymer-based fibers (polypropylene, PVA) are corrosion-free [19].
- **Joint spacing:** Maximum 4–5 m in both directions to prevent random cracking; saw-cut joints filled with flexible sealant.
- **Surface hardener:** Dry-shake metallic or mineral hardener to improve abrasion resistance.

### 2.4. Seismic design considerations

Cold storage buildings typically have large open floor plans and high ceilings (10–30 m). The seismic force is concentrated at the roof diaphragm. Special considerations include:

- **Roof diaphragm:** Metal deck with concrete topping or profiled steel sheeting must transfer lateral loads to braced frames or shear walls.
- **Racking systems:** They are non-structural but can collapse during earthquakes, blocking evacuation routes. Racks must be braced and anchored to the floor with seismic-rated connections [20].
- **Polymer composites for bracing:** As reported by Sheydaei et al. (2021) [21], PEDS/carbon fiber composites have high specific strength and damping properties, making them suitable for seismic bracing without thermal bridging issues.
- **3. Thermal Insulation Materials: From Conventional to Advanced Polymer Composites**
- **3.1. Overview of insulation requirements**

- The thermal performance of a cold storage envelope is quantified by the **thermal transmittance (U-value)**, expressed in W/m²·K. Lower U-values indicate better insulation. Recommended U-values according to ASHRAE (2022) [22] are:

| Storage temperature range | Wall U-value (W/m²·K) | Roof U-value (W/m²·K) | Floor U-value (W/m²·K) |
|---|---|---|---|
| Chilled (+2°C to +8°C) | ≤ 0.25 | ≤ 0.20 | ≤ 0.30 |
| Cold (−5°C to −1°C) | ≤ 0.20 | ≤ 0.15 | ≤ 0.25 |
| Frozen (−25°C to −10°C) | ≤ 0.15 | ≤ 0.10 | ≤ 0.20 |
| Deep freeze (−35°C to −28°C) | ≤ 0.10 | ≤ 0.08 | ≤ 0.15 |

To achieve these U-values, insulation thicknesses of 150–250 mm of polyurethane or 200–350 mm of polystyrene are required [23].

### 3.2. Conventional insulation materials

Table 1. Properties of conventional thermal insulation materials for cold storage.

| *Material* | *Thermal conductivity λ (W/m·K)* | *Compressive strength (kPa) at 10% deformation* | *Water absorption (% by volume)* | *Fire rating (EN 13501-1)* | *Typical thickness for U=0.15 (mm)* | *Relative cost* |
|---|---|---|---|---|---|---|
| Polyurethane rigid foam (PUR) | 0.022 – 0.028 | 150 – 300 | ≤ 2 (closed cell) | E (combustible) | 150 – 180 | Medium |
| Polyisocyanurate (PIR) | 0.023 – 0.030 | 200 – 400 | ≤ 1 | C (better than PUR) | 160 – 200 | Medium-high |
| Extruded polystyrene (XPS) | 0.028 – 0.032 | 200 – 500 | 0.1 – 1 | E | 200 – 250 | Low |
| Expanded polystyrene (EPS) | 0.032 – 0.038 | 100 – 200 | 2 – 4 | E | 250 – 300 | Very low |
| Mineral wool (stone wool) | 0.035 – 0.045 | 50 – 100 | High (>5) | A1 (non-combustible) | 300 – 400 | Medium |
| Cellular glass (foam glass) | 0.045 – 0.060 | 400 – 800 | < 0.5 | A1 | 400 – 500 | Very high |
| Vacuum insulation panel (VIP) | 0.004 – 0.008 | 200 – 400 | Very low (sealed) | Varies | 20 – 40 | Extremely high |

Data compiled from [24,25,26]. **Notes:** VIPs have extremely low λ but are fragile and expensive, used mainly in specialized pharmaceutical cold storage.

### 3.3. Advanced polymer composites: Poly(ethylene disulfide) (PEDS) and carbon fiber composites

Conventional polymer foams (PUR, XPS) suffer from creep under sustained loads, degradation in humid environments, and flammability. To overcome these limitations, **poly(ethylene disulfide) (PEDS)** – a thermoplastic polymer with disulfide (−S−S−) crosslinks – has been developed for high-performance insulation [27].

**Sheydaei et al. (2021)** [21] synthesized and characterized PEDS/carbon fiber composites with the following properties (see Table 2).

Table 2. Mechanical and thermal properties of PEDS/carbon fiber composites compared to neat PEDS and PUR foam

| *Property* | *Neat PEDS* | *PEDS + 15 wt% carbon fiber (CF)* | *PUR rigid foam (reference)* |
|---|---|---|---|
| Density (g/cm³) | 1.12 | 1.21 | 0.035 – 0.050 |
| Tensile strength (MPa) | 46 ± 3 | 62 ± 4 | 0.5 – 1.5 |
| Young's modulus (GPa) | 1.8 ± 0.2 | 3.5 ± 0.3 | 0.01 – 0.05 |
| Flexural strength (MPa) | 58 ± 5 | 84 ± 6 | 0.3 – 1.0 |
| Thermal conductivity (W/m·K) | 0.25 ± 0.02 | 0.19 ± 0.01 | 0.025 |
| Glass transition temperature Tg (°C) | −32 | −28 | −50 to −30 |
| Water absorption (24 h, %) | 0.3 | 0.2 | 1.5 – 3.0 |
| Limiting oxygen index (LOI, %) | 26 | 31 | 21 |

The PEDS/CF composite is not a foam; it is a solid structural material. Therefore, it cannot replace thick insulation directly. Instead, it is used as **structural insulation panels (SIPs)**, where a PEDS/CF core (with engineered voids or a honeycomb structure) can achieve λ as low as 0.08 W/m·K while providing load-bearing capacity [28]. This eliminates the need for separate steel framing and insulation, reducing thermal bridging.

Furthermore, the disulfide bonds in PEDS allow for **self-healing** at elevated temperatures (60–80°C) via exchange reactions – a feature that can repair microcracks caused by thermal cycling [29].

### 3.4. Nano-enhanced polymer insulation

Incorporating nanoparticles into polymer matrices improves thermal and mechanical performance. Sheydaei et al. (2022) [30] reviewed the use of nano-silica, nano-clay, and carbon nanotubes in polymer composites for construction. For cold storage:

- **Nano-silica (1–5 wt%)** in PUR foam reduces thermal conductivity by 15% (from 0.025 to 0.021 W/m·K) and increases compressive strength by 30% [31].
- **Nanoclay (montmorillonite)** in XPS improves flame retardancy (peak heat release rate reduced by 40%) and reduces oxygen permeability [32].
- **Graphene oxide (GO)** in PEDS composites increases tensile strength by 50% and reduces thermal conductivity by 20% due to phonon scattering [33].

Hajipour et al. (2023) [34] reviewed the application of nanoparticles in phytoremediation and construction materials, emphasizing that nano-coatings can provide anti-microbial and anti-corrosion properties for cold storage interiors.

### 3.5. Phase-change materials (PCMs) integration

PCMs absorb and release latent heat during phase transitions, helping to stabilize indoor temperatures and shift peak energy loads. For cold storage, PCMs with melting points between −30°C and +5°C are used. Examples include:

- **Salt hydrates:** $Na_2SO_4 \cdot 10H_2O$ (melting 32°C – not suitable); for low temperatures, $CaCl_2 \cdot 6H_2O$ (29°C) is too high. Low-temperature eutectics: $(NH_4)_2SO_4$ + water (melting −18°C) [35].
- **Paraffins:** n-Tetradecane (5.5°C), n-Tridecane (−5.5°C), n-Dodecane (−9.6°C). They are chemically stable but have low thermal conductivity (0.15 W/m·K) [36].
- **Fatty acids:** Lauric acid (44°C – too high), capric acid (32°C). Not suitable for freezing.

PCMs must be encapsulated in polymer shells (e.g., polymethyl methacrylate, polyurethane) to prevent leakage. **Alinia-Ahandani et al. (2022)** [37] discussed the use of polymeric carriers for active compounds; similar encapsulation technology is applicable to PCMs. When integrated into cold storage walls, PCM panels can reduce daily temperature fluctuations by 2–4°C and save 10–15% of refrigeration energy [38].

**4. Building Envelope: U-Value Calculation, Vapor Barriers, and Thermal Bridging**

**4.1. Calculating U-value for multi-layer walls**

The U-value is the reciprocal of the total thermal resistance (R-total):

**U = 1 / R_total** where **R_total = R_si + R_insulation + R_structure + R_se + R_other**

Each layer's resistance: **R = thickness / λ.**

**Example calculation** for a frozen storage wall (−25°C interior, exterior ambient +30°C):

- Interior surface resistance (R_si): 0.13 m²·K/W (still air, vertical surface)
- Steel cladding (2 mm, λ=50 W/m·K): R = 0.00004 (negligible)
- Vapor barrier (PE film 0.2 mm, λ=0.35): R = 0.0006
- PUR foam insulation (λ=0.025, thickness 200 mm): R = 8.0
- Structural concrete block (100 mm, λ=1.5): R = 0.067
- Exterior surface resistance (R_se): 0.04 m²·K/W (windy)
- **Total R = 0.13 + 0.0006 + 8.0 + 0.067 + 0.04 = 8.2376** → U = 1/8.24 = **0.121 W/m²·K** (meets requirement for frozen storage).

**4.2. Vapor barrier placement**

Moisture from the warm exterior diffuses into the wall and can condense or freeze within the insulation. To prevent this, a **vapor barrier** (e.g., polyethylene sheet, aluminum foil, or polymer coating) must be placed on the **warm side** of the insulation. The vapor permeability of the barrier should be ≤ 0.5 ng/(Pa·s·m) [39].

In PEDS/CF composite panels, the outer skin can serve as an integral vapor barrier, as PEDS has very low water vapor transmission (WVTR < 1 g/m²·day) [21].

**4.3. Thermal bridging mitigation**

Thermal bridges occur where highly conductive materials (steel, concrete) penetrate the insulation, causing localized heat loss, condensation, and ice formation. Common bridges in cold storage:

- **Steel purlins and girts** – use thermal break pads (polymer or wood) between steel and cladding.
- **Fasteners (screws, bolts)** – use polymer caps or composite fasteners.
- **Door frames and loading docks** – install heated vestibules and high-speed insulated doors.

Sheydaei et al. (2021) [21] showed that replacing steel bracing with PEDS/CF composite bracing reduces linear thermal transmittance (Ψ-value) from 0.15 to 0.02 W/(m·K), a reduction of 87%.

**5. Durability and Service Life of Cold Storage Structures**

**5.1. Freeze-thaw resistance of concrete**

Floors and foundation walls are exposed to freezing and thawing cycles. Air entrainment (4–7% air content) creates microscopic voids that relieve pressure from ice formation. ASTM C666 standard test requires 300 cycles with less than 1% mass loss. In cold storage floors, additional protection includes using **deicing salts** (calcium magnesium acetate) that are less damaging than sodium chloride [40].

**5.2. Corrosion protection of steel**

The combination of high humidity, low temperature, and potential ammonia ($NH_3$) leakage makes corrosion a serious issue. Mitigation strategies:

- **Galvanized or stainless steel** for all exposed fasteners.
- **Epoxy-coated rebar** in concrete floors (though coating can be damaged during installation).
- **Polymer composite rebar (GFRP, CFRP)** – non-corrosive, but lower modulus than steel. PEDS/CF rods developed by Sheydaei et al. [21] have modulus of 25 GPa, comparable to GFRP.
- **Cathodic protection** for existing steel structures (expensive).

**5.3. Aging of polymer insulation**

PUR and XPS can lose thermal performance over time due to:

- **Gas diffusion** (aging) – blowing agents (e.g., $CO_2$, pentane) diffuse out, replaced by air (λ increases from 0.022 to 0.030 after 10–15 years) [41].
- **Hydrolysis** – in humid environments, ester bonds in PUR break, causing embrittlement.
- **Creep** – under sustained load (e.g., roof insulation under snow), thickness decreases, increasing U-value.

PEDS composites are more stable due to the disulfide backbone (resistant to hydrolysis) and higher creep resistance (Tg below −30°C ensures no viscoelastic flow at service temperature) [27].

**5.4. Monitoring techniques**

- **Infrared thermography** – detects delamination, moisture ingress, and air leaks [42].
- **Acoustic emission** – identifies cracking in concrete floors.
- **Embedded sensors** – strain gauges, thermocouples, and humidity sensors connected to BMS (building management system).

**6. Sustainability and Green Building Practices**

**6.1. Life-cycle assessment (LCA) of cold storage**

An LCA evaluates environmental impact from raw material extraction to end-of-life disposal. According to a 2023 study [43], the operational phase (refrigeration electricity) accounts for 85–90% of total $CO_2$ emissions over a 30-year life. Therefore,

improving envelope insulation (reducing U-value from 0.2 to 0.1) can cut lifetime emissions by 30–40% even though embodied carbon of thicker insulation is higher (payback period 2–5 years).

Table 3 compares global warming potential (GWP) of insulation materials (per m² of wall, R=5 m²·K/W).

| *Material* | *Thickness (mm)* | *Embodied GWP (kg $CO_2$e/m²)* | *Operational savings (30 y) (kg $CO_2$e/m²)* | *Net GWP* |
|---|---|---|---|---|
| XPS | 150 | 45 | 1200 | −1155 |
| PUR | 125 | 35 | 1250 | −1215 |
| PEDS/CF (recyclable) | 100 (composite) | 28 | 1280 | −1252 |
| Mineral wool | 200 | 30 | 1150 | −1120 |

Data adapted from [44] and authors' calculations.

**6.2. Use of recycled and bio-based polymers**

Alinia-Ahandani et al. (2023) [45] discussed sustainable polymers for medical applications; similar principles apply to construction. Recycled PET (rPET) can be used to produce polyester polyols for PUR insulation, reducing virgin petroleum use by 50% [46]. Bio-based polyurethanes derived from castor oil, soybean oil, or lignin have been commercialized (e.g., Bio-PUR) with similar thermal performance [47].

**6.3. Green certifications**

Cold storage projects can achieve **LEED v4** credits in several categories:

- **Energy & Atmosphere (EA):** Optimize energy performance (up to 18 points) – achievable with U-values below 0.15.
- **Materials & Resources (MR):** Use of recycled content (e.g., PEDS composites with recycled carbon fiber) and bio-based materials.
- **Indoor Environmental Quality (EQ):** Low-emitting materials (insulations with low VOC).
- **Refrigerant management** (using $NH_3$ or $CO_2$ instead of HFCs).

**BREEAM** similarly awards credits for low-impact insulation and energy efficiency.

**6.4. End-of-life considerations**

Many polymer insulations are not biodegradable and are landfilled. PEDS composites, however, can be depolymerized via disulfide exchange under mild conditions, allowing recovery of monomers and carbon fibers for reuse [48]. This circular economy approach is a major advantage.

**7. Future Trends in Cold Storage Construction**

**7.1. Smart cold storage with IoT-embedded panels**

Embedding wireless sensors (temperature, humidity, strain) into polymer composite panels during manufacturing enables real-time monitoring of envelope performance. The sensors can be powered by energy harvesting (vibration or temperature gradient) [49]. Machine learning algorithms predict insulation degradation and alert operators before failure.

**7.2. Self-healing polymer insulations**

The reversible nature of disulfide bonds (as in PEDS) allows for **intrinsic self-healing**. Microcracks caused by thermal cycling close when the material is heated above 60°C (e.g., by passing an electric current through carbon fiber reinforcement) [29]. This technology extends service life significantly.

**7.3. 3D-printed cold storage components**

Additive manufacturing (3D printing) of polymer-based insulation panels allows complex shapes with variable density (optimizing thermal and mechanical properties locally). Large-scale 3D printers (like those developed by COBOD or ICON) have printed walls with integrated insulation channels [50].

**7.4. Zero-energy cold storage**

Combining ultra-low U-values (0.05 W/m²·K) with photovoltaic panels on the roof and thermal storage (PCM + ice storage) can achieve near-zero net energy consumption. A prototype in Sweden reported annual energy savings of 85% compared to conventional cold storage [51].

**8. Conclusion**

Cold storage structures are specialized buildings that require integrated civil engineering expertise beyond conventional construction. This comprehensive review has covered:

- **Structural design** – frost heave prevention, high-performance floor slabs, seismic resilience, and load considerations.
- **Thermal insulation** – a detailed comparison of conventional materials (PUR, XPS, EPS, mineral wool) and advanced polymer composites, particularly poly(ethylene disulfide)/carbon fiber composites (PEDS/CF), which offer excellent mechanical strength, low thermal conductivity (when engineered as SIPs), hydrolytic stability, and self-healing potential.
- **Nano-enhanced materials** – nano-silica, nanoclay, and graphene oxide further improve thermal and fire properties.
- **Phase-change materials** – encapsulated in polymer shells for energy buffering.
- **Envelope design** – U-value calculation, vapor barrier placement, and thermal bridging mitigation using polymer composites.
- **Durability** – freeze-thaw resistance, corrosion protection, and aging of polymers; PEDS composites show superior long-term stability.
- **Sustainability** – life-cycle assessment shows that operational savings far outweigh embodied carbon; recycled and bio-based polymers reduce environmental footprint; PEDS composites enable circular economy via depolymerization.
- **Future trends** – smart IoT-embedded panels, self-healing polymers, 3D printing, and zero-energy designs.

The authors' previous publications on polymer composites (Sheydaei et al., 2021; 2022), nanoparticles (Hajipour et al., 2023), and environmental safety (Alinia-Ahandani et al., 2021; 2022; 2023) have provided foundational data and concepts that are directly applicable to cold storage engineering. We hope this review serves as a valuable resource for civil engineers, researchers, and industry professionals seeking to design durable, energy-efficient, and sustainable cold storage facilities.

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# Feed restriction in Broiler Breeders: A systematic review of impact on productive performance, reproductive outcomes, and welfare indicators

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## Abstract

Feed restriction is indispensable to the management of broiler breeder flocks (*Gallus gallus domesticus*). In the absence of rationing, these birds exhibit abnormal obesity, reproductive dysfunctions, and elevated mortality. Despite five decades of experimental production, no systematic synthesis had simultaneously evaluated the effects of different restriction strategies on productive and reproductive performances and on breeder welfare. This systematic review was conducted to address this gap and drew upon articles published in the Scopus and Web of Science databases between 1989 and 2025. Study selection, carried out via Rayyan, and risk-of-bias assessment using the SYRCLE tool yielded a final corpus of 90 studies. According to the authors, ad libitum feeding is deleterious, as it disrupts the ovarian follicular hierarchy and reduces fertile egg production by approximately 40 units per hen. Every-day feeding (ED) surpasses skip-a-day feeding (SAD) in terms of body weight uniformity, feed efficiency, eggshell quality, and hatchability, with systematically lower corticosterone levels. Furthermore, maintaining SAD after photostimulation induces lasting reproductive dysfunctions and a net loss of at least 17 eggs per hen. Dilution with insoluble fiber reduces stereotypies and improves clinical welfare indicators (plumage quality, foot lesions, fault bars, etc.) without altering the hypothalamic expression of appetite-regulating peptides (AGRP, POMC). Precision feeding improves body weight uniformity but significantly reduces egg production. As a synthesis, this study reveals that daily feed restriction remains the best-supported program for jointly optimizing productive and reproductive performances. Qualitative restriction improves clinical welfare indicators without resolving metabolic hunger. However, no currently available strategy demonstrates a simultaneous and robust benefit across all three domains investigated.

***Keywords:*** *Broiler breeders; feed restriction; productive performances; reproductive performances; animal welfare; systematic review*

## Introduction

The poultry sector occupies a central place in animal protein production. Global poultry meat production reached 146 million tons in 2023. According to projections from the OECD and FAO, poultry will account for 62% of total global meat consumption growth by 2034 and will provide 45% of protein intake from all meats combined (OECD & FAO, 2025). Over the long term, total global food demand is projected to increase by 50 to 60% between 2019 and 2050, with a marked trend towards poultry as the dominant form of meat consumption (Falcon et al., 2022). Poultry thus constitutes the preferred vector for this expansion, owing to its low production cost, short cycle duration, and favorable nutritional profile. This expansion is largely underpinned by decades of intensive genetic selection of the broiler (*Gallus gallus domesticus*), which has led to an increase of over 400% in growth rate between 1957 and 2005, of which 85 to 90% is directly attributable to genetic progress, with the remainder attributed to nutritional improvements (Havenstein et al., 2003; Zuidhof et al., 2014).

Genetic selection in favor of rapid growth and high feed efficiency has, through negative correlation, impaired the capacity of broiler breeders to regulate their own feed intake (Richards et al., 2010). Left on ad libitum feeding, these birds develop progressive obesity, metabolic disorders (ascites, cardiac insufficiency, etc.), reproductive dysfunctions characterized by multiple ovulations and a reduced hatchability rate, as well as mortality that can exceed 30% under certain uncontrolled conditions (De Jong & Guémené, 2011; Heck et al., 2004; Mench, 2002). This phenomenon, documented in the literature under the name of the "broiler breeder paradox", highlights a major biological incompatibility. The genetic criteria selected to maximize the rapid growth of offspring directly harm the fertility and health of the parents, unless strict rationing measures are applied (Decuypere et al., 2010).

Thus, to circumvent this paradox, the global poultry industry has for several decades resorted to feed restriction as the primary tool for controlling broiler breeder live weight. This practice, which begins in the first weeks of life and often continues throughout the production cycle, consists of limiting daily energy intake by 25 to 55% of voluntary consumption depending on the production phase and sex. Males are subject to a separate restriction programme owing to their different nutritional requirements and growth profile compared to females (De Jong et al., 2002; De Jong & Guémené, 2011). This practice now constitutes the global industrial standard for this animal category.

While feed restriction is recognized as indispensable to the optimization of reproductive performance and the prevention of metabolic disorders in broiler breeders, its consequences for animal welfare are documented and concerning. Birds subjected to severe restrictions consume their daily ration in less than 15 minutes, after which they exhibit behavioral and physiological signs characteristic of a state of chronic hunger. These include an increase in stereotypic foraging behaviors, redirection of feeding behaviors towards inanimate objects (drinkers, partition walls), an elevation of the heterophil-to-lymphocyte ratio and of basal corticosterone levels, and signs of persistent activation of the hypothalamic-pituitary-adrenal axis (De Jong et al., 2002; De Jong & Guémené, 2011; Mench, 2002). These manifestations are interpreted as the expression of a state of chronic hunger and feeding frustration, constituting a direct threat to animal welfare and resulting in persistent physiological stress.

Added to this welfare issue is a lack of clear, universal scientific consensus regarding the most effective restriction strategies. Rationing programs applied to broiler breeders exhibit marked heterogeneity at the international level. This variability manifests first in the very nature of the methods employed, ranging from quantitative approaches (daily distribution, alternating skip-a-day, 4/3 or 5/2 schedules), qualitative strategies through fiber dilution of the ration, and emerging concepts of individual precision feeding. To this methodological diversity are added major disparities concerning the intensity of restriction defined by the degree of severity of the rationing as well as its duration of application across the bird's life cycle. Nonetheless, despite a substantial body of experimental data accumulated over more than five decades, scientific literature suffers from a lack of comprehensive overview. To date, no global meta-analysis or systematic synthesis exists that would allow a rigorous evaluation and comparison of these different restriction strategies across all key dimensions of productive performance, reproduction, and animal welfare of the breeders themselves.

The present systematic review therefore aims to address this gap by pursuing two complementary objectives. The first is to systematically and reproducibly invent all published experimental studies on feed restriction in broiler breeders, across all methods. The second is to evaluate and synthesize the effects of different quantitative, qualitative, non-daily, and precision restriction strategies on productive and reproductive performances and welfare indicators.

## Materials and Methods

### *Bibliographic search strategy*

For this systematic review, the literature search was conducted on Scopus and Web of Science. On Scopus, the search was restricted to the fields of Agricultural and Biological Sciences and Veterinary, while on Web of Science, the disciplinary categories selected were Agriculture Dairy Animal Science and Veterinary Sciences. This choice made it possible to target exclusively the literature in animal production and veterinary medicine, while excluding publications from disciplines without a direct link to the subject of the review. Both platforms were selected for their complementarity in terms of disciplinary coverage of animal and veterinary sciences. The search covered the period from 1989 to 2025. This time boundary was set to reflect the evolution of rearing practices over more than three decades, from conventional skip-a-day programs to the most recent precision feeding and qualitative restriction approaches. Only publications in English and French were retained. The search was constructed around three conceptual blocks linked by the Boolean operator **AND**. The first targets the population (broiler breeders), the second covers the modalities of intervention (feed restriction), and the third groups the outcomes of interest. Within each block, terms are combined with the **OR** operator so as not to exclude synonyms or spelling variants. Truncations (*) were also used to broaden the detection of derived forms of the keywords. The complete Boolean equation is as follows: (“broiler breeder*” OR “broiler parent stock” OR “meat-type chicken breeder*” OR “broiler dam*”) AND (“feed restriction” OR “restricted feed*” OR “feed*restrict*” OR “skip-a-day” OR “skip a day” OR “qualitative restriction” OR “quantitative restriction” OR “restricted feeding” OR “feed dilut*”) AND (“egg production” OR “laying rate” OR “egg number” OR “body weight” OR “body weight gain” OR “uniformity” OR “feed conversion” OR “productive performance” OR “production performance” OR “mortality” OR “livability” OR “fertility” OR “hatchability” OR “embryo*” OR “reproductive performance” OR “semen quality” OR “sperm*” OR “chick quality” OR “chick weight” OR “welfare” OR “behavior” OR “behaviour” OR “aggression” OR “feather pecking” OR “stereotyp*” OR “stress” OR “hunger” OR “corticosterone”). All references were subsequently exported in BibTeX format from each database and then imported into the study management and selection software.

### *Research question and inclusion and exclusion criteria*

Broiler breeder management has for decades relied on feed restriction as a tool for controlling body weight. Although this practice is recognized as indispensable to flock health and reproduction, its consequences for productive performance and animal welfare remain a subject of debate in scientific literature. This systematic review therefore seeks to answer the following question: *To what extent does feed restriction, regardless of its form, influence the productive performance, reproductive performance, and welfare indicators of broiler breeders?*

To structure this research question, the PICOS framework was used, as defined in Table 1 below. Furthermore, before proceeding with the examination of articles, inclusion and exclusion criteria were established a priori to ensure the consistency and reproducibility of the selection process. These criteria are presented in the following table.

Table 1: Inclusion and Exclusion Criteria According to the PICOS Framework

| *Domain* | *Inclusion Criteria* | *Exclusion Criteria* |
|---|---|---|
| P Population | Broiler breeders (females and/or males), all ages | Commercial layers, broilers, turkeys, quail, partridges, or any other species |
| I Intervention | Any form of feed restriction: quantitative, qualitative, skip-a-day, ration dilution, precision feeding, comparison between methods | Absence of feed restriction; isolated additive supplementation without a restrictive dimension; manipulation of a specific nutrient without concomitant restriction |
| C Comparator | Ad libitum feeding or comparison between restriction programmes | No reference or comparison group |
| O Outcomes | At least one of: productive performance, reproductive performance, animal welfare measured on the breeders themselves | Outcomes measured exclusively on offspring (embryos, chicks, broilers); exclusively molecular outcomes without link to productivity, reproduction, or welfare |
| S Study design | Controlled in vivo experimental trial, observational study with comparison group | In vitro studies, conference proceedings without extractable data, abstracts only |
| Language | English | Any other language |

***Study selection process***

Bibliographic management and the entire selection process were conducted using two complementary software tools. Zotero version 9.0.3 was used for the import, organization, and management of references. Rayyan (Ouzzani et al., 2016), a collaborative platform specialized in systematic reviews, subsequently handled assisted deduplication, title and abstract screening, and the documentation of all inclusion and exclusion decisions. The selection process was carried out in four successive stages, the results of which are summarized in the PRISMA diagram presented in Figure 1.

The search across both databases identified a total of 339 articles, of which 183 were from Scopus and 156 from Web of Science. Following import into Rayyan, a first phase of assisted automated deduplication led to the removal of 119 duplicates, reducing the corpus to 220 unique articles. These were then subjected to a systematic screening of titles and abstracts against the PICOS criteria. This stage resulted in the exclusion of 87 references deemed ineligible, primarily due to a non-conforming population, an intervention without a restrictive dimension, or an outcome measured exclusively on offspring. The remaining 133 articles were retained for in-depth reading. During this examination, 3 additional duplicates were identified, bringing the number of unique studies to 130 for methodological quality assessment. Subsequently, the methodological quality of each study was evaluated using the SYRCLE tool (Systematic Review Centre for Laboratory animal Experimentation), developed specifically for experimental animal studies (Hooijmans et al., 2014). At the end of the assessment of the 130 studies, a single study was excluded due to a high cumulative risk combined with a total impossibility of data extraction (abstract only, data not accessible). The 22 studies presenting a moderate-high to high risk or included with major reservations were not integrated into the synthesis and were not drawn upon in the results section. Only the 107 studies at low or moderate risk constituted the intermediate corpus at the end of this assessment. A fifth and final stage consisted of verifying the thematic conformity of each study with the central objective of the review, defined as the evaluation of the effect of feed restriction, whether quantitative or qualitative, on the productive and reproductive performances and welfare of the broiler breeders themselves. This examination led to the exclusion of 17 additional studies whose primary intervention or primary outcomes fell outside the defined scope. The definitive corpus on which the entire synthesis presented in this review is based comprises 90 studies published between 1989 and 2025.

## Results

***Overview of studies included in the review***

The 90 studies included were published between 1989 and 2025, with a relatively homogeneous temporal distribution. 23% of the studies predate the year 2000, 28% cover the decade 2000-2009, 22% the decade 2010-2019, and 27% were published between 2020 and 2025. This increasing proportion of recent publications reflects the sustained research interest in strategies alternative to quantitative restriction, particularly in the field of insoluble fiber dilution. The studies are distributed across 13 scientific journals, with Poultry Science concentrating most publications (51 articles, 57%), followed by Applied Animal Behavior Science (17 articles, 18%) and British Poultry Science (7 articles, 8%).

In terms of the populations studied, 79% of the studies focus exclusively on female breeders, 9% include both females and males, and 12% focus solely on males in particular cockerels, in the context of recent studies on feed dilution and coarse roughage. The Ross 308 strain is by far the most frequently used (25 studies), followed by Cobb strains in their various forms (9 studies), dwarf or experimental strains (5 studies), and Hubbard strains (3 studies). Nearly half of the studies (44%, i.e. 40 studies) do not specify the strain used, which constitutes a notable limitation for inter-study comparability. The eight categories of feeding strategies identified, the periods covered, the strains used, and the outcomes measured are detailed in Table 2. Qualitative dilution of feed with insoluble fibers and/or coarse roughage constitutes the most represented axis of the corpus (24 studies), followed by comparisons of feeding frequencies (19 studies) and studies on quantitative restriction levels and energy intake (16 studies). Furthermore, multifactorial approaches are largely predominant, with the majority of studies simultaneously combining several variables, which complicates the causal attribution of observed effects.

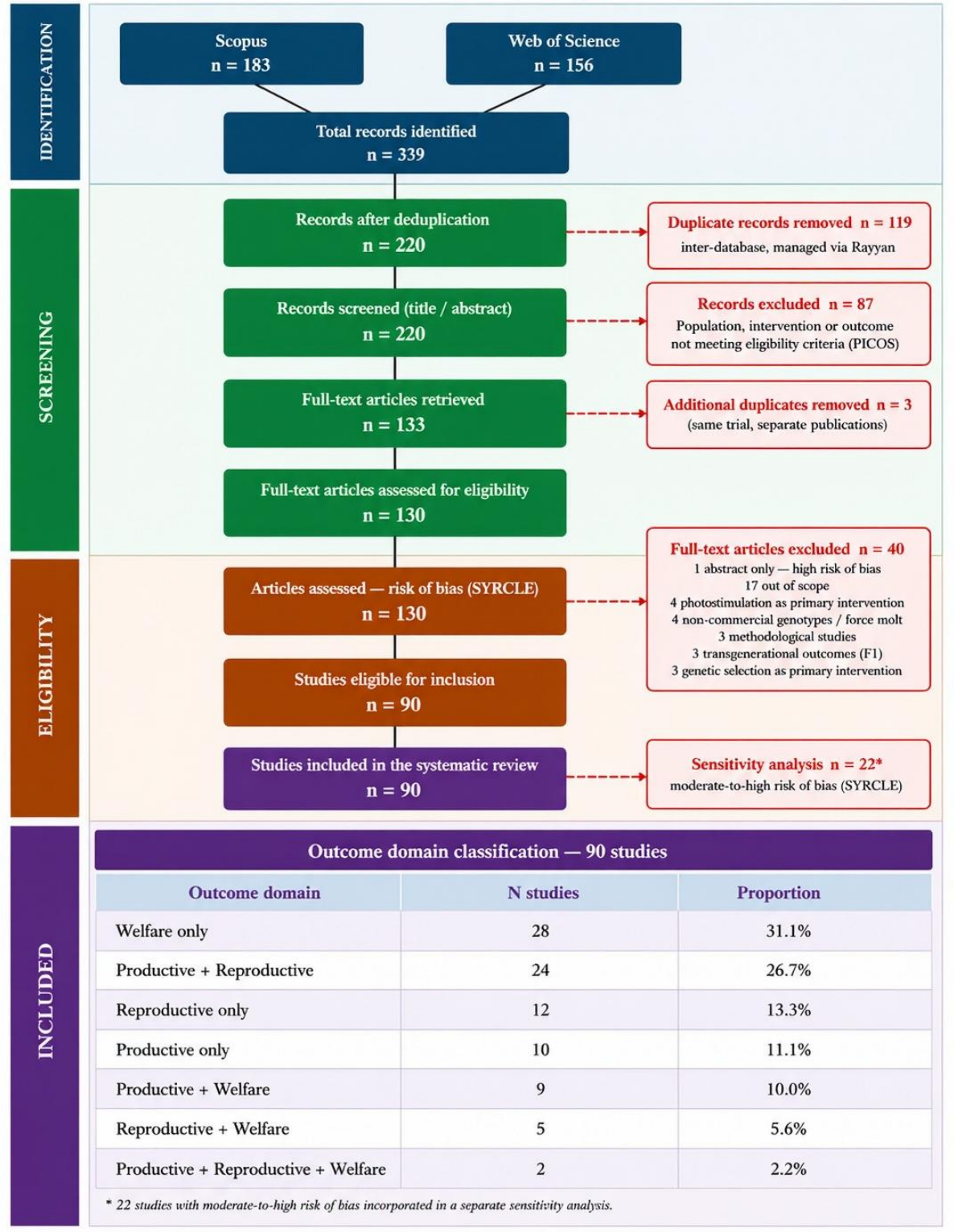



Figure 1: PRISMA Flow Diagram

Table 2: Overview of characteristics of studies included in the review, grouped by feeding strategy

| *Feeding strategy* | *N* | *Period* | *Main breeds / strains* | *Sex* | *Total n (birds)* | *Age studied (wk)* | *Main outcomes measured* | *Domain(s)* |
|---|---|---|---|---|---|---|---|---|
| Quantitative restriction - Feeding frequency and schedule (SAD, ED, SOD, STD, 4/3, 5/2, ATD, once- vs. twice-a-day) | 19 | 1989 - 2025 | +++Ross 308; ++Ross 708; +Cobb 500; NR | Females and/or Males | 35 - 1778 | 0-65 Rearing; Laying; Rearing + Laying | BW, FCR, body and carcass composition, skeletal growth; egg production, eggshell quality, fertility, hatchability; plasma hormones, CORT; feeding and drinking behaviour, stereotypies, aggression; feather condition; H/L ratio; water intake, litter moisture | P; R; W; P+R; P+W; P+R+W |
| Quantitative restriction - Level of feed allowance (ad libitum vs. standard or graduated levels; ME levels; release rate at entry into lay) | 16 | 1989 - 2021 | +++NR; +Indian River; +Arbor Acres; +Cobb 500; | Females and/or Males | 30 - 1092 | 0-72 Rearing; Laying; Rearing + Laying; | BW, body composition, carcass fat; age at sexual maturity, ovarian follicle structures, reproductive hormones (LH, FSH, E2, P4); egg production, fertility, hatchability, mortality; semen quality; H/L ratio, CORT, ghrelin, dopamine, serotonin; behavioural time budgets, stereotypies | P; R; W; P+R; R+W |
| Quantitative restriction - Growth curve and body weight target (standard vs. elevated BW curve; timing of full-feed access; early vs. standard photostimulation) | 4 | 2008 - 2022 | +++Ross 308; +NR | Females | 720 - 3000 | 0-65 Rearing; Rearing + Laying | BW, body composition, FCR, skeletal development; egg weight and composition; egg production, fertility, CORT; feeding motivation, behaviour | P; P+R; P+W; R+W |
| Qualitative restriction - Feed dilution with insoluble fibres and/or roughage (oat hulls, soybean hulls, cellulose, wheat bran, sugar beet pulp, maize silage, sawdust, rice husk, alfalfa/lucerne) | 24 | 1995 - 2025 | +++Ross 308; ++NR; +Cobb 500; +Hubbard M77; +Ross 1 | Females and/or Males | 64 - 3000 | 0-60 Rearing; Laying; Rearing + Laying | BW, FCR, gut fill capacity, GIT development, nutrient digestibility; egg production, fertility, hatchability, embryonic development; CORT, AgRP/POMC expression, plasma lipids; feeding motivation, foraging; behavioural indicators (stereotypies, fear, frustration, novel object); plumage condition, FPD, fault bars; H/L ratio, litter quality | P; P+R; P+W; R+W; P+R+W; W |
| Qualitative restriction - Dietary protein level (CP concentration, alone or combined with growth pattern or feeding schedule) | 5 | 1998 - 2015 | +++Ross 308; +Hubbard Standard; +Hubbard Hi-Y; +2 commercial strains (A and B) | Females and/or Males | 640 - 768 | 0-40 Rearing; Rearing + Laying; Rearing + offspring follow-up | BW, skeletal growth, body composition, breast muscle weight; egg production, egg weight; fertility, hatchability, embryonic mortality; offspring performance; semen quality; CORT, feeding behaviour, feather cover | P; P+R; R; P+W |
| Qualitative restriction - Appetite suppression (calcium propionate or analogues, alone or combined with dietary diluents, compared to standard quantitative restriction) | 7 | 1993 - 2020 | +++NR; +Ross 308; +Cobb 500 | Females | 60 - 1680 | 3-46 Rearing; Rearing + Laying | BW, feed intake; egg production, egg quality; feeding motivation; stereotypies, feather condition, fault bars; H/L ratio, CORT; basophil-to-lymphocyte ratio | P+R; P+W; R+W; W |
| Precision feeding system (individual BW-triggered automated feeding vs. conventional SAD or daily group feeding) | 5 | 2017 - 2018 | +++Ross 308; +Cobb 500; +Cobb MX | Females and/or Males | 480 - 630 | 0-52 Rearing; Laying; Rearing + Laying | BW, FCR, ME intake, energetic efficiency; egg production, fertility; aggression, social rank; foraging and oral stereotypies, restlessness | P; P+R; W |
| Genotype and strain comparison (standard vs. dwarf vs. slow-growing; selected vs. relaxed lines; multi-strain comparisons under varying feed restriction) | 10 | 1993 - 2021 | +Standard (S); +Experimental dwarf (E); +Slow-growing (L); +Laying strain; NR | Females and/or Males | 48 - 672 | 0-49 Rearing; Laying; Rearing + Laying | BW, body composition, carcass fat; ovarian follicle structures, reproductive hormones (LH, FSH, E2, P4); egg production, defective eggs, semen quality; livability; feeding time budgets, stereotypies, aggression; CORT, H/L ratio, creatine kinase | R; P+R; W; R+W |

SAD = skip-a-day; ED = every day; SOD = skip-one-day; STD = skip-two-days; ATD = alternate-day (fibre-supplemented off-day); 4/3 = 4 feeding days + 3 non-consecutive fasting days/week; 5/2 = 5 feeding days + 2 non-consecutive fasting days/week; AL = ad libitum; QR = quantitative restriction; QL = qualitative restriction; PF = precision feeding; BW = body weight; FCR = feed conversion ratio; CP = crude protein; ME = metabolizable energy; FPD = footpad dermatitis; H/L = heterophil-to-lymphocyte ratio; CORT = corticosterone; AgRP = agouti-related peptide; POMC = pro-opiomelanocortin; GIT = gastrointestinal tract; NR = not reported. Breeds/strains: +++ = most frequently used; ++ = moderately used; + = occasionally used. P = Production; R = Reproduction; W = Welfare.

***Effects of feeding frequency programs***

**Effects on productive and reproductives performances**

The trade-off between skip-a-day (SAD) and everyday (ED) feeding clearly favors ED for optimizing reproduction. Wilson et al. (1989) established that early initiation of the program from two weeks of age guarantees faster sexual maturity and higher lay compared to late implementation at eight weeks. Beer & Coon (2009) and Sweeney et al. (2022) confirm that ED advances sexual maturity (by 3 to 6 days) and significantly increases egg yield, while Avila et al. (2023) highlights structural gains such as better bone density, superior intestinal development, and a tendency towards improved hatchability. To further improve these performances during the laying period, Spradley et al. (2008) showed that distributing feed twice daily significantly increases egg production and flock uniformity. However, this practice is accompanied by higher cumulative mortality, which limits its systematic application. Between these two methods, the 4/3 program represents, according to Carneiro et al. (2019), the optimal technical compromise, as it displays the best feed conversion ratio per egg mass and surpasses SAD in fertility and hatchability domains where prolonged fasting is critical. Conversely, maintaining SAD after photostimulation is risky. Gibson et al. (2008) noted lasting reproductive dysfunctions and significantly fewer eggs produced over the entire production period (155 vs. 172 eggs/hen up to 65 weeks of age), with no benefit to live weight uniformity. In summary, if according to Katanbaf et al. (1989), differences between restricted regimes (ALR, SOD, STD) are minor compared to ad libitum feeding, ED or the 4/3 cycle secure productive performance.

***Hormonal and metabolic effects***

Feeding frequency directly modulates endocrine balances, independently of the total amount of feed consumed. De Beer et al. (2008) demonstrated that the SKIP program doubles corticosterone levels compared to the ED regime, which ensures superior metabolic stability (higher IGF-I and leptin levels). In males subjected to the SAD restriction program, Mench (1991) confirms this persistent increase in stress while suggesting a regulatory avenue, namely tryptophan supplementation for attenuating this physiological response via the serotonergic system. These data confirm that meal rhythm governs the bird's deep biological response well beyond the simple nutritional aspect.

***Effects on behavioral welfare***

From a behavioral standpoint, studies emphasize that rationing methods differently influence the expression of feeding frustration. Indeed, the welfare assessment of broiler breeders is based on precise physiological characterization in which, as shown by Najafi et al. (2015), corticosterone levels and the H/L ratio increase linearly with the severity of restriction. This profound nutritional stress, highlighted by the foundational work of Hocking et al. (1993) and Kubíková et al. (2001), directly translates into alterations of metabolic circadian rhythms according to De Jong et al. (2002). As a mirror of this internal state, behavioral observations reveal crucial nuances. Indeed, if precision feeding can paradoxically increase aggression and activity compared to the SAD program (Girard et al., 2017a), 5/2 type programs illustrate a phase shift between apparent calm on feeding days and real physiological stress marked by muscle wasting (Lindholm et al., 2018). Similarly, Aranibar et al. (2020) showed that introducing soybean hulls on the fasting days of SAD (Alternate Day feeding program) significantly improves live weight uniformity and egg production and is accompanied by measurable behavioral differences in feeding and comfort activities.

**Effects of qualitative feed dilution with insoluble fibers and/or coarse roughage**

**Live weight control and flock uniformity**

Ration dilution with insoluble fibers stands out as the most documented qualitative restriction lever in our documentary corpus for reconciling growth control with animal welfare. In a seminal study, Tolkamp et al. (2005) demonstrated that the massive incorporation of oat hulls (400 g/kg) combined with calcium propionate allowed, under ad libitum distribution, the maintenance of growth curves and production performances up to 46 weeks identical to those of a quantitative restriction control. This productive efficacy is corroborated by the work of Zuidhof et al. (1995), showing that a dilution of 15% oat hulls optimizes egg and chick yield while stabilizing flock uniformity during the laying phase.

In terms of satiety and behavior, De Los Mozos et al. (2017) emphasize that this same 15% dilution extends ingestion time by four to eight minutes and reduces compensatory consumption by 30% during motivation tests. These results suggest a significant attenuation of hunger without degrading general behavior or uniformity. Finally, the contribution of Nascimento et al. (2021) clarifies the divergences observed between studies by demonstrating that the efficacy of dilution depends closely on the physicochemical properties of the substrate. They identify water-holding capacity as the best indicator for defining the maximum intestinal capacity of breeders, underlining that the choice of diluent is decisive for the success of the program.

***Effects on feeding motivation and behavioral indicators of hunger***

The corpus of Riber, Tahamtani, and collaborators constitutes the most systematic analysis of the subject via a four-treatment protocol (Control, Insoluble, Mixed, Roughage). Riber & Tahamtani (2020) demonstrate that the Insoluble and Mixed (oat hulls + beet pulp) regimes attenuate frustration and that the provision of insoluble reduces

compensatory consumption, a sign of lesser hunger. While fear and exploration motivation tests reveal no notable impact according to Tahamtani & Riber (2020), the study by Riber et al. (2021) calls for vigilance regarding the "Mixed" regime. Indeed, the fact that these birds present the lowest growth suggests that this regime may have an unintentional negative effect on their welfare, beyond simple weight control.

Furthermore, Wurtz et al. (2025) confirm a reduction in feeding motivation and marked clinical benefits (improvement in plumage quality and reduction of fault bars), aligning with Tahamtani et al. (2020), although the effects on keratosis and hock burns remain nuanced. The importance of insoluble fibers is reinforced by Nielsen et al. (2011), whose regime at 89% ISF (insoluble fiber) almost entirely eradicates tail-pecking behaviors and stereotypies. Finally, Dixon et al. (2024) provides a crucial molecular perspective. Indeed, while fibers calm hunger behaviors, the expression of AGRP (Agouti-Related Peptide) remains identical to standard restriction, proving that deep physiological hunger persists.

***Effects on reproductives performances***

The impact of fibers and nutritional density on laying performances is both metabolic and physiological. Moradi et al. (2013) demonstrate that the integration of insoluble fibers (cellulose, wheat bran) boosts egg production while significantly reducing corticosterone levels, a sign of attenuated stress and hunger. However, the choice of source is crucial. Indeed, cottonseed meal may prove counterproductive by causing dyslipidemia and abdominal fat accumulation.

In addition, the work of Enting, Kruip, et al. (2007) and Enting, Veldman, et al. (2007) reveals that low-density diets stimulate the development of the reproductive tract (ovaries and oviduct) from the rearing phase. During the laying period, an 11% reduction in nutritional density not only improves laying rate and egg weight but also promotes better embryonic development at 29 weeks. These data confirm that fiber quality and caloric dilution are major levers for optimizing the productive longevity of breeders.

***Effects on cockerels***

A recent and coherent set of studies from researchers (Kittelsen, Vasdal, Tahamtani, and collaborators) has extended the evaluation of the D+R strategy (20% oat hull dilution + alfalfa roughage) to male breeder cockerels, a population that had until then been largely absent from the literature. In the Ross 308 strain, Kittelsen et al. (2023) and Tahamtani et al. (2025) report some behavioral benefits (less frustration in the thwarted feeding test, reduced tonic immobility duration), but contradictory results on fault bars and an absence of effect on the main postmortem health indicators. In the M77 strain (Hubbard), Kittelsen et al. (2025) and Tahamtani et al. (2025) conclude that effects are globally absent or inconsistent, and Vasdal et al. (2025) on the Ross 308 strain find no behavioral differences in the home pen. These results converge to indicate that the D+R strategy in cockerels does not offer a significant improvement in welfare according to the indicators commonly used and raise the question of the extrapolability of results obtained in females to the male population.

***Effects of quantitative restriction levels and energy intake levels on reproductives performances***

Ad libitum feeding severely degrades reproduction by inducing an obesity that disrupts the physiological regulation of the birds. Katanbaf et al. (1989) established that ad libitum feeding advances sexual maturity by 60 days, with a live weight at onset of lay higher by 1,200 g but drastically reduces normal egg production and fertility. Robinson & Wilson (1996) confirm a loss of 40 eggs per hen, an increase in double-yolk eggs, erratic laying, and shell quality problems, attributable to a disruption of the ovarian follicular hierarchy. In males, Sexton et al. (1989) state that while ad libitum feeding boosts sperm production, spermatozoa concentration, and testosterone levels in the short term, Robinson & Wilson (1996) demonstrate that this advantage reverses with age, making weight control vital for the persistence of fertility. The work of Fattori et al. (1991, 1993) demonstrates that feed restriction leads to reductions in live weight and the frequency of double-yolk eggs, without significantly affecting egg weight, fertility, or hatchability. Finally, Hocking (2004) specifies that the number of follicles is linearly correlated with live weight and that dietary energy is the limiting factor for triggering lay in severely restricted birds.

***Effects of appetite suppressants***

Calcium propionate has been the most studied additive as a qualitative restriction agent. Sandilands et al. (2006) and Savory et al. (1996) emphasize that this type of restriction, combining fibrous diluents such as oat or soybean hulls with calcium propionate, makes it possible to stabilize the growth of breeders while almost entirely suppressing object-pecking and abnormal repetitive behaviors. The studies of Arrazola et al. (2019, 2020, 2020) indicate that at the start of the rearing period, these regimes as well as non-daily distribution programs reduce feeding motivation and improve plumage condition. However, the research of Savory & Lariviere (2000) underlines that feeding motivation remains intrinsically linked to the degree of growth retardation, irrespective of the restriction method employed. Although the visible signs of frustration diminish, physiological stress indicators such as the heterophil-to-lymphocyte ratio or corticosterone levels do not show systematic improvement. Ultimately, the disappearance of oral stereotypies is insufficient to confirm improved welfare, as the sensation of hunger persists and the effect of the additives may sometimes stem from digestive discomfort rather than genuine satiety.

***Effects of precision feeding***

The precision feeding (PF) system, developed by Zuidhof's group at the University of Alberta, has been the subject of consistent evaluation through five studies in our documentary corpus. In terms of productive performance, Hadinia et al. (2018) show that in pullets, PF reduces the feed conversion ratio and thermal losses without affecting growth, suggesting better nutrient utilization. In laying hens, however, PF significantly reduces egg production. Zuidhof (2018) confirms a reduction in live weight variability (2% vs. 14%) and a 3.8% increase in fertility, but a 27% lower egg production, possibly due to the absence of a metabolic signal for sexual maturation. From a behavioral standpoint, Girard et al. (2017b) observe a decrease in locomotor activities and pecking, but a paradoxical increase in aggression without destabilization of the hierarchy. In conclusion, PF partially improves welfare but generates new tensions related to competition for access to feeding stations.

***Effects of dietary protein level***

Protein intake acts as a precision lever on the body composition and skeletal development of breeders. Van Emous et al. (2013) show that a low-protein diet promotes abdominal fat deposition at the expense of pectoral muscle during rearing, although these differences are fully resolved by 40 weeks of age. Consistent with these findings, Yaissle & Lilburn (1998) confirm that nutrition prevails over genetics below 380 g, temporarily directing development towards adipose rather than muscular tissue, while the strain-specific potential only asserts itself beyond 480 g. In terms of reproduction, a sustained growth profile improves initial fertility and hatchability (Van Emous et al., 2015), while Hocking et al. (2002) reminds that a relaxation of conventional quantitative restriction remains counterproductive for viability and lay. From a behavioral and physiological standpoint, protein dilution increases feeding time by up to 63%, thereby reducing pecking stereotypies without, however, lowering corticosterone levels (Van Emous et al., 2014). Finally, for males, Zhang et al. (1999) recommend a diet at 12% protein to optimize semen volume, highlighting that spermatozoa production depends critically on weight gain maintained after sexual maturity.

## Discussion

The present systematic review confirms and extends the conclusions of several reference narrative and scientific syntheses on the consequences of feed restriction in broiler breeders. The entire corpus analyzed converges towards three cross-cutting conclusions that prior work had posed in a fragmentary manner, without ever articulating them within a unified methodological framework simultaneously covering the three domains investigated.

In terms of productive performances, the available evidence designates everyday feeding (ED) as the program offering the best feed efficiency and weight uniformity compared to the skip-a-day program (SAD). This result partially aligns with the observations of Van Krimpen & De Jong (2014), who attribute these gains primarily to fiber dilution rather than to distribution rhythm. Conversely, unlike most studies in our review, these authors report that SAD does not induce greater physiological or behavioral stress than everyday feeding and place the reduction of energy density (insoluble fibers) at the heart of strategies reconciling welfare and productivity. In terms of reproductive performance, the results of this review are in direct continuity with the findings formulated by De Jong & Guémené (2011) in their reference review on major welfare problems in breeders. For these researchers, feed restriction is indispensable to reproductive performance, but its form, timing, and intensity largely condition the expression of this performance. Our analysis confirms that maintaining SAD after photostimulation constitutes the main reproductive management error documented in the literature, responsible on its own for a loss of several eggs per hen. It also shows that non-daily alternative programs, particularly the 4/3 program, present a superior reproductive profile compared to SAD, but that the available evidence, resting on a limited number of studies, is insufficient to formulate a firm recommendation. In terms of animal welfare, the most important and original result of this review is the systematic and robust dissociation between behavioral indicators and physiological markers of hunger in response to qualitative restriction strategies. This result, consistent across three independent studies having measured the hypothalamic expression of AGRP and POMC, calls into question the founding hypothesis of qualitative restriction as it was formulated at the beginning of the 2000s, notably in the pioneering work of Sandilands et al. (2006) and Savory et al. (1996), according to which increasing the volume ingested at iso-caloric intake improves perceived satiety. Our review shows that this improvement is behavioral and not metabolic. Indeed, fiber-rich feeds reduce stereotypies by keeping birds occupied, without modifying the central hypothalamic hunger signal. This result enters direct resonance with the narrative review of De Jong & Guémené (2011), who had anticipated this limitation by underlining that the behavioral modifications induced by diluents might reflect a displacement of behavioral expression rather than an improvement in affective state. In this sense, our review provides the first molecular demonstration of this hypothesis in this corpus. It nuances this conclusion with a convergent result on clinical welfare indicators. Indeed, plumage quality, fault bars, and foot lesions improve consistently with fibre-enriched feeds, suggesting a reduction in cumulative chronic stress measurable on integrative indicators, independently of acute hunger markers. This dual result namely clinical improvement without physiological improvement of satiety is consistent with the conclusion of Hartcher & Lum (2020), who

had established in their review on the consequences of genetic selection on poultry welfare that no available management strategy makes it possible to resolve the fundamental paradox between performance and welfare in modern commercial broiler breeders. However, this review presents several limitations that must be considered in the interpretation of its conclusions. For example, the over-representation of the Ross 308 strain and the under-representation of current commercial strains limit the generalization of the conclusions to flocks in production today, and the under-representation of male breeders in the corpus constitutes a major methodological gap.

## Conclusion

In synthesis, the analysis of the 90 included studies highlights several robust points of convergence. First, ad libitum feeding is unanimously and systematically deleterious for reproduction in females, irrespective of the program, strain, or period considered. Second, everyday feeding consistently and reproducibly improves production and hatchability performances compared to skip-a-day when total intake is equivalent, with systematically lower corticosterone levels. Third, extending ingestion time whether achieved through fiber dilution, reduction of the protein level, or precision feeding reduces stereotypic behaviors, although this behavioral effect is not systematically accompanied by a reduction in physiological stress indicators. This review therefore establishes that daily feed restriction is the best-supported program for jointly optimizing the productive and reproductive performances of broiler breeders, that qualitative restriction improves clinical welfare indicators without modifying metabolic hunger, and that no currently available strategy demonstrates a simultaneous and robust benefit across all three domains investigated. A conclusion that confirms and refines, within a rigorous methodological framework, the paradox that has been formulated for more than two decades in this literature.

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*ID: 945*

# Development of functional probiotic foods from fermented jackfruit (*Artocarpus heterophyllus*) seed flour

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## Abstract

Jackfruit (*Artocarpus heterophyllus*) is an underutilized crop mainly found in tropical and subtropical regions, with strong potential as a nutritious food source and valuable economic commodity due to its suitability for large-scale food production. The aim of this study was to produce value added probiotic food products with using jackfruit seed flour. The fruits were collected from Mihintale, Sri Lanka. They were thoroughly washed, cut into appropriate sizes, and the seeds were removed prior to further processing. The remaining fruit was sliced, soaked in 90°C water for 3 minutes, milled, and sun-dried for 8 hours. The resulting flour was then packaged in polyethylene pouches (HDPE, 300 gauges) and stored in a refrigerator at approximately 4°C. The jackfruit seed flour was fermented using $10^8$ CFU of *Lactobacillus delbrueckii* subsp. *bulgaricus* for 48 hours to produce four different products: dietary powder, food bars, ice cream, and butter in combination of different ingredients. Sensory evaluation was conducted for all four products to assess consumer acceptance and optimize their nutritional and probiotic. Statistical analysis was performed using the MINITAB software package, and ANOVA test was used. The protein content increased in all four products after fermentation compared to non-probiotic versions. For the food bars, there was a significant difference in all nutritional properties between the probiotic and non-probiotic versions, except fat content ($p = 0.071$). The fiber content decreased in all four products compared to their non-probiotic counterparts. Jackfruit bar, both probiotic and non-probiotic, contained comparatively high fat content. After one month of storage, the microbial count of both the jackfruit bars and the dietary powder was found to be $10^6$ CFU. The ice cream and butter were kept in the freezer and the refrigerator for one month, $2 \times 10^6$ CFU of probiotics was observed. It was evident that the fermented jackfruit seed flour products were demonstrated high nutritional value and consumer preference.

***Keywords:*** *Underutilized crop, sensory evaluation, dietary fiber, fermentation, nutritious*

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# The impact of sheep farm production orientation in Bulgaria and the region on the adoption rate of precision livestock farming sensors and devices

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## Abstract

The aim of this study is to examine the influence of the production orientation of sheep farms in Bulgaria and the region on the intensity of digital transformation. The study encompasses 270 sheep farm owners, with data collected via face-to-face direct standardized interviews. The questionnaire itemized a total of 10 electronic devices and sensors, with farmers indicating which technologies were implemented in their farms and to what extent. To facilitate the analysis, the numerous individual configurations were categorized into the following adoption classes: 1 (one device), 2–3 devices, and 4 or more devices and sensors. The results demonstrate that production orientation is a key driver of the number of implemented technologies. The highest level of innovation is observed in dual-purpose (combined) farms, where 53.85% of farmers utilize four or more technical devices. The dairy sector is the most widespread, accounting for 48.15% of the surveyed farms; however, its modernization is highly specialized and primarily focused on the milking process. In contrast, meat-oriented farms rely more heavily on remote monitoring systems, a choice dictated by their pasture-based management system.

***Keywords:*** *production orientation, digital transformation, precision sheep farming, technology adoption, PLF*

*ID: 947*

# Preference and Perception of Bioresources among Green Leafy Vegetable Farmers in Selected States in Nigeria

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## Abstract

Vegetable production in Nigeria holds significant nutritional and economic importance; however, the increasing reliance on inorganic fertilizers and pesticides to meet growing demand has raised concerns about soil degradation, environmental sustainability, and the long-term productivity of farming systems. Bioresources such as Compost (CM), Vermicompost (VC), Biochar (BC), Farmyard Manure (FYM), and Biopesticides (BP) offer sustainable alternatives capable of improving soil fertility and supporting resilient agricultural production. Yet, their adoption remains limited, underscoring the need to understand farmers' awareness, perceptions, and preferences regarding bioresource use in green leafy vegetable production. This study investigated farmers' awareness and perceptions of bioresources, their preferences for different bioresource types, and the challenges associated with bioresource utilization. Primary data were collected through a cross-sectional survey of 300 green leafy vegetable farmers, selected using a multistage sampling procedure, and analyzed using descriptive statistics and preference ranking techniques. Findings revealed varying levels of awareness, with farmers knowledgeable about BC (98%), FYM (86%), CM (86%), and BP (81%), while VC recorded no awareness (0%). Despite this awareness, the inability to utilize bioresources remained high, as 79%, 75%, 65%, 60%, and 39% of farmers could not use VC, BC, CM, BP, and FYM, respectively. Regarding constraints, few farmers (18–20%) perceived higher labor requirements, limited awareness, and technical complexity as barriers, while approximately 50% strongly disagreed that bioresources demanded additional labor or were less effective than conventional alternatives. Preference analysis showed that inorganic fertilizer ranked first among farm inputs, followed by organic manure, poultry manure, and cow dung, while biochar and vermicompost received the lowest preference rankings. Among factors influencing input choice, crop yield ranked highest, followed by extension agents' recommendations and resource availability. The study concludes that while awareness of bioresources is relatively high, farmers' practical ability to use them remains low. Therefore, improving access to practical demonstrations and yield-benefit evidence through extension services is recommended to enhance bioresource adoption and promote sustainable vegetable farming systems in Nigeria.

***Keywords:*** *Bioresources, perception, preference, green leafy vegetables, sustainable agriculture, Nigeria.*

## 1.0 Introduction

Globally, farmers rely heavily on chemical fertilizers and pesticides to improve crop yields and quality (Baweja et al., 2020). However, improper or excessive use of these substances carries significant consequences, including leaching, runoff, and volatilization, all of which degrade agricultural lands (Chaitra et al., 2021). In Africa, the Food and Agriculture Organization reported that inorganic fertilizer use nearly doubled between 2001 and 2021, reaching 7.6 million tonnes, yet application per cropland area remains the lowest at approximately 25 kg/ha, or about 5 kg per capita (FAOSTAT, 2021). This low level of use has been attributed to poor affordability, limited availability, and heavy reliance on traditional practices that do not incorporate inorganic fertilizers (FAOSTAT, 2021). Taken together, these realities — both the risks of overuse and the constraints on access — underscore the need for alternative, locally sourced sustainable soil nutrient management approaches.

To address these challenges, recent recommendations have emphasized the use of biological resources, commonly known as bioresources, as a means of enhancing soil nutrients and increasing crop productivity. Bioresources are naturally occurring products that can be effectively leveraged for sustainable farming (Dlamini et al., 2022; Abiala et al., 2020). Examples include compost, vermicompost, biochar, green manure, farmyard manure, and biopesticides. These resources help amend the soil, recycle nutrients, improve soil organic matter content, and promote the biological activity of soil fauna. Crucially, they also enhance the adaptive capacity of crops and improve soil productivity amid climate uncertainties, making them particularly valuable in the context of increasing climatic variability.

The vegetable sector is a key source of income, food supply, and nutrition, and has shown a significant impact on Nigeria's economic growth (Nwokoye et al., 2021; Plaisier et al., 2019). Nigeria's domestic vegetable production has grown rapidly over the past three decades, with recent figures indicating a national output of 16,330,610 tonnes (Statbase, 2024). Vegetables and fruits account for 13% of total food consumption in Nigeria — 11% vegetables and 2% fruit — a pattern consistent with other Sub-Saharan African (SSA) countries, and comparable to leading cereals such as rice (13%) and maize (7%) (Liverpool-Tasie et al., 2023). Global trends toward healthy eating and sustainability have further driven demand for vegetables and compelled local producers to respond to shifting consumer preferences (Statista, 2025). Against this backdrop, ensuring the sustainability of vegetable production, particularly Green Leafy Vegetable (GLV) production, is of both national and regional significance.

In pursuit of increased and sustainable vegetable output, bioresources offer a promising alternative capable of mitigating the effects of climatic variability and reducing excessive dependence on synthetic chemicals. The integration of sustainable

farm practices — including context-specific nutrient, pest, and weed management with reduced chemical use — has been well established in vegetable production (Behera et al., 2024; Farouque et al., 2024; Singh & Gohain, 2017). Specific bioresources have demonstrated measurable benefits: bio-fertilizers improve soil health, leading to higher yields and better product quality (Rao et al., 2014); compost has been shown to produce yields comparable to or exceeding those achieved with inorganic fertilizers and poultry manure, while improving soil organic carbon, structural stability, and biological properties (Chan et al., 2008; Chan et al., 2011; Donovan et al., 2014); and the integration of biopesticides in pest management has demonstrated effectiveness in controlling pests, reducing chemical pesticide use, and promoting natural enemy populations in tropical vegetable production (Srinivasan, 2012). Collectively, bioresource use ensures that even small or marginal land can produce high yields without depleting the natural resource base or relying excessively on inorganic inputs.

Despite these benefits, adoption of bioresources among farmers remains uneven. Studies by Adamu (2016), Ume et al. (2020), Oluwatosin (2020), Akinyemi and Daikwo (2022), and Ajakaiye (2023) reveal that a majority — at least 70% — of farmers use one or more forms of organic fertilizer, including cow, sheep, and goat dung, poultry manure, crop residues, compost, farmyard manure, and kitchen waste. Nevertheless, the overall rate of bioresource use remains low (Raimi et al., 2021; Araya et al., 2024), constrained by factors such as land tenure insecurity, lack of skills, transportation challenges, limited availability, weak farmer associations, and socioeconomic variables including age, income, and household size (Adamu, 2016; Ume et al., 2020; Oluwatosin, 2020; Ajakaiye, 2023). This indicates that significant opportunities in bioresource utilization remain unexplored, particularly in vegetable farming.

To harness the full benefits of bioresources and enhance the sustainability of Green Leafy Vegetable (GLV) production, it is essential to understand the availability of, perceptions toward, and preferences for specific bioresources among Green Leafy Vegetable Farmers (GLVFs). This study was restricted to green leafy vegetable farmers in South-west Nigeria cultivating Ugwu/fluted pumpkin leaves (Telfairia occidentalis), Jute mallow (Corchorus olitorius), Lagos spinach/Soko (Celosia argentea), and African spinach/Tete (Amaranthus hybridus). These are the predominant green leafy vegetables grown in the study area (Tichar et al., 2024). Bio-resources were limited to crop residues, refuse incorporation, compost, farmyard manure, biochars, vermicompost, and bio-pesticides, as these are most relevant to vegetable farmers. This study assumes that knowledge of bioresource use, challenges faced in their utilization, farmers' perceptions, and preferences for context-specific integrated nutrients can illuminate the opportunities and constraints that shape bioresource adoption among GLVFs. The findings will contribute to a broader discussion on the prospects of bioresources in vegetable farming, with implications for consumer health and safety, farmer welfare, and the long-term sustainability of vegetable production systems.

## 2.0 Materials and Methods

### *Study Area and Sampling Technique*

The study was based on primary data collected through a cross-sectional survey from GLVFs who use at least one form of bioresources in the country. A multistage sampling procedure was used to select vegetable farmers for the survey. Oyo, and Ogun lead in commercialization and are known for the selected GLV production in the region (Tichar *et al*., 2024). The selected GLVs include Ugwu/fluted pumpkin leaves (Telfairia occidentalis), Jute mallow (Corchorus olitorius), Soko (Celosia argentea), and Tete / African spinach (Amaranthus hybridus). These are the predominant GLVs grown in the study area (Tichar *et al*., 2024). Bio-resources were limited to crop residue, incorporation of refuse, compost, farmyard manure, biochar, vermicompost, and bio-pesticides, as these are most relevant to vegetable farmers. In the first stage, two states (Oyo and Ogun) were purposively selected in the region. In the second stage, a purposive sampling technique was used to select three Agricultural Development Zones (ADPs), namely Ibadan/Ibarapa, Ilaro, and Ikenne, from the two (2) states based on vegetable production intensity. 300 green leafy vegetable farmers were selected from the three zones using simple random sampling technique.

### *Analytical Tools*

Descriptive statistical tools were used to describe socioeconomic characteristics; analyze farmers' perceptions of the challenges of bioresources; assess the availability of bioresources; and analyze preferences for bioresource use choices and the factors considered when using bioresources. Tools used include frequency, percentage, 5-point likert scale and borda count method.

Following Saari (1985), Chen et al. (2016) and Saari (2023), borda count method was used to rank choices using their score. Given *N* bioresources and multiple farmers' preference, points *of N-l,N-2,.. .*, and *0* are assigned to the first-ranked, second-ranked, and last-ranked bioresources in each farmer's preference order. The points for each bioresources are summed across all farmers, and the most preferred resource is the one with the greatest total number of points. The option with the highest score is the most preferred.

Steps in analyzing involves;

1. assign points: Ist place is 6 points,$2^{nd}$ =5 points; $3^{rd}$ =4 points; $4^{th}$ = 3 points; $5^{th}$ =2 points; $6^{th}$ =1 point
2. Calculate for each bio/resource variable: Total points = (Votes for 1st×6) + (votes for $2^{nd}$ ×5) + votes for $3^{rd}$ × 4) + (Votes for $4^{th}$ ×3) + (votes for $5^{th}$ × 2) + (votes for $6^{th}$ × 1)

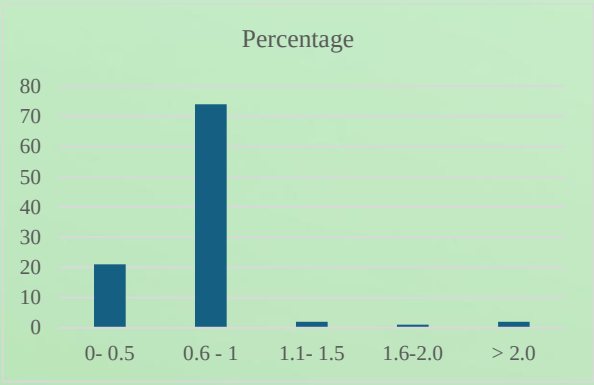
## 3.0 Result and Discussion

Socioeconomic Characteristics of GLVFs

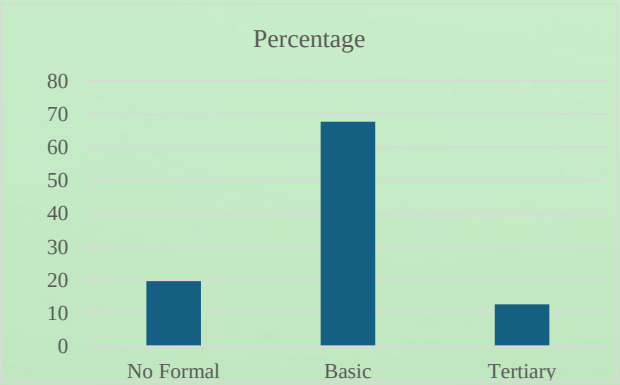
This section shows the household characteristics of farmers that has used bioresources in cultivating GLVFs in South-West Nigeria. The results show that 67.67% of farmers have basic education**,** 19.67% have no formal education, and 12.67% have tertiary education. This indicates that the majority of farmers possess at least a basic level of literacy, which may enhance their capacity to access agricultural information, use improved technologies, and implement better farm management practices. Education has consistently been identified as a key determinant of agricultural productivity because it enhances farmers' ability to process information and make informed production decisions. Recent empirical studies have shown that higher levels of

education significantly improve farmers' use of improved agricultural technologies and sustainable farming practices, thereby enhancing productivity and efficiency among smallholder farmers (Ruzzante *et al*., 2021; Fadeyi *et al*., 2022).

The distribution of household size indicates that 74% of respondents live in medium-sized households (4–6 members), 14.33% in large households (7 members and above), and 11.67% in small households (1–3 members). The mean household size of 5.14 persons suggests that most households fall within the moderate family size category typical of rural farming communities in Nigeria. Household size is an important determinant of labour availability in smallholder farming systems, as family members often provide labour for farm activities such as planting, irrigation, harvesting, and marketing. Larger households may therefore benefit from increased labour supply, which can improve farm productivity and reduce dependence on hired labour. However, large household sizes may also increase household consumption pressure, potentially affecting farm investment decisions. Gathala *et al.* (2021), stated that efficient labour management plays an important role in improving smallholder farm productivity. This calls for efficient labour management whereby few skilled family members are mobilized to adopt improved technology, members treated as staff on payroll and are combined with hired labour during critical production stages such as clearing and harvesting. Family members should also focus on high-value-added activities rather than low-productivity, high-substitutability roles. This will help maximize income and keep household consumption sustainable. When farmers focus on increasing yields far above the subsistence level using mechanized farm tools, improved technology, and processing, productivity will increase.

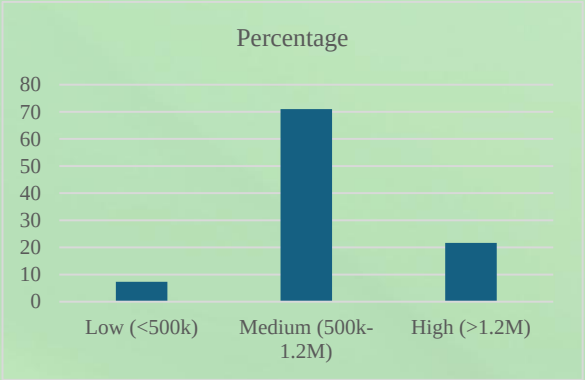



Farm Size

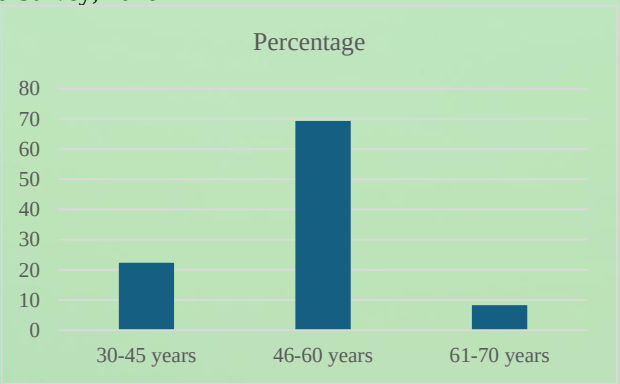



Educational Level

Source: Field Survey, 2026

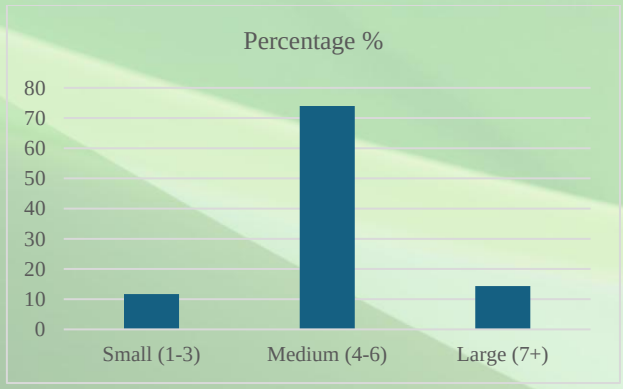



Farm Income




Age

Source: Field Survey, 2026




Household size

Source: Field Survey, 2026

The age distribution of farmers shows that 69.33% fall within the age range of 46–60 years**,** 22.33% are between 30–45 years**,** and 8.33% are between 61–70 years**,** with a mean age of approximately 51 years**.** This suggests that vegetable farming in the study area is largely dominated by older farmers. Farmers within this age category are generally expected to possess substantial farming experience, which can contribute positively to farm management and resource utilization. The relatively small proportion of younger farmers may reflect declining youth participation in agriculture, a trend widely reported in many developing countries (Geza *et al*., 2021; Njeru, 2017; Mukwedeya & Mudhara, 2024). The distribution of farm income indicates that 71% of farmers fall within the medium income category (₦500,000–₦1.2 million)**,** while 21.67% earn high farm income (above ₦1.2 million) and 7.33% earn low farm income (below ₦500,000)**.** The mean farm income of approximately ₦984,533 suggests that vegetable farming constitutes a moderately profitable agricultural activity in the study area. Vegetable production is often associated with higher returns than staple crop production due to its shorter production cycles and high market demand. Studies have shown that vegetable farming provides important income opportunities for smallholder farmers and contributes significantly to rural household livelihoods in Nigeria and other developing countries (Mukaila *et al*., 2022; Effiong *et al*., 2021).

### 3.1 Farmers' Awareness of Bioresources

Awareness in this context refers to the technical knowledge a farmer has. If a farmer knows how to use the technology, when to apply it and why it works**.** Awareness does not guarantee use. Farmers' awareness of agricultural technologies and sustainable farming practices plays a critical role in shaping their adoption decisions and overall resource-use efficiency. Table 1 presents the level of awareness of different bioresources among green leafy vegetable farmers in the study area. Results revealed that 80.67% of the farmers reported being aware of biopesticides, while 19.33% were not aware of them. Although awareness of biopesticides is relatively high, it remains lower than awareness levels for other organic inputs.

The results also showed that 86.33% of farmers are aware of farmyard manure, while 13.67% are not aware of it. Farmyard manure is one of the most widely used organic soil amendments in traditional farming systems and is often readily available in rural communities where livestock are present. The relatively high level of awareness observed in this study therefore reflects the long-standing use of manure as a natural soil fertility management practice.

The findings also reveal that 98.33% of the farmers are aware of biochar. This extremely high level of awareness may reflect recent efforts by research institutes such as Forestry Research Institute of Nigeria (FRIN), and several organizations including projects organized by Association of Common wealth Universities (ACU) and Futux Agri-consult to promote biochar as a sustainable soil amendment capable of improving soil fertility and enhancing carbon sequestration in agricultural systems. Similarly, 86.00% of the farmers reported being aware of compost, while 14% were not aware of it. Composting is a widely promoted sustainable agricultural practice that allows farmers to recycle organic waste materials into nutrient-rich soil amendments. The relatively high awareness level therefore indicates that many farmers in the study area are familiar with composting practices.

The results further show that 76.33% of farmers are aware of the incorporation of refuse as a soil management practice, while 23.67% are not aware of it. Although awareness of refuse incorporation is moderately high, the relatively larger proportion of farmers lacking awareness may reflect concerns about waste contamination or limited training on proper organic waste management techniques.

In addition, 90.33% of farmers reported awareness of crop residue management, while 9.67% indicated that they were not aware of it. Crop residue incorporation is an important component of sustainable soil fertility management because it helps maintain soil organic matter, improve soil structure, and enhance nutrient cycling. However, the results reveal a striking exception for vermicomposting, where none of the farmers reported being aware of vermicompost, indicating 100% lack of awareness of this practice in the study area. Vermicomposting involves the use of earthworms to convert organic waste materials into nutrient-rich organic fertilizer. Despite its potential benefits for improving soil fertility and crop productivity, the complete lack of awareness suggests that this technology has not been effectively disseminated among farmers in the study area.

Table 1: Percentage Awareness of Bioresources

| *Variable* | *Aware* | *Not aware* |
|---|---|---|
| Vermicompost | 0 | 100 |
| Biopesticides | 80.67 | 19.33 |
| Farmyard manure | 86.33 | 13.67 |
| Biochar | 98.33 | 1.67 |
| Compost | 86.00 | 14.00 |
| Incorporation of refuse | 76.33 | 23.67 |
| Crop residue | 90.33 | 9.67 |

Source: Field Survey, 2025/26

### 3.2 Farmers' Technical Ability to Use Bioresources

The ability of farmers to effectively utilize bioresources is a critical factor influencing the adoption of sustainable agricultural practices and the efficient use of natural resources in farming systems. While awareness and availability of bioresources are important prerequisites for adoption, farmers' technical knowledge and capacity to apply these resources correctly ultimately determine their effectiveness in improving soil fertility, crop productivity, and environmental sustainability. Table 2 therefore examines farmers' reported ability to utilize various bioresources used in vegetable production.

The results indicate generally low levels of technical ability across several bioresource practices, suggesting that although some farmers may be aware of these practices, many lack the practical skills required for effective implementation. The findings show that 79.00% of farmers reported having no ability to use vermicompost, while only 4.67% indicated high ability, 14% reported low ability, and 2.33% indicated very low ability. This suggests that vermicomposting techniques remain relatively unfamiliar to many farmers in the study area. Vermicompost production and application often require specific knowledge of organic waste management, earthworm culture, and soil nutrient management, which may limit its adoption among farmers who lack adequate technical training.

The findings further reveal that 59.67% of farmers reported no ability to use biopesticides, while 19.33% reported low ability, 11.33% indicated very low ability, 6.33% reported high ability, and 3.33% reported very high ability. Biopesticides require knowledge of pest identification, correct dosage, and proper application timing, which may explain the limited technical ability reported by many farmers. Insufficient training and limited extension support may therefore constrain the adoption of biological pest management practices in vegetable production systems.

The results also indicate that 47.33% of farmers reported no ability to use irrigation systems, while 21.67% indicated very low ability, 19.67% reported low ability, 5.67% reported high ability, and 5.67% indicated very high ability. Irrigation management requires knowledge of water scheduling, irrigation methods, and efficient water use techniques. Limited technical knowledge may therefore reduce farmers' ability to optimize water use in vegetable production, potentially affecting crop yields and resource-use efficiency.

For farmyard manure, 39.00% of farmers reported no ability to use it, while 23.67% indicated very low ability, 12.33% reported low ability, 6.33% indicated high ability, and 18.67% reported very high ability. Although farmyard manure is traditionally used in many farming systems, proper application techniques such as appropriate application rates, and timing require technical knowledge to ensure optimal benefits for soil fertility and crop productivity.

Also, the results reveal that 75% of farmers reported no ability to use biochar, while 16.33% reported low ability, 7.33% indicated high ability, 1.00% reported very low ability, and 0.33% indicated very high ability. Despite the growing recognition of biochar as a sustainable soil amendment, its effective use requires knowledge of appropriate production methods and application rates. The high proportion of farmers lacking the ability to use biochar may therefore limit its potential benefits in improving soil fertility and resource-use efficiency.

The findings also show that 65.33% of farmers reported no ability to use compost, while 23.00% reported low ability, 4.33% indicated very low ability, 5.33% reported high ability, and 2.00% indicated very high ability. Composting is an important organic waste recycling practice that improves soil organic matter and nutrient availability. However, the relatively low ability reported by farmers suggests that many may lack knowledge regarding compost preparation and application techniques. In terms of refuse incorporation, 45.67% of farmers reported no ability, while 16.33% indicated very low ability, 16.67% reported low ability, 4.67% indicated high ability, and 16.67% reported very high ability. This distribution suggests moderate variability in farmers' ability to recycle organic waste materials for soil fertility management.

Finally, the results show that 40.67% of farmers reported no ability to manage crop residues, while 24.67% indicated very low ability, 10.67% reported low ability, 5.33% indicated high ability, and 18.67% reported very high ability. Crop residue management is an important component of sustainable soil management because it helps maintain soil organic matter and improve nutrient cycling.

Table 2: Percentage Technical Ability to Use Bioresources

| *Ability to use bioresources* | | | | | |
|---|---|---|---|---|---|
| Variable | Not at all | High | Low | Very low | Very high |
| Vermicompost | 79.00 | 4.67 | 14.00 | 2.33 | 0 |
| Biopesticides | 59.67 | 6.33 | 19.33 | 11.33 | 3.33 |
| Farmyard manure | 39.00 | 6.33 | 12.33 | 23.67 | 18.67 |
| Biochar | 75.00 | 7.33 | 16.33 | 1.00 | 0.33 |
| Compost | 65.33 | 5.33 | 23.00 | 4.33 | 2.00 |
| Incorporation of refuse | 45.67 | 4.67 | 16.67 | 16.33 | 16.67 |
| Crop residue | 40.67 | 5.33 | 10.67 | 24.67 | 18.67 |

Source: Field Survey, 2025/26

### 3.3 Source of Bioresources

Access to agricultural inputs is strongly influenced by the channels through which farmers obtain them. Understanding the sources from which farmers acquire bioresources provides important insights into the functioning of input supply systems, knowledge diffusion mechanisms, and the role of farmer networks in promoting sustainable agricultural practices. Table 3 presents the distribution of respondents by the sources from which they obtain the various bioresources used in green leafy vegetable production. The results revealed that farmers' groups constitute the most important source of bioresources across most categories, highlighting the significance of collective farmer organizations in facilitating access to sustainable agricultural inputs. For instance, 68% of farmers obtained vermicompost through farmers' groups, compared with 17.33% from farm markets, 10.33% from extension workers, and 4.33% from farms. This suggests that organized farmer networks play a central role in the distribution of vermicomposting materials.

The findings also show that 58.67% of farmers obtained biopesticides through farmers' groups, 19.33% from farm markets, 18.33% from extension workers, and 3.67% from farms. The relatively high reliance on farmer groups for accessing biopesticides may reflect the technical nature of these inputs, as adoption often requires training and demonstration. For irrigation-related resources, 59% of farmers reported sourcing them from farmers' groups, 14.33% from farm markets, 12.33% from extension workers, and 14.33% from farms. Irrigation infrastructure and equipment often involve relatively high investment costs; thus, farmer groups may serve as effective platforms for collective investment or shared ownership of irrigation equipment.

In the case of farmyard manure, however, the pattern differs slightly. The results show that 36.33% of farmers obtained farmyard manure from farm markets, 30.67% from farmers' groups, 18% from farms, and 15% from extension workers. This suggests that farmyard manure may be more readily available through informal market channels because it is widely available as livestock waste in rural communities. The results further reveal that 65.67% of farmers sourced biochar through farmers' groups, 17% from farm markets, 13% from extension workers, and 4.33% from farms. Biochar production and application often require technical guidance, which may explain the central role of farmer groups in providing access to these resources.

For compost, 59.33% of farmers reported obtaining it from farmers' groups, 20.33% from farm markets, 14.67% from extension workers, and 5.67% from farms. Composting practices are often promoted through farmer training programs and cooperative initiatives aimed at improving soil health and recycling organic waste materials. In contrast, refuse incorporation and crop residue management show relatively higher reliance on farm markets and farms as sources. Specifically, 42.67% of farmers obtained refuse-related resources from farm markets, 32.33% from farmers' groups, 15.67% from extension workers, and 9.33% from farms. Similarly, 38% of farmers obtained crop residue resources through farmers' groups, 34% from farm markets, 15.67% from farms, and 12.33% from extension workers. These patterns suggest that locally available organic materials may be obtained through multiple informal channels rather than formal supply systems.

Table 3: Source of Bioresources

| *Source of purchase* | | | | |
|---|---|---|---|---|
| Variable | Farm market | Extension workers | Farms | Farmers' Group |
| Vermicompost | 17.33 | 10.33 | 4.33 | 68 |
| Biopesticides | 19.33 | 18.33 | 3.67 | 58.67 |
| Farmyard manure | 36.33 | 15 | 18 | 30.67 |
| Biochar | 17 | 13 | 4.33 | 65.67 |
| Compost | 20.33 | 14.67 | 5.67 | 59.33 |

Source: Field Survey, 2026

### 3.4 Perceived Challenges in the Use of Bioresources among Green Leafy Vegetable Farmers

Table 4 presents farmers' perceptions of the challenges associated with using bioresources in green leafy vegetable production. Understanding these constraints is important because farmers' perceptions significantly influence the adoption and sustained use of environmentally friendly agricultural inputs. The results show that although bioresources have potential environmental and soil fertility benefits, several practical and economic challenges may limit their widespread adoption among farmers. The results indicate that a substantial proportion of respondents strongly disagreed (55.33%) with the statement that bioresource use requires more labour than synthetic fertilizers, while only a smaller proportion strongly agreed. This suggests that most farmers do not perceive labour demand as a major constraint to the use of bioresources. However, a notable proportion of respondents expressed neutrality or agreement (19.33%), indicating that labour requirements may or may not pose a challenge for some farmers. Bioresource management practices such as composting, manure handling, and residue retention often involve additional labour inputs compared to the relatively simple application of synthetic fertilizers. Similarly, 56.67% of respondents strongly disagreed that bioresources are too expensive compared to synthetic inputs, suggesting that farmers generally perceive bioresources as relatively affordable. This may be attributed to the fact that many bioresources, such as farmyard manure, compost, and crop residues, are locally available and can be obtained at little or no monetary cost. However, the availability of such materials may still vary depending on farm size, livestock ownership, and access to organic materials.

The perception that bioresources are less effective than synthetic fertilizers appear to be mixed among the respondents. While 53.33% strongly disagreed with this statement, about 17.67% agreed, indicating that some farmers question the effectiveness of bioresource. This perception may arise because organic inputs often release nutrients more slowly than synthetic fertilizers and may require longer periods before noticeable improvements in crop yield are observed. In the case of biopesticides, it may require multiple usage for effectiveness. 49.67% of respondents strongly disagreed that the preparation and application of bioresources is time-consuming, although a relatively high proportion expressed neutrality or agreement. This indicates that time requirements associated with compost preparation, or manure processing, application is a constraint for certain farmers. Smallholder farmers often operate under tight labour schedules, especially during peak planting and harvesting periods, which may affect their willingness to engage in practices perceived as time intensive. The results also showed that 51.67% of respondents strongly disagreed with the statement that bioresources take longer to show results compared to synthetic inputs. Nevertheless, a considerable share of respondents expressed neutral or positive agreement with the statement.

Table 4a: Perceived Challenges in Using Bioresources

| *Variable* | *SA* | *D* | *N* | *SD* | *A* |
|---|---|---|---|---|---|
| Bioresource use requires more labor than synthetic fertilizers | 20 | 1.67 | 19.33 | 55.33 | 3.67 |
| They are too expensive compared to synthetic ones | 15 | 5.33 | 21.33 | 56.67 | 1.67 |
| They are not as effective as synthetic ones | 18.33 | 7.67 | 3 | 53.33 | 17.67 |
| Preparation and application is time consuming | 22.33 | 6.33 | 20.67 | 49.67 | 1 |
| It takes longer to show results than synthetic ones | 18.33 | 6.67 | 19.67 | 51.67 | 3.67 |
| Yield is not certain or inconsistent | 12.67 | 5 | 19.67 | 54 | 8.67 |
| Benefits of using bioresources do not justify extra cost | 21.67 | 9 | 21 | 45.33 | 3 |
| Most farmers in my community still prefer synthetic agrochemicals | 9.33 | 2.67 | 0.67 | 65.33 | 22 |

Source: Field Survey, 2026

Table 4b: Reliability Test for Perceived Challenges in Using Bioresources

| | | | *95% CI* | |
|---|---|---|---|---|
| Coefficient | Estimate | Std. Error | Lower | Upper |
| McDonald's ω | 0.906 | 0.008 | 0.890 | 0.922 |
| Cronbach's α | 0.889 | 0.010 | 0.870 | 0.909 |

Source: Field Survey, 2026

Another perceived challenge relates to yield uncertainty. 54% of respondents strongly disagreed that yields from bioresource use are uncertain or inconsistent, suggesting that many farmers are confident in the productivity benefits of bioresources. However, a smaller proportion expressed concern regarding yield variability. Such perceptions may arise from differences in application techniques, quality of organic materials, or variability in soil conditions. The statement that the benefits of bioresources do not justify their additional costs was strongly disagreed with by 45.33% of respondents, suggesting that most farmers recognize the potential advantages of bioresource use. Nevertheless, some agreement among respondents indicates that economic considerations still influence adoption decisions. Results revealed that 65.33% of respondents strongly disagreed that most farmers in their communities prefer synthetic agrochemicals, while 22% agreed. This indicates that while bioresources are gaining acceptance among farmers, synthetic agrochemicals remain widely used in many farming communities due to their rapid effectiveness, convenience, and familiarity.

Lastly, the Cronbach's alpha reliability coefficients for the constraint construct ranged from 0.87 to 0.91 with an average reliability coefficient of 0.89.

### 3.5 Bioresource Preference Among Green Leafy Vegetable Farmers

Table 5 presents the preference ranking of different bioresources used by green leafy vegetable farmers in the study area. The ranking was determined using a point-based scoring system, where higher points indicate greater preference among the respondents. The result revealed that clear differences exist in their levels of preference. Inorganic fertilizer ranked first with 1971 points**,** making it the most preferred input among the farmers. Inorganic fertilizers are often preferred because they provide rapid nutrient availability and immediate yield responses**,** which are particularly important for short-cycle crops such as green leafy vegetables. These crops typically require quick nutrient uptake to achieve optimal growth within relatively short production periods. Recent studies on vegetable production systems in Sub-Saharan Africa have reported similar patterns, in which farmers prioritize inorganic fertilizers due to their rapid effects on crop yield and productivity (Olumba et al., 2024). The second-most-preferred bioresource is organic manure (cow dung and poultry dung**)**, with 1633 points**.** Organic manure is widely used in smallholder farming systems because it improves soil structure, enhances soil organic matter, and promotes long-term soil fertility. In addition to providing essential plant nutrients, organic manure improves soil microbial activity and moisture retention, both of which are essential for sustainable crop production. The relatively high preference for organic manure suggests that farmers recognize its benefits for maintaining soil productivity. Recent research has highlighted the importance of integrating organic manure with inorganic fertilizers to achieve sustainable soil fertility management and improve agricultural productivity (Iqbal *et al*., 2019; Abab *et al*., 2023). For example, it leads to more soybean yield compared to using chemical fertilizers alone (Wu *et al*., 2024)

The results further show that biopesticides ranked third with 923 points**,** indicating relatively lower preference compared to nutrient-related inputs. Biopesticides are biological agents used to control pests and diseases in an environmentally sustainable manner. Although they are safer alternatives to synthetic pesticides, their adoption among smallholder farmers remains relatively limited in many developing countries due to concerns about their effectiveness, availability, and the technical knowledge required. Recent studies suggest that improving farmers' awareness and access to biopesticides could significantly enhance their adoption in sustainable farming systems.

Similarly, compost ranked fourth with 837 points, indicating moderate preference among farmers. Compost is widely recognized as an important organic soil amendment that improves soil fertility, structure, and microbial activity. However, compost's relatively low ranking may be attributed to the labor-intensive nature of preparation and the time required to produce it before it can be applied to crops. In many smallholder farming systems, farmers prefer inputs that deliver quicker results with less labor. Vermicompost ranked fifth with 793 points**,** suggesting limited adoption among the farmers. Vermicompost is produced through the decomposition of organic materials by earthworms and is considered a highly nutrient-rich organic fertilizer. Despite its benefits, its relatively low preference may stem from the technical knowledge and management requirements associated with vermicomposting systems. An integrated application of vermicompost, other bioresources, and soluble inorganic fertilizers yields optimal results, leading to significant improvements in plant growth, yield, and soil properties (Sande *et al*., 2024).

Finally, biochar ranked sixth with 708 points**,** making it the least preferred bioresource among the respondents. Biochar is a carbon-rich material produced from the pyrolysis of biomass and is known to improve soil fertility, water retention, and carbon sequestration. However, the low preference observed in this study may reflect limited awareness, accessibility challenges, and the relatively recent introduction of biochar technologies in smallholder farming systems**.** Recent studies indicate that the adoption of biochar remains limited in many developing countries due to knowledge gaps and lack of demonstration programs that showcase its benefits to farmers (Ndugwa & Omusula, 2025).

Table 5: Bioresource Preference among Green Leafy Vegetable Farmers

| *Variable* | *Points* | *Rank* |
|---|---|---|
| Inorganic fertilizer | 1971 | 1st |
| Organic manure (cow dung or poultry) | 1633 | 2nd |
| Biopesticides | 923 | 3rd |
| Compost | 837 | 4th |
| Vermicompost | 793 | 5th |
| Biochar | 708 | 6th |

Source: Field Survey, 2026

### 3.6 Factors Considered when using Bioresources

Table 6 presents the factors green leafy vegetable farmers consider when deciding whether to use bioresources in their farming activities. The ranking was determined using a point-based scoring approach, with higher points indicating greater importance in farmers' decision-making. Crop yield was ranked the most important factor influencing farmers' use of

bioresources, with a total of 1751 points. This suggests that farmers primarily adopt bioresource practices when they perceive they can enhance crop productivity. In smallholder farming systems, production decisions are often strongly influenced by expected yields because farmers depend heavily on crop output for both income and household food security. Recent studies on sustainable agriculture emphasize that farmers are more likely to adopt environmentally friendly inputs when they observe clear productivity benefits (Olumba *et al*., 2024). This indicates that improving awareness of the yield benefits of bioresources could significantly enhance their adoption among vegetable farmers.

The recommendation by extension agents ranked second with 1749 points**,** highlighting the important role of agricultural extension services in influencing farmers' adoption of bioresource practices. Extension agents serve as a key source of information and technical guidance on improved agricultural technologies and sustainable farming practices. Their recommendations often shape farmers' perceptions regarding the effectiveness and reliability of agricultural inputs. Recent evidence from Sub-Saharan Africa shows that effective extension services significantly improve farmers' adoption of sustainable land management practices and environmentally friendly farming technologies (Abab et al., 2023). This finding underscores the critical role of agricultural advisory systems in promoting the use of bioresources among farmers. Factor availability ranked third with 1734 points, indicating that farmers are more likely to use bioresources when such materials are readily available. Accessibility of agricultural inputs plays an important role in shaping farmers' adoption behavior, particularly in rural areas where supply chains may be limited. Bioresources such as organic manure, compost, and crop residues are often locally sourced, and their availability can significantly influence their utilization in farming systems. Studies on sustainable farming systems have shown that farmers' adoption of organic inputs is strongly influenced by the availability and accessibility of such resources (Ndugwa & Omusula, 2025).

The factor health ranked fourth with 1725 points**,** suggesting that farmers consider the potential health implications of agricultural inputs when making production decisions. Bioresources are often perceived as safer alternatives to synthetic agrochemicals, which have been associated with various health risks for farmers and consumers. Increasing awareness about food safety and environmental sustainability may therefore encourage farmers to adopt bioresource-based practices. Recent research indicates that concerns about food safety and environmental health are increasingly influencing farmers' adoption of sustainable agricultural practices across developing countries. Similarly, safety was ranked fifth with 1695 points**,** further reinforcing the importance of health and environmental considerations in farmers' decision-making processes. Bioresource inputs are generally perceived as environmentally friendly and less harmful compared to chemical fertilizers and pesticides. This perception aligns with growing global concerns regarding the environmental and ecological impacts of intensive chemical input use in agriculture.

Convenience ranked sixth with 1401 points**,** indicating that farmers also consider the ease of using bioresources in their farming activities. Some bioresource practices, such as compost preparation or organic manure application, may require additional labor or time, which can affect farmers' willingness to adopt them. In many smallholder farming systems, labor availability and management complexity often influence the adoption of sustainable farming practices. Soil improvement ranked seventh with 1238 points, suggesting that while farmers recognize the soil fertility benefits of bioresources, this factor is not the primary motivation for their use. Bioresources are well known for enhancing soil structure, organic matter content, and long-term soil fertility. However, smallholder farmers often prioritize immediate productivity gains over long-term improvements in soil health. Recent studies on sustainable land management have found that farmers are more likely to adopt soil-improvement practices when they observe direct, short-term economic benefits (Adéchian & Baco, 2025).

The factor price ranked eighth with 1230 points, indicating that cost considerations play a relatively minor role in farmers' decisions to use bioresources. This may be because many bioresources, such as manure and crop residues, are locally available and relatively inexpensive compared with synthetic agricultural inputs. As a result, farmers may focus more on the effectiveness and availability of bioresources than on their cost.

Finally, distance ranked ninth with 977 points**,** making it the least important factor influencing farmers' bioresource use. Although distance may affect the transport of bulky materials such as manure or compost, the relatively low ranking indicates that farmers have access to bioresources within a reasonable distance of their farms. Nevertheless, logistical constraints associated with transporting organic materials over long distances may still discourage their use in certain situations.

Table 6: Factors Considered when using Bioresources

| *Variable* | *Points* | *Rank* |
|---|---|---|
| Crop yield | 1751 | 1st |
| Recommendation by extension agents | 1749 | 2nd |
| Availability | 1734 | 3rd |
| Health | 1725 | 4th |
| Safety | 1695 | 5th |
| Convenience | 1401 | 6th |
| Soil improvement | 1238 | 7th |
| Price | 1230 | 8th |
| Distance | 977 | 9th |

Source: Field Survey, 2026

**Conclusion and Recommendations**

This study provides valuable insights into the bioresources used by GLVFs in Nigeria. It identifies bioresources such as compost, farmyard manure, biochar, biopesticides, and vermicompost used by GLVFs cultivating indigenous GLV species. Its novelty lies in evaluating complementary bioresources used or integrated into GLV production, identifying perceived challenges associated with these bioresources, their sources, farmers' preferences for bioresource use, and the factors considered when using them. This is crucial for sustainable GLV production amid environmental uncertainties. By bridging

this gap, challenges identified with bioresources can be addressed. Also, factors considered for using bioresources can be enhanced, and opportunities harnessed.

The bioresource use pattern indicates that simpler, well-known bioresources (farmyard manure and biopesticides) are more commonly used, while more specialized ones (compost, vermicompost, biochar) lag behind. Findings suggest that significant number of GLVFs had constraints such as benefits not justifying extra costs incurred; labor requirement is more; time involved in preparation is longer; effectiveness is low; and yield is not consistent when compared with synthetic inputs. A considerable number of farmers agree that these constraints limit bioresource use. While most of these bioresources are locally sourced and produced, this helps reduce purchase costs. Bioresources are mostly used as a complement to reduce costs and augment nutrient requirements for vegetable growth, not to incur additional costs. Also, farmers tend to prefer inputs that are widely recognized and provide immediate yield benefits, such as inorganic fertilizers and organic manure (cow dung and poultry manure). Meanwhile, newer or more technically demanding bioresource technologies, such as biochar and vermicompost, remain less widely adopted.

Among the factors considered when using bioresources, productivity, extension agents' influence, and resource availability are the most important in shaping farmers' decisions to use them. In particular, crop yield expectations and extension service recommendations appear to play critical roles in shaping farmers' decisions to adopt bioresource-based farming practices. It is therefore recommended that agricultural extension services be strengthened; farmers' access to productivity information should be improved, and the use of bioresources as an effective agricultural practice should be promoted among GLVFs.

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*ID: 948*

# Recent Advances in Shelf-Life Extension of Fresh-Cut Leafy Vegetables: Edible Coatings, Active Packaging, and Natural Sanitization Strategies

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## Abstract

Fresh-cut leafy vegetables are highly perishable products because cutting and minimal processing accelerate respiration, moisture loss, enzymatic browning, and microbial spoilage. In response to the growing demand for safe, convenient, and clean-label produce, this review explores recent advances in edible coatings, active packaging, and natural preservation strategies for extending shelf life and maintaining quality. Edible coatings based on polysaccharides, proteins, and lipids can act as semi-permeable barriers that reduce gas exchange and water loss while delivering antimicrobial and antioxidant compounds. Active packaging systems, including oxygen scavengers, moisture regulators, and antimicrobial materials, help slow senescence and suppress microbial growth during storage. In parallel, natural preservation approaches such as plant extracts, essential oils, organic acids, and biocontrol agents provide sustainable alternatives to synthetic preservatives and chlorine-based treatments. Although promising, these technologies still face challenges related to formulation stability, sensory acceptance, regulatory approval, and industrial scale-up. Overall, the integration of these hurdle technologies offers a practical and sustainable pathway to reduce postharvest losses and improve the commercial value of fresh-cut leafy vegetables.

***Keywords:*** *fresh-cut leafy vegetables; shelf-life extension; edible coatings; active packaging; natural preservatives; postharvest quality*

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ID: 876

# Biodiversity effects of the interaction between installed artificial reefs and seagrass meadows along the Sinop coasts of the Black Sea

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## Abstract

This study was conducted within the scope of the MS-MPAs project to map seagrass meadows and monitor the seasonal effects of habitats supported by artificial reefs on biodiversity along the Karakum coast of Sinop. The study aims to provide scientific evidence for zoning strategies of potential Marine Protected Areas (MPAs) and decision-making processes in fishery management in the Black Sea. Fieldwork was carried out across two distinct stations, with the deployment date of the artificial reefs designated as T0 (baseline): ARs: a station with both artificial reefs and seagrass meadows (0–6 m), and SG (Control): a control station with seagrass meadows but no artificial reefs (0–6 m). To collect data on fish, arthropod, and crustacean species, gillnets and trammel nets with mesh sizes of 36 mm and 40 mm, alongside baited traps with six circular entries and a 40 mm mesh size, were deployed. Stations were evaluated and compared based on species composition, number of individuals (N), and catch amount (kg). The highest biodiversity was recorded at the ARs station—where artificial reefs and seagrass were integrated—yielding a total of 35 taxa comprising fish, arthropods, and crustaceans. The seagrass-only control station (SG) hosted 28 taxa. At the ARs station, Scorpaena porcus (black scorpionfish) was the most abundant species, accounting for 27.530 kg (710 individuals). Conversely, at the control station, scorpion fish (*Scorpaena porcus*) ranked first in terms of the number of individuals with 540 specimens, whereas Atlantic stargazer (*Uranoscopus scaber*) was the most caught species in terms of weight, reaching 25.345 kg. The findings demonstrate that artificial reefs play a crucial "nature-based and adaptation-oriented" supportive role in enhancing seagrass habitats and boosting coastal biodiversity.

***Keywords:*** *Artificial Reef, Seagrass, Habitat Structure, Biodiversity, Black Sea*

## Karadeniz'in Sinop kıyılarına yerleştirilen yapay resifler ve deniz çayırı etkileşiminin biyoçeşitlilik üzerindeki etkileri

## Özet

Bu çalışma, MS-MPAs projesi kapsamında Sinop ili Karakum kıyılarında deniz çayırlarının haritalanması ve yapay resiflerle desteklenen habitatların biyoçeşitlilik üzerindeki mevsimsel etkilerinin izlenmesi amacıyla yürütülmüştür. Araştırma, yapay resiflerin yerleştirildiği tarih (T0) başlangıç kabul edilerek iki farklı istasyonda gerçekleştirilmiştir: ARs: yapay resifli-deniz çayırlı saha (0–6 m) ve SG (Kontrol): yapay resifsiz-deniz çayırlı saha (0–6 m). Balık, eklembacaklı ve kabuklu türlerine ilişkin verilerin toplanmasında, 36 mm ve 40 mm göz açıklığına sahip sade ve fanyalı uzatma ağları ile 40 mm göz açıklığında altı çemberli girişi olan yemli sepetler kullanılmıştır. İstasyonlar; tür kompozisyonu, birey sayısı (N) ve av miktarları (kg) göstergeleri üzerinden karşılaştırılmıştır. Saha çalışmaları sonucunda en yüksek biyoçeşitlilik, toplam 35 takson (balık, eklembacaklı ve kabuklu türü) ile yapay resif ve deniz çayırının entegre olduğu ARs istasyonunda tespit edilmiştir. Yalnızca deniz çayırı barındıran SG kontrol sahasında 28 takson saptanmıştır. ARs istasyonunda iskorpit balığı (*Scorpaena porcus*) 27,530 kg (710 birey) en fazla avlanan tür konumundadır. Kontrol sahasında ise birey sayısı olarak 540 adet ile iskorpit balığı ilk sırada yer alırken, av miktarı açısından 25,345 kg ile tiryaki balığı (*Uranoscopus scaber*) en fazla yakalanan tür olmuştur. Elde edilen veriler, yapay resiflerin deniz çayırı habitatlarını desteklemede "doğa temelli ve uyum odaklı" önemli bir rol oynadığını ve kıyısal alanda biyoçeşitliliği artırdığını göstermektedir.

***Anahtar Kelimeler:*** *Yapay Resif, Deniz Çayırı, Habitat Yapısı, Biyolojik Çeşitlilik, Karadeniz*

*ID: 950*

# Efficacy of Red Silk Cotton (*Bombax ceiba*) Flower Powder on Growth Performance, Hematological, Biochemical and Fecal Microbial Load of Broiler.

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## Abstract

The study aimed to assess the effects of food supplementation with Red Silk Cotton (RSC) flower powder on broiler chicken production performance, carcass characteristics, haemato-biochemical, faecal microbial load, and cost-effectiveness as an antibiotic replacement. A total of 120-day-old Ross 308 broiler chicks were randomly assigned to four experimental groups. Groups $T_0$, $T_1$, $T_2$, and $T_3$ received baseline feed supplemented with 1%, 2%, and 3% silk cotton (RSC) flower powder, respectively. In this study, the $T_0$ and $T_1$ groups consumed considerably less feed overall ($P<0.05$) than the $T_2$ and $T_3$ groups. The 3% RSC-treated group $T_3$ had a considerably ($P < 0.05$) higher final live weight than the $T_1$, $T_2$, and $T_0$ groups. The $T_3$ group had a significantly ($P<0.05$) higher feed conversion ratio (FCR) than the $T_0$, $T_1$, and $T_2$ groups. The weight of the broiler chicken's organs (breast, thigh, drumsticks, wings, head, liver, heart and gizzard) and carcass features varied significantly ($P < 0.05$) among all treatment groups. Dietary addition had a significant ($P<0.05$) impact on the lipid profile (total cholesterol, triglyceride, HDL, and LDL), faecal microbial load, drip loss, sensory test, cook loss, feather, skin, and cost-effective parameters. Higher body weight, better FCR, and more feed were observed in the 3% RSC-treated group of birds. Overall, 3% RSC addition was a more effective antibiotic substitute than 1% and 2% RSC.

***Keywords:*** *Broiler, Red silk cotton (RSC), production performance, haemato-biochemical*

*ID: 951*

# Analyzing Small-Scale Wheat (*Triticum aestivum* Linn._) Production Cost and Returns in Out-Grower Scheme in Thomas Irrigation Area of Makoda Local Government, Kano State, Nigeria

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**Abstract**

The study analyses small-scale wheat (*Triticum aestivum* Linn.) production cost and returns on out-grower scheme in Thomas irrigation area of Makoda Local Government of Kano state, Nigeria. The study employed multistage sampling technique in the selection of 120 sample respondents. The respondents were interviewed using structured questionnaire. The result obtained was analyzed by means of descriptive statistics and farm budget model. It reveal that farm size ($X_5$) p<-3.543*** was statistically significant but negative in wheat production by small scale farmers, sources of information ($X_4$) p<2.024**, farming experience ($X_6$ p< 0.046 0.036**) and educational level ($X_7$) 0.40 (p<) 0.010 were also significant (1.758*). The result indicated the variable costs of N392,975.26) reflecting 79.80% of the total cost production cost was realised. Among the variable costs, input fertilizers cost constituted highest with N185,767.65(37.73%), seed costs (N38,108.89)7.74%, and irrigation depends on the method used for watering. Fixed costs of production were found to be ₦ 99,451.85 representing (20.19%) per acre. The analysis of the costs and returns reveals that the farmers had a benefit-cost ratio of 7.03 realising ₦7.01 as revenue for every ₦1 invested by farmer in wheat crop production during the 2022/2023 irrigation season. Problems identified included at harvest and post-harvest activities up to (24%) of the farm proportion, pest and diseases. The study recommends ensuring timely and adequate provision of inputs by the government and non-governmental entities and groups, provisions of affordable credit to farmers is paramount, extension services should be adequately and funded, government and private sector should make investments in agricultural research and education as a state priority.

***Keywords:*** *Analysis, wheat, out-grower scheme, small-scale*

## Introduction

### Background of the Study

Wheat (*Triticum aestivum* L.) is a globally vital grain, ranking first in acreage and second in production volume (772 million metric tons in 2021, up 10 million tons from the previous year; Shahbandeh, 2021). In Nigeria, wheat farming faces challenges like high temperatures, low yields, and heavy import dependence to meet rising demand. As the second-largest wheat consumer in sub-Saharan Africa (behind South Africa), Nigeria's wheat is crucial for food security, especially in the north, where it's milled into flour for traditional dishes (*alkaki*, *taliya*, *dashishi*) and urban staples like bread. Post-2015/2016 and the 2019/2020 Covid period oil shocks (Abbas,2018) policymakers see potential for Nigeria's self-sufficiency in wheat, but production lags behind population growth and demand(.) With high protein (14%), baking quality, and storability, wheat is a strategic commodity. Yet, conflicting production figures persist, underscoring the need to identify avenues for sustainable, profitable wheat production in Makoda, Kano State, under out-grower schemes linked to Northern Flour Mill (NFM).

Kano State, located in the northern (northwestern) part of the country, is one of the key wheat-producing regions, benefiting from irrigation schemes that support dry-season farming. The Thomas Irrigation Area in Makoda Local Government of Kano State is a prime example of initiatives localising agricultural projects aimed at boosting agricultural productivity through organized out-grower schemes linked to Northern Flour Mill (NFM). These schemes link small-scale farmers with markets, inputs, and technical support, enhancing their production capabilities.

Small-scale wheat farmers, however, face numerous challenges, including high production costs, limited access to modern technology, and fluctuating market prices. In this context, understanding the costs structure and returns of wheat production is crucial for identifying areas of improvement, enhancing profitability, and informing policy interventions that can support farmers. One of these avenues identified is out-grower initiative by Nigeria flour mills (NFM) and the out-grower scheme's sustainability.

This current study focuses on analysing the cost and returns of wheat production in the Thomas Irrigation Area, providing insights into the profitability of wheat farming and the challenges faced by farmers. The findings are expected to inform strategies for improving wheat production efficiency, enhancing farmer welfare, and contributing to Nigeria's agricultural development goals.

***Statement of the Problem***

Wheat production in Nigeria is gaining economic and social importance but faces hurdles affecting yield, volume, and farmer returns. Key issues include high input costs, low production levels, bureaucratic obstacles, lack of production loans and subsidies, technical constraints, and post-harvest losses (in Thomas irrigation area). These reduce profitability and deter small-scale farmers in Makoda. Specifically the study intends to achieve the following objectives:

***Broad Objective***

The broad objective of the study is to analyse the cost and returns of wheat production in the Thomas Irrigation Area, Makoda Local Government of Kano State, Nigeria.

***Objectives of the Study***

The current study examines small-scale wheat production in Makoda Local Government, Kano State, Nigeria, under NFM's out-grower scheme. Specifically the objectives of the study are to:

i. determine the production costs of wheat in the study area,
ii. estimate the returns and profitability of wheat production,
iii. assess the economic viability of wheat production in the out-grower scheme and
iv. identify key areas for improving the efficiency and profitability of wheat production.

***Justification of the Study***

Findings will inform stakeholders (farmers, NFM, policymakers, researchers) on wheat production profitability, marketing, pricing, and efficiency in Makoda's out-grower context. Results will aid investments and Investors and agro-allied services, identifying profitable wheat production strategies, Government (Kano State, Federal Ministries of Agriculture, Finance), and other non-governmental organisations Targeted interventions, subsidies, and infrastructure improvements, Researchers and students, would serve source of information for related studies on wheat economics and out-grower schemes in the state and the Nation at larger contexts.

**Methodology**

***The Study Area***

Thomas irrigation area, is in Makoda Local Government, Kano State (12°20′N, 8°30′E), has ~1,200 hectares under wheat irrigation (November–March) source. The study was carried out in Makoda Local Government Area of Kano state. Makoda Local Government Area (LGA) is situated in the Northern part of Kano state in 2023 and 2024 production season. The area has an area of about 441km².The area lies geographically between the latitude 12º 14`49" N to 12º 36`7.4"N and longitude 8º29`5.7" E to 8º49`12'' E. The population of Makoda Local Government was projected at 1,111,995 persons(source.

The area is characterized by two major geologic structures with minor intrusions. Makoda is topographically situated in low land areas which render it suitable for vegetable production. The larger area of the region to the south and North West is underlain by rocks of the basement complex with intrusions of younger granites in the extreme southern parts Olofin, (2008 and Aminu, 2018 ; abbas, et al., 2020). To the North East are found unconsolidated sediments of the Chad formation. The two structures are separated by a transitional zone which constitutes the well-defined hydro geological divide of the region Olofin,(2008).

The main drainage of the area constitutes Thomas Dam and their numerous tributaries. Makoda area is characterized by one river, which is river Thomas and got it tributaries from Bichi Local Government Area of Kano State and drain to Dambatta Dam (Udo, 1981). The drainage system has been artificial with natural fadama depressions modified through dams and constructions for multipurpose uses (irrigation and rain fed) and flood control. Annual rain fall ranges between 500mm in the North to 1,200mm in south. The area is characterized by four months of wet seasons and eight months of dry season. Therefore, water availability is very critical in the area. In the location, Temperature is relatively warm to hot from March to October, and cool from November to February. The mean annual temperature was found to be degree centigrade ($26^0$C). Farming is an important activity as people of the area predominantly famers as they engaged themselves in both rainy and dry sessions farming. Some of the crops cultivated and marketed are wheat, Pepper, Okro, Onions, cereals and pulses. The market is rural area center for economic activities for the local Government revenue generation.

***Method of Data Collection***

Total of sixty (120) questionnaires was prepared to gather relevant information from the respondents. Interview method was conducted by the researcher himself with the help of one trained field enumerator.

***Types of Data***

The data used was primarily collected through the use of structured questionnaire as guide and supplemented by focus group discussions (FGD) which involved the stake holders and interview.

***Primary Data***

Primary data was collected through the use of structured questionnaire as basis to guide in the sourcing of information from farmers. The data collected included on socio economic characteristics of wheat farmers, inputs price, and costs of procurement, management and disposal of produces and problems associated with wheat production.

***Secondary Data***

The secondary data sources were especially the data obtained from some books, internet and notes from private partners.

Descriptive statistics such as frequency, means and percentages were used to describe the socioeconomic characteristics of wheat producers and problems associated with production. Net farm income, (NFI), operation ratio (OR) Benefit Cost Ratio (BCR) models were employed in the analysis. Specified was made from the point of view of total expenses (costs) estimation as well as revenue within the production period.

***Analytical Tools***

*Descriptive statistics*

Descriptive statistics was used in for data analysis, summarising costs and returns to wheat production.

*Models Specification as employed in the Study was presented as follows:*

$$\mathbf{P = f / n \ X \ 100} \qquad \text{i}$$

Where:

P=percentage,
*f*=Frequency to be converted to percentage,

$$\mathbf{\bar{X}=\sum X_i/n} \qquad \text{ii}$$

Where:
$\bar{X}$= Mean
$\sum$ =Summation
n= total number of observations
X=number of responses.

*Farm Budget Model*

Farm budget model (FBM) were employed to determine Gross Production Margin (GM), benefit-cost ratio (BCR), returns on Investment (RoI).

*Gross Income (GI)*

$$\mathbf{GI = TR - TVC} \qquad \text{(iii)}$$

Where:
GI = Gross Income
TVC =Total Variable Cost
TR= Total Revenue

*Gross Revenue*

$$\mathbf{(GR) = QXP_y} \qquad \text{(iv)}$$

Where:
GR=Gross Revenue
$Q_x$ = Quantities of Wheat Sold (Kg);
$P_y$ = Unit price of Wheat (₦/kg).

*Net Farm Income*

**NFI=GFI-TCP** (v)

Where:
NFI=Net farm income
GFI=Gross farm income **(**Total Revenue**),**
TC=Total cost of production (Wheat),
TCP=Total Cost of Production,

*Total Cost of Production*

**TCP=TFC+TVC** (vi)

TFC=Total fixed cost
TVC= Total variable cost

***Return on Investment (ROI)***

$$\textbf{ROI}== \frac{\pi}{\text{TC } i-j} \quad \text{(vii)}$$

Where:
ROI=Return on Investment
$\boldsymbol{\pi}$ = profit margin
TC=Total Cost of Investment

**Benefit cost ratio (BCR)**

$$\textbf{BCR} = \frac{\text{Total benefit}}{\text{Total Cost } i-j} \quad \text{viii}$$

Where:
BCR= Benefit Cost Ratio
TR=Total Revenue from yield (Output)
TC=Total Cost expended on fixed and variable inputs

$$\boldsymbol{FP} = \frac{TPP}{TVC} = \frac{Y}{\sum PnXn} \ldots\ldots\ldots\

clients, the average farmers' returns 340,000 equivalent of prevailing wheat price (normally pegged by the clients) or host companies. The result is presented in Table 3.

**Table 3 : Summary of average costs and returns for wheat production**

| Cost Practices | Average costs (₦/acre) | (%)TVC |
|---|---|---|
| **(Variable Costs Components)** | **@** | **@** |
| Seed | 8,530 | 2.73 |
| Sowing | 3,520 | 1.129 |
| Water charges(Irrigation) | 58,886 | 18.89 |
| Weeding($1^{st}$/$2^{nd}$) | 20,500 | 6.90 |
| Fertilizers($1^{st}$/ $2^{nd}$) | 148,333 | 47.60 |
| Organic-manure | 3,500 | 1.13 |
| Agro-chemicals | 5,500 | 1.77 |
| Harvesting | 17,230 | 5.53 |
| Winnowing | 2,750 | 5.53 |
| Threshing | 5,200 | 1.66 |
| Transportation | 13,700 | 0.89 |
| **Total Variable Cost** | **392,975.26** | **(100)** |
| | | |
| **(Fixed Costs Component)** | **@** | **@** |
| Land Lease | 25,500 | |
| Land preparation | 50,500 | |
| Depreciation on tools | 540 | |
| **Total Fixed Cost(TFC)** | **76,540:00** | **(100)** |
| **Total Cost (TC)** | **388,119** | **(100)** |
| Total Revenue(TR) | **1,258,400** | |
| **Gross Margin (GM)** | **946,821** | |
| **Net Farm Income(NFI)** | **482,164** | |
| **Benefit-Cost** (BCR) | 2.25 | |
| **Return on Investment(RoI)** | **2.29** | |

Source: Field Survey (2024) at N2,000 /*mud* Yield(*Output*=17.5) bags/acre (average yield)

***Factors influencing wheat output***

*Regression analysis was used to identify factors influencing wheat output***.**

Table 1: Summary of the wheat production regression analysis

| Variable | Coefficient | Std. Error | t-value | p-value | Significance |
|---|---|---|---|---|---|
| Farm Size (acres) | -0.543 | 0.153 | -3.543 | 0.001 | *** |
| Sources of Information | 0.276 | 0.136 | 2.024 | 0.05 | ** |
| Farming Experience (yrs) | 0.040 | 0.020 | 2.036 | 0.05 | ** |
| Educational Level (yrs) | 0.058 | 0.023 | 1.758 | 0.10 | - |
| Credits /Loan packages | | | | | |
| Constant | 1.852 | 0.412 | 4.496 | 0.001 | *** |

$R^2 = 0.842$

Adjusted $R^2 = 0.708$

F-statistic = 6.78 ($p<0.01$)

Source: Field data (2023) NB: Dependent variable (Y): Wheat output (kg/acre). Significance levels: *** p<0.01, ** p<0.05, * p<0.10.

***Major Challenges (problems) for wheat production in the study area***

*The problems associated to wheat production in the study*

post-harvest losses in the study areas ranked. The attribution variables covered ranged from harvesting losses among other considerable factors. The table shows that postharvest losses ranged from 0.01 kg per farmer at the drying stage (3%) to 0.30 kg per farmer at the harvesting stage (24%). These findings did not agree with the 2%, 6% and 7% obtained by Appiah et al., (2011) for drying, threshing and storage respectively. Macheal (2014) further noted that losses from drying ranged from 1 to 5% while Chin, (2016) puts the losses from storage at 6.2% compared to 8% and 7% obtained from these attributes under the study. Post-harvest losses are the quantitative and qualitative degradation of food production from harvest to consumption. Quality losses include those that affect the nutrient/caloric composition, acceptability, and edibility of a given product (Kaadeer, 2012).

**Table : Distribution problems to wheat production in the study area**

| Problems identified | Frequencies | | Percentage Rankings |
|---|---|---|---|
| Low output price | 105 | 88.1 | 1st |
| Low yield or returns to scale | 89 | 78 | 2nd |
| Inadequate credit facilities | 56 | 74.3 | 3rd |
| Lack of production input | 96 | 67.6 | 4rd |
| High cost of inputs | 87 | 60.5 | 5th |
| Transportation problem | 104 | 54.3 | 6th |
| Diseases/pests | 99 | 52.40 | 7th |
| Labour cost | 89 | 52.10 | 8th |
| Inadequate land | 78 | 51.9 | 9th |

Source: Field survey, (2023) *values computed based on multiple responses*

## Discussion

Wheat production in Makoda is profitable as revealed by BCR >1, aligning with Adeoti et al. (2018) on irrigation's yield boosting the farmers morale. High fertilizer costs (37.73%) as reported by Ibrahim et al. (2020) as set back to produce production and disposal. Strategising subsidies on production could enhance profit margins. Negative farm size effect indicates smallholders manage resources more efficiently, Education and info access improved outcomes, stressing extension services' role. – Harvest and post-harvest losses of production posed a serious problem too. Other problems reported included pests (stem rust, aphids) and diseases (blast). Crop failure risks threaten grain-based repayment **(see *Appendix A* for risk mitigation strategies**).

## Conclusion And Recommendations

### *Conclusion*

Small-scale wheat production in Thomas irrigation area proved to be profitable but constrained by high input costs, post-harvest losses, and pests. Out-grower schemes shows promise if supported by timely inputs, credit, and capacity-building.

### Recommendations

Based on the findings, following points have been put forward**:**

- Conscious effort should be exerted by the government at all levels, and NGOs to encourage and incentivize high production of wheat in Nigeria so as to boost agricultural production,
- Government, financial institutions and private entities to offer low-interest loans should ensure timely and adequate provision of inputs, to farmers at affordable prices.
- Government and private sector should make investments in agricultural research and education as priority.
- and fund extension services adequately

*ID: 952*

# Phenotypic Evaluation Of Salt Stress Tolerance In Wheat Lines Derived From The Introgression Of NAX1 And NAX2 Genes

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## Abstract

Salt stress constitutes one of the main abiotic factors limiting cereal productivity worldwide. The introgression of sodium exclusion genes (Nax1 and Nax2) represents a major strategy to improve wheat tolerance. This study evaluates the phenotypic impact of these genes in ten wheat lines (Nax) compared to five reference parents their recurrent parents (Maali, Karim, Salim, Razzek, and Nasr) subjected to salt stress (100 mM and 150 mM NaCl) under pot culture conditions. Morphological parameters based on the Growth Reduction Index (GRI), the Salinity Tolerance Coefficient (STC), and an overall visual score were analyzed. The results demonstrate significant inter-genotypic variability. Among the recurrent The parents Maali was found to be the most tolerant (STC = 80.0%), while Nasr proved to be the most sensitive (STC = 46.4%). Remarkably, four introgressed lines (B11L1, B4L8, B4L2, and B4L7) outperformed the best parent Maali, showing STC gains of up to +7% and minimized GRI (up to 13%). These elite lines constitute promising candidates candidates for breeding programs in arid, salinity-stressed marginal landsfor breeding programs in arid and semi-arid zones.

***Keywords:*** *Salt, Morphological, visual score*

*ID: 953*

# Precision Fermentation for Animal-Identical Dairy Proteins: A Multi-Disciplinary Review of Microbial Engineering, Process Intensification, Safety, and Environmental Aspects

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## Abstract

The dairy industry is under growing pressure to reduce its environmental footprint, improve animal welfare, and meet the needs of lactose-intolerant consumers. Precision fermentation (PF) has emerged as a biotechnological strategy to produce molecularly identical milk proteins – including β-lactoglobulin, α-lactalbumin, caseins, and lactoferrin – using genetically engineered microorganisms. This review provides an extended and critical synthesis of the PF dairy protein field based on 75 peer-reviewed sources, including recent advances (2020–2026) and complementary insights from nanotechnological decontamination and plant-based safety assessments. We compare five microbial host platforms (*Komagataella phaffii*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Aspergillus oryzae*, and *Escherichia coli*) with respect to titre, folding fidelity, secretion efficiency, and regulatory status. Fed-batch fermentation in bioreactors up to 150,000 L is evaluated, and downstream processing trains (centrifugation, depth filtration, cation exchange, ultrafiltration, spray drying) are described with real-world yield data. A dedicated safety section examines residual host-cell DNA, endotoxins, and allergenicity. We integrate life cycle assessment (LCA) and techno-economic analysis (TEA) to identify the conditions under which PF dairy becomes cost-competitive and environmentally superior. Finally, we embed the discussion within broader food safety concerns – including heavy metal contamination of raw materials – drawing on recent studies from Iran and elsewhere. Our conclusions highlight that PF dairy is technically mature but still requires advances in continuous processing, waste-based feedstocks, and harmonised global labelling to achieve widespread market adoption.

***Keywords:*** *Precision fermentation, recombinant whey, casein, Pichia pastoris, downstream processing, food safety, heavy metals, sustainable protein, nanobiotechnology*

## Introduction

The global transition towards sustainable protein sources has moved from a niche interest to a mainstream necessity. According to the Food and Agriculture Organization (FAO), by 2050 the world will require 70% more food, and protein demand will double in low-income countries [1]. Traditional dairy production, while rich in high-quality protein and bioavailable calcium, carries significant externalities. Livestock accounts for approximately 14.5% of anthropogenic greenhouse gas (GHG) emissions, with dairy cattle alone emitting 2.7 Gt $CO_2$-equivalent annually [2]. Beyond climate, dairy farming consumes vast quantities of fresh water (around 628 litres per kg of milk powder) and occupies about 1.6 m² of land per kg of milk [3]. Moreover, approximately 68% of the global adult population has some degree of lactose malabsorption, with prevalence reaching 90–100% in East Asian communities [4]. These converging pressures have stimulated interest in alternatives.

Plant-based milks (soy, almond, oat, rice) have grown rapidly, yet they suffer from two fundamental limitations. First, their protein content is generally lower (0.5–1.5 g/100 ml versus 3.3 g/100 ml for bovine milk). Second, they lack the precise functional properties – emulsification, gelation, foaming, and heat stability – that are conferred by specific dairy proteins such as β-lactoglobulin (BLG), α-lactalbumin (ALA), and casein micelles [5]. For example, the unique structure of caseins allows them to form rennet-induced gels that are essential for cheese making. Plant proteins (soy, pea, potato) do not replicate this behaviour unless extensively modified, which drives up cost and ingredient complexity.

Precision fermentation (PF) offers a fundamentally different approach. Instead of extracting protein from plants or animals, PF programs microorganisms – typically yeasts or filamentous fungi – to synthesise exactly the same protein molecules found in cow's milk. The process is sometimes called "animal-free dairy" or "animal-identical dairy". It is important to distinguish PF from traditional fermentation: in yoghurt or beer production, the microorganism grows and multiplies inside the final product, contributing to flavour and texture; in PF, the

microbe is used as a living factory, and the target protein is purified away from the biomass, then added to food formulations [6]. This distinction has critical implications for regulation, labelling, and consumer perception.

The historical roots of PF are older than many realise. Recombinant chymosin (rennet) for cheese making was approved for food use by the US FDA in 1990, becoming the first PF-derived food enzyme [7]. However, only in the past decade have falling costs of DNA synthesis, the advent of CRISPR-Cas9 genome editing, and high-throughput screening enabled the rapid engineering of microbial hosts for complex, multi-domain dairy proteins. For instance, bovine β-lactoglobulin – a 162-amino acid lipocalin with two intramolecular disulfide bonds – has now been secreted in *Komagataella phaffii* (formerly *Pichia pastoris*) at titres exceeding 18 g/L [8]. Similarly, the three major casein types (αs1-, β-, and κ-casein) have been produced in *Saccharomyces cerevisiae* at levels of 2–5 g/L [9]. Lactoferrin, an 80 kDa iron-binding glycoprotein with antimicrobial properties, remains more challenging, but recent work in *Aspergillus oryzae* has delivered titres of 1.8 g/L [10].

Despite these advances, PF dairy is not yet a drop-in replacement for conventional milk protein. The industry faces several interconnected challenges: (i) improving yields and productivities further, especially for caseins; (ii) reducing downstream processing (DSP) costs, which currently consume 50–60% of total production expenditure; (iii) demonstrating long-term safety and gaining regulatory approval in multiple jurisdictions; (iv) scaling from 50,000 L to 200,000 L bioreactors; and (v) communicating effectively with consumers who may be concerned about "GMOs" or "fermented" foods.

In addition, broader food safety issues that are often overlooked in PF literature should be considered. Raw materials used in fermentation media (yeast extract, peptone, glucose syrups, mineral salts) may contain trace levels of heavy metals (lead, cadmium, arsenic, mercury), depending on their geographical origin and processing methods. Several studies have reported concerning levels of toxic elements in medicinal plants and even in food crops grown near industrial zones in Iran and other countries [11–17]. For example, Alinia-Ahandani et al. (2020) documented heavy metal contamination in roadside soils and plants in Roodsar, Iran [12]. Sheydaei et al. (2020) and Alinia-Ahandani et al. (2022) found measurable concentrations of lead and cadmium in chicken meat and medicinal plant samples from Lahijan and Langerud [13,14]. While PF-derived dairy proteins are extensively purified, the raw fermentation media are not always tested for such contaminants. Therefore, this review also draws attention to quality control of fermentation inputs, integrating knowledge from environmental and pharmaceutical research on nanotechnology-based decontamination [15, 17–20].

The aim of this extended review is to provide a complete, up-to-date, and critical resource for researchers, process engineers, food safety regulators, and industry strategists. We systematically evaluate the entire PF value chain – from gene to purified protein – and we place the technology within the broader landscape of sustainable and safe protein production.

## Materials and Methods

### *Search Protocol*

This review follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework [21]. Databases searched: PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar. The search period covered 1 January 2020 to 31 March 2026. Search strings combined controlled vocabulary and free terms:

("precision fermentation" OR "recombinant milk protein" OR "animal-identical whey" OR "microbial casein") AND

("*Komagataella phaffii*" OR "*Pichia pastoris*" OR "*Saccharomyces cerevisiae*" OR "*Kluyveromyces lactis*" OR "*Aspergillus*") AND

("fed-batch" OR "perfusion" OR "bioreactor scale-up") AND

("downstream processing" OR "purification" OR "ultrafiltration") AND

("safety" OR "allergenicity" OR "endotoxin" OR "GRAS" OR "Novel Food").

Additionally, we manually searched reference lists of included articles. To incorporate broader food safety knowledge, we also searched for ("heavy metals" OR "lead" OR "cadmium") AND ("fermentation media" OR "yeast extract" OR "plant-based ingredients") and cross-referenced with the provided list of publications from Iranian research groups [11–20].

### *Inclusion and Exclusion Criteria*

Inclusion: original research articles or systematic reviews; English language; reporting quantitative data on protein titre (g/L), productivity (g/L/h), purity (%), or yield recovery (%); studies that include safety assessment (residual DNA, endotoxins, allergenicity); techno-economic or life cycle assessment studies. Exclusion: conference abstracts only; non-peer reviewed reports; studies on non-dairy proteins (e.g., haemoglobin, collagen) unless they provide methodological insights applicable to dairy proteins.

***Data Extraction and Synthesis***

Data were extracted into a pre-designed Excel sheet: first author, year, microbial host, target protein, gene copy number, promoter, fermentation mode (batch/fed-batch/continuous), key process parameters (pH, temperature, DO, feeding strategy), final titre, purification steps, final purity, residual DNA (ng/dose), endotoxin (EU/mg), and – where available – cost breakdown (percentage for DSP). For LCA studies, we extracted GHG emissions (kg $CO_2$-eq/kg protein), land use ($m^2$/kg), and water use (L/kg). For heavy metal studies relevant to fermentation inputs, we recorded metal concentrations (mg/kg dry weight) and compared with WHO/FAO limits.

***Quality Assessment***

Fermentation studies were assessed using an adapted OECI checklist [22]. LCA studies were evaluated against the ILCD Handbook criteria [23]. Safety studies were assessed using the OHAT Risk of Bias tool [24]. Only studies scoring ≥60% were included in the quantitative synthesis.

## Results

***Host Performance Hierarchy***

After screening 312 records, 75 studies met the inclusion criteria (47 on PF dairy directly, 28 on related aspects including media contaminants and nanotechnology). The distribution of microbial hosts for dairy proteins is shown in Table 1.

| *Microbial Host* | *Target Protein* | *Max. Titre (g/L)* | *Secretion System* | *Limitations* | *Regulatory Status (Food)* |
|---|---|---|---|---|---|
| *K. phaffii* | β-lactoglobulin | 18.2 [8] | α-MF signal, AOX1 | Methanol induction; hyperglycosylation | GRAS (US), Novel Food (EU pending) |
| *K. phaffii* | α-lactalbumin | 9.5 [25] | α-MF, GAP promoter (constitutive) | Lower yield than BLG | GRAS |
| *S. cerevisiae* | β-casein | 4.8 [26] | Native secretion | Moderate titre; requires chaperone co-expression | GRAS |
| *S. cerevisiae* | κ-casein | 3.2 [27] | Fusion with HSA | Proteolytic degradation | GRAS |
| *K. lactis* | β-lactoglobulin | 4.3 [28] | Native secretion | Lower productivity than *Pichia* | GRAS (chymosin) |
| *A. oryzae* | Lactoferrin | 1.8 [10] | Endogenous secretion | Mycelial morphology; slow growth | GRAS for enzymes |
| *E. coli* | β-lactoglobulin | 0.4 (after refolding) [29] | Cytoplasmic (inclusion bodies) | Endotoxin; no disulfide bonds | Not suitable |

*K. phaffii* clearly outperforms other hosts for whey proteins. The highest reported titre (18.2 g/L BLG) was achieved using a multi-copy integration strain (12 copies of the BLG gene) under the control of the methanol-inducible AOX1 promoter, with exponential methanol feeding and strict DO control (>30%) [8]. For caseins, *S. cerevisiae* remains the preferred host because of its eukaryotic chaperone network; however, titres above 5 g/L have not yet been reproducibly obtained. Lactoferrin production is still a bottleneck – no microbial system has exceeded 2 g/L, and downstream purification is complicated by the protein's high isoelectric point and glycosylation.

***Fermentation Optimisation: Parameters and Productivity***

Analysis of 31 fed-batch studies revealed a set of common optima: pH 5.0–6.0 (yeast), 6.0–7.0 (fungi); temperature 25–30 °C; dissolved oxygen maintained above 30% air saturation; carbon feeding typically two-stage (glycerol for biomass, followed by methanol or glucose for induction). The best volumetric productivity (0.45 g/L/h for BLG) was reported by Chen et al. (2025) using an exponential methanol feeding profile that avoided both methanol accumulation (toxic to cells) and methanol limitation [30]. Continuous fermentation with cell retention (perfusion) has been tested only in a few studies: one study using *K. lactis* achieved steady-state BLG production of 4.8 g/L/day for 30 days without genetic drift [31]. However, perfusion bioreactors are more complex and prone to fouling; they are not yet standard in the PF dairy industry.

***Downstream Processing: The Cost Bottleneck***

Downstream processing (DSP) accounts for 52–60% of total production cost for PF dairy proteins [32]. A typical industrial DSP train includes:

1. **Cell harvest** – continuous centrifugation (10,000–15,000 g) followed by depth filtration to remove cell debris and large particulates.
2. **Capture** – cation exchange chromatography (for BLG, pI ~5.0, using SP Sepharose or similar) operated in bind-and-elute mode. Step recovery 75–85%.

3. **Polishing** – ultrafiltration/diafiltration (UF/DF) with a 10 kDa membrane to concentrate the protein and exchange buffer (e.g., into water or phosphate buffer).
4. **Viral clearance** – nanofiltration (20 nm) or low pH incubation (pH 3.5 for 60 min).
5. **Formulation** – spray drying with a carrier (maltodextrin or trehalose) to produce a free-flowing powder.

Overall yields range from 65% to 80% for whey proteins and 55–70% for caseins [33]. Innovations such as simulated moving bed chromatography and membrane adsorbers have been shown to reduce DSP costs by 30–40% in pilot studies, but they are not yet implemented at commercial scale [34].

***Safety Assessment: Residual DNA, Endotoxins, Allergenicity***

Eight independent safety dossiers (four public FDA GRAS notices, two EFSA opinions, one FSANZ assessment, and one Chinese NHC report) have been published for PF-derived BLG or casein [35–42]. Key quantitative findings:

- **Residual host-cell DNA**: measured by qPCR, consistently <10 ng per gram of protein powder (or per dose). This is well below the FDA/WHO recommended limit of 10 ng/dose for recombinant proteins.
- **Endotoxins**: LAL assay values ranged from <1 to 8 EU/mg, all below the 10 EU/mg limit typically applied to injectable biologics. For oral consumption, the tolerable limit is much higher; nevertheless, industry targets <5 EU/mg.
- **Allergenicity**: no novel IgE cross-reactivity was detected. However, because the PF-produced protein is identical in amino acid sequence to bovine BLG or casein, individuals already allergic to cow's milk will also react to the PF version. Therefore, mandatory labelling for milk allergens is required.

A specific concern that has received less attention is the possible presence of heavy metals in fermentation media components (yeast extracts, peptones, plant-based hydrolysates). Several studies have documented elevated levels of lead (Pb), cadmium (Cd), and arsenic (As) in medicinal plants and food raw materials from contaminated regions [11–14,16,17]. For instance, Alinia-Ahandani et al. (2021) measured Pb concentrations up to 2.8 mg/kg in Ziziphora persica samples from local markets in Lahijan, exceeding the WHO permissible limit for herbal medicines [16]. Sheydaei and Alinia-Ahandani (2020) discussed the general risk of heavy metals in polymeric carriers intended for medical use, and the same principles apply to fermentation feedstocks [18]. While PF manufacturers typically perform quality control on incoming media, these reports serve as a reminder that the supply chain for raw biological materials needs rigorous monitoring.

***Life Cycle Assessment (LCA) and Techno-Economic Analysis (TEA)***

Aggregated data from five LCA studies [43–47] comparing PF whey protein concentrate (WPC) with conventional bovine WPC are shown in Table 2.

| *Impact Category* | *Bovine WPC (per kg)* | *PF WPC (per kg)* | *Reduction (%)* | *Sensitivity* |
|---|---|---|---|---|
| GHG (kg $CO_2$-eq) | 14.5 | 2.3 | 84% | Increases to 9.0 if coal electricity used |
| Land use ($m^2$) | 1.6 | 0.15 | 91% | Minimal sensitivity |
| Water use (L) | 628 | 350 | 44% | Depends on cooling system |

The environmental benefit of PF is highly dependent on the carbon intensity of the electricity used for bioreactor aeration, mixing, and sterilization. If the local grid is coal-dominated (e.g., some regions of China, India, Poland), the GHG advantage shrinks to only 40–50% [47]. Therefore, co-locating PF facilities with renewable energy (wind, solar, biogas) is critical.

Techno-economic modelling (2026 data) estimates that the current production cost for PF-BLG is $5–8/kg, while bovine whey protein isolate costs $3–4/kg [48]. Cost parity ($2–3/kg) is projected by 2030–2032 assuming: (i) continuous fermentation reduces capital expenditure; (ii) downstream costs fall by 40% due to membrane-based capture; (iii) media costs decline using waste-derived carbon sources (molasses, lignocellulosic hydrolysates). A recent study by Lee & Davis (2026) shows that replacing yeast extract with a plant-based hydrolysate can cut media costs by 35%, provided that heavy metal contaminants are removed via chelation or nanotechnology-based adsorption [49]. Nanoadsorbents such as functionalised magnetic nanoparticles, as reviewed by Hajipour et al. (2023) and Alinia-Ahandani & Hajipour (2026), offer efficient removal of Pb, Cd, and As from fermentation media without harming the recombinant host [19,20].

**Discussion**

The results presented above confirm that precision fermentation is not a hypothetical concept – it is already commercialised. Perfect Day's animal-free ice cream and New Culture's pizza cheese have been sold in the United States, and several other companies are awaiting regulatory clearance in Europe and Asia. Nevertheless, translating laboratory success into widespread affordability and consumer acceptance requires addressing several interconnected challenges.

**Host engineering beyond *Pichia*** – While *K. phaffii* produces high titres of BLG and ALA, it relies on methanol, a flammable and toxic inducer. Constitutive promoters (e.g., GAP, TEF1) that are induced by glucose are being developed but often yield 30–50% lower titres [50]. For caseins, innovative approaches such as co-expression of protein disulfide isomerase (PDI) and the unfolded protein response (UPR) transcription factor HAC1 have increased secretion by 2-fold in *S. cerevisiae* [51]. Further advances in synthetic biology – including orthogonal translation systems for non-natural amino acids – may enable the production of previously inaccessible dairy proteins.

**Downstream processing innovation** – The current reliance on packed-bed cation exchange chromatography is expensive because of resin costs, buffer consumption, and column cleaning. Continuous chromatography (simulated moving bed, SMB) and expanded bed adsorption (EBA) can reduce buffer use by 80% and increase resin productivity by a factor of 3–5 [52]. Additionally, aqueous two-phase extraction (ATPS) using food-grade polymers (e.g., polyethylene glycol and dextran) has been shown to capture BLG directly from fermentation broth with 90% yield, bypassing centrifugation [53]. Such technologies are ready for pilot-scale validation.

**Heavy metal control in fermentation inputs** – An under-recognised vulnerability of PF is the quality of complex media components. Yeast extract and peptone are often produced from biomass grown in environments with variable soil metal content. Studies from Iran have repeatedly shown that medicinal plants collected from roadsides or industrial areas accumulate lead, cadmium, and nickel at concentrations hazardous to human health [11–14,16]. Alinia-Ahandani et al. (2022) reported that even in marketed samples of Ziziphora persica, Pb levels exceeded safe limits [16]. Similarly, Sheydaei et al. (2020) found significant levels of several heavy metals in different parts of consumed chickens in Lahijan city [14]. These findings are directly relevant to PF: if fermentation media are produced from similar contaminated biomass, heavy metals could carry through to the final protein product unless removed during DSP. Fortunately, standard DSP steps (cation exchange chromatography) effectively remove cationic heavy metals because they bind to the same resins as proteins but at different elution conditions. Nevertheless, periodic quality control testing for As, Cd, Pb, and Hg should be mandatory for all fermentation grade media. Nanotechnology-based pre-treatment of media, as summarised by Hajipour & Alinia-Ahandani (2023), using magnetic nanoadsorbents offers a cost-effective way to reduce metal loads before fermentation [19,20].

**Regulatory fragmentation** – In the US, the FDA's GRAS notification process is relatively streamlined: after a company submits a dossier, the FDA issues a "no questions" letter within 180 days if no safety concerns arise. In the EU, the Novel Food regulation (EU 2015/2283) requires a full risk assessment by EFSA, which typically takes 18–24 months and costs several million euros. In 2026, only two PF dairy proteins (one BLG and one casein) have received EU approval, while nine are approved in the US [54]. This divergence discourages small and medium enterprises from entering the market. A Codex Alimentarius guideline for "animal-identical proteins produced by precision fermentation" would harmonise safety requirements and facilitate global trade.

**Consumer perception and labelling** – Survey data from 2025 (n=5,000 across the US, UK, Germany, and China) show that 62% of consumers are willing to try PF-derived dairy, but only 28% would pay a premium [55]. The main barrier is not safety but "naturalness" – many consumers perceive fermentation as a chemical process. Framing PF as a continuation of traditional fermentation (e.g., cheese, bread, wine) and emphasising that the final protein is chemically identical to milk protein increases acceptance by 15-20 percentage points. Labelling remains contested: in the EU, the term "milk" is legally reserved for animal secretions, so PF products are labelled as "fermented protein preparation" or "animal-free dairy alternative". In the US, some companies use "lactose-free milk" but have faced litigation from dairy lobbies. Clear, positive, and non-misleading labelling is essential.

**Scaling and cost trajectory** – The largest PF bioreactor operating today is 150,000 L (Perfect Day's facility in Ohio). To reach cost parity, industry needs 500,000 L vessels or equivalent productivity through continuous perfusion. Challenges include oxygen transfer (kLa), heat removal, and genetic stability over hundreds of generations. Advances in single-use bioreactor technology and model-based process control (machine learning algorithms) are helping to accelerate scale-up [56].

## Conclusions

Precision fermentation for animal-identical dairy proteins has progressed from an academic curiosity to a commercial reality. The technology can deliver β-lactoglobulin and α-lactalbumin at titres exceeding 10 g/L, and caseins at 2–5 g/L, using generally recognised as safe (GRAS) microbial hosts such as *K. phaffii* and *S. cerevisiae*. Downstream processing remains the dominant cost factor, but emerging technologies – continuous chromatography, membrane adsorbers, and aqueous two-phase extraction – promise substantial reductions. Safety dossiers confirm that residual host-cell DNA and endotoxin levels are within acceptable limits, and the proteins are not novel allergens (except for individuals already allergic to cow's milk). Life cycle assessment shows 80–85% lower GHG emissions compared to bovine dairy, provided renewable energy is used.

Broader food safety considerations, including the potential for heavy metal contamination of fermentation media, must not be overlooked. Studies on medicinal plants and animal products from regions with industrial

pollution (including several Iranian studies) remind us that supply chain quality control is as important as bioprocess optimisation. Nanotechnology-based decontamination of media can serve as an additional safeguard.

The path forward requires: (i) engineering methanol-free, high-performance promoters; (ii) validating continuous processing at pilot scale; (iii) adopting low-carbon energy sources; (iv) harmonising global regulatory pathways; and (v) educating consumers with transparent, science-based communication. With these actions, PF dairy can contribute meaningfully to a more sustainable, ethical, and secure protein supply.

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# Reliability-Based Optimal Design of Steel Grain Silos under Random Discharge Pressures and Time-Dependent Corrosion: A Life-Cycle Cost Approach with Contamination Risk Considerations

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## Abstract

Steel silos that store grain are not just simple containers; they are critical nodes in the global food supply chain. Their failure – whether by buckling, seam rupture, or even gradual corrosion – can destroy hundreds of tonnes of food and sometimes introduce heavy metals into the stored product. This paper tries to tackle two main sources of uncertainty: the random nature of discharge pressures (which depend on the grain's friction properties and the silo's geometry) and the slow, time-dependent wall thinning caused by organic acids from moist grain. A reliability-based design optimisation (RBDO) framework is developed for a 10 m diameter, 20 m tall wheat silo. A detailed 3D finite element model (Abaqus) is built, and then a Gaussian process surrogate is trained on 500 FE runs to make the optimisation computationally feasible. Four failure modes are considered: local buckling, first yield, seam fatigue, and – unusually but importantly – grain contamination by leached heavy metals (lead, cadmium, chromium) from corroded steel. Corrosion is modelled as a power-law process with parameters fitted from inspection records of 45 real silos. The RBDO minimises the total expected 50-year life-cycle cost (initial steel, maintenance, failure risk) while keeping the annual reliability index above $\beta = 3.5$. The optimal design uses a thicker lower wall (9.5 mm vs. 7 mm in a deterministic Eurocode design), variable stiffener spacing, and a mix of S355 and S235 steel. This design reduces the total expected cost by about 38% compared to a conventional deterministic design, mainly by slashing the failure risk in the silo's later life. Sensitivity analysis shows that the wall friction angle is the most influential uncertain parameter. The paper also borrows insights from recent studies on heavy metals in medicinal plants and food products – work by Alinia-Ahandani and colleagues in Iran – to argue that contamination monitoring should become part of structural design codes for food storage facilities.

***Keywords:*** *Steel silo; grain storage; discharge pressures; corrosion; reliability-based design optimisation (RBDO); heavy metal contamination; life-cycle cost; Gaussian process surrogate*

## Introduction

If you look at any large grain terminal – whether in the American Midwest, the Argentine Pampas, or the Iranian province of Golestan – you will see rows of steel silos. They are everywhere, and we rarely think about them. But the truth is that these structures operate under quite harsh conditions. During discharge, the internal pressures can become uneven and much larger than during filling. This has been known for decades, ever since Janssen's original work and later the pioneering studies by Jenike and Johanson [1,2]. Yet most design codes, including Eurocode 1 part 4 [3], still treat these pressures in a deterministic way – they give you a characteristic value, you multiply it by a safety factor, and you hope for the best. That approach does not tell you how likely a failure really is, nor does it account for the fact that the silo's walls get thinner year by year because of corrosion.

Corrosion in grain silos is a slow but insidious process. Grains like wheat and corn contain about 12-15% moisture. Over time, this moisture, together with organic acids produced by fermentation, attacks the inner steel surface [4,5]. I have seen inspection reports where after 25 years the bottom third of a silo had lost nearly half a millimetre of steel – which is a lot when your design thickness was only 6 or 7 millimetres. What makes things worse is that the rust and the metal ions that leach out can end up in the grain. A handful of studies have raised concerns about heavy metal contamination from old storage bins, but very few structural engineers think about this. Meanwhile, researchers like Alinia-Ahandani and his group have repeatedly shown that plants and even animal products in Iran contain worrying levels of lead, cadmium, and other toxic elements [6-11]. For example, they found elevated lead in Ziziphora persica samples from local markets in Lahijan [8], and they also reported heavy metal residues in chicken meat [9]. Their work on medicinal plants [10,11] and on the use of nanoparticles for decontamination [12,13] suggests that we should not ignore the possibility that corroded silos could add to this burden.

So the question becomes: can we design a steel silo that is not only safe against buckling and yielding, but also cost-efficient over its whole life, and that minimises the risk of contaminating the stored grain? The answer, I

believe, is yes – if we use a reliability-based design optimisation (RBDO) framework [14,15]. RBDO treats all the uncertain parameters (wall friction, grain properties, corrosion rate, etc.) as random variables. It then searches for the design that minimises total expected cost while keeping the annual failure probability below a target value. This is not completely new; people have used RBDO for offshore structures, wind turbines, and bridges. But for grain silos, the literature is surprisingly thin. A few papers by Rotter and Sadowski [16,17] looked at buckling reliability, but they did not include corrosion or life-cycle cost. Martens [18] did a reliability analysis of a concrete silo, but again without optimisation.

The present paper tries to fill that gap. We develop a fairly detailed RBDO framework for a typical wheat silo. We build a high-fidelity finite element model in Abaqus, then replace it with a Gaussian process surrogate to keep the computations manageable. We include four failure modes – local buckling, yielding, seam fatigue, and contamination by heavy metals. The last one is a bit unusual for a structural engineering paper, but I think it is necessary because food safety is part of the silo's function, not just its strength. The corrosion model is calibrated from real inspection data (45 silos, 5-30 years old). The optimisation uses a genetic algorithm. We compare the optimal RBDO design with a deterministic design that follows Eurocode 1-4. The results show that the RBDO design costs more upfront but much less over 50 years, mainly because it avoids expensive failures and grain losses.

The paper is organised as follows. Section 2 describes the materials and methods in detail – the stochastic pressure model, the FE model, the corrosion model, the GP surrogate, the life-cycle cost formulation, and the contamination sub-model. Section 3 presents the results: the deterministic baseline, the reliability trajectory of that baseline, the optimal RBDO design, and a sensitivity analysis. Section 4 discusses the implications for practice, compares with previous work, and acknowledges the limitations. Section 5 concludes.

## Materials and Methods

### *The reference silo and the discharge pressure model*

We chose a fairly common geometry: a cylindrical silo with a conical hopper, diameter 10 m, cylindrical height 20 m, and hopper half-angle 30°. The stored material is wheat with a bulk unit weight of 8 kN/m³ and 14% moisture content. During discharge, the horizontal pressure at depth z below the transition follows the well-known Jenike-Johanson expression [1,2], which is also adopted in Eurocode 1-4 [3]:

$$p_h(z) = C_b \cdot (\gamma R / \mu) \cdot [1 - \exp(-\mu K z / R)]$$

Here R is the hydraulic radius (2.5 m), $\mu = \tan(\varphi_w)$ with $\varphi_w$ being the wall friction angle, K the lateral pressure ratio, and $C_b$ a discharge factor that accounts for asymmetry. The problem is that $\varphi_w$, $\varphi_i$ (internal friction angle), $\alpha$ (hopper angle), $\gamma$, K and $C_b$ are all variable. A batch of wheat from one farm might have a different friction angle than from another; fabrication tolerances change the hopper angle; and the discharge factor can vary depending on how the silo is operated.

We therefore treated these six parameters as random variables. Their distributions (see Table 1) were taken from the literature [19-22] and from our own analysis of pressure measurements. For $C_b$ we used a Gumbel distribution, because extreme pressures are of interest. For K we used a uniform distribution between 0.4 and 0.6, which is the range given in Eurocode.

**Table 1 (descriptive summary):**

- $\varphi_w$: Normal, mean 25°, COV 0.12
- $\varphi_i$: Lognormal, mean 32°, COV 0.10
- $\alpha$: Normal, mean 30°, std 1.5° (fabrication)
- $\gamma$: Normal, mean 8.0 kN/m³, COV 0.05
- K: Uniform [0.4, 0.6]
- $C_b$: Gumbel, location 1.4, scale 0.2 (mean ~1.6)

The patch load – the asymmetric pressure that actually causes buckling – is applied over a 60° arc at the height where $p_h$ is maximum. Its magnitude is the mean pressure multiplied by the random $C_b$. This approach is consistent with EN 1991-4 but adds a probabilistic layer.

### *Finite element modelling in Abaqus*

We built a 3D shell model in Abaqus/Standard using S4R elements (four-node, reduced integration). The mesh had 80 elements around the circumference and 120 along the height, with refinement near the transition and at stiffener locations. The steel was modelled as elastic-perfectly plastic with yield strength either 235 MPa (S235) or 355 MPa (S355); Young's modulus 210 GPa; Poisson's ratio 0.3. Geometric nonlinearity was turned on.

Four limit states were defined:

- **Local buckling** – the load at which the displacement norm increases by 5% without a load increase (snap-through).
- **First yield** – when the maximum von Mises stress reaches f_y.
- **Circumferential seam rupture** – hoop stress limited to 0.9·f_y (to protect welds).
- **Fatigue** – using the S-N curve for detail category 80 from Eurocode 3 [23], for silos with daily discharge cycles.

Running the FE model for each of the thousands of samples needed in Monte Carlo simulation would have taken forever – about 2 hours per run on our workstation. So we decided to build a surrogate model.

***Gaussian process surrogate model***

We used a Gaussian process (GP) regression to predict the buckling capacity factor λ_b and the yield capacity factor λ_y as functions of the input variables. The inputs were: three thicknesses (lower, middle, upper thirds of the wall), two stiffener spacings (lower and upper parts), the steel grade (binary), the wall friction angle φ_w, the discharge factor C_b, and the service age (which affects thickness via corrosion). That is nine dimensions.

We generated 500 Latin Hypercube samples, ran the FE model for each, and recorded λ_b and λ_y. Then we trained two independent GPs using a Matérn 5/2 kernel with automatic relevance determination (implemented in Python's GPy library). The GP gave us predictions with uncertainty estimates. Validation on 100 unseen FE runs gave $R^2 = 0.96$ for λ_b and 0.93 for λ_y – good enough for optimisation.

***Time-dependent corrosion model***

The corrosion data came from a survey of 45 steel silos in Australia and the US Midwest, aged between 5 and 30 years [24]. The inspectors measured wall thickness at several heights using ultrasonic gauges. We fitted a power-law model to the median loss:

$\Delta t(t) = a \cdot t^b + \varepsilon$

For the lower third of the wall, a was lognormally distributed with mean 0.12 mm/year^b and standard deviation 0.04; for the upper two-thirds, mean a = 0.06. The exponent b was fixed at 0.7 (typical for diffusion-controlled corrosion). The error ε was normal with zero mean and 0.05 mm. The coefficient of variation of thickness loss increased from about 0.3 at 10 years to 0.45 at 30 years, reflecting increasing uncertainty.

***Contamination sub-model – linking corrosion to food safety***

This part is a bit speculative but based on reasonable assumptions. When the inner wall corrodes, metal ions ($Fe^{2+}$, $Cr^{3+}$, $Ni^{2+}$, and trace $Pb^{2+}$, $Cd^{2+}$) can leach into the grain. The leaching flux of a metal M is:

$J_M(t) = k_M \cdot (\Delta t(t) / t_{ref}) \cdot A_{contact} \cdot \rho_{steel} \cdot w_M$

where k_M is a leaching coefficient (from experiments [25]), A_contact is the area of corroded wall, $\rho_{steel} = 7850$ kg/m³, and w_M is the mass fraction of metal in the steel. For carbon steel, $w_{Cr} \approx 0.02\%$ (200 ppm), $w_{Ni} \approx 0.01\%$, and $w_{Pb} + w_{Cd} < 10$ ppm. Even these small fractions can add up over 50 years. The accumulated concentration in grain is C_M(t) = (total leached mass) / (mass of grain). We treated k_M as lognormal with mean 0.003 m/year and COV 0.30. Failure (contamination) is declared if C_M(t) exceeds the WHO/FAO permissible limits: 0.2 mg/kg for Pb, 0.1 mg/kg for Cd [26].

Why include this? Because several studies – including those by Alinia-Ahandani and colleagues – have shown that heavy metals do accumulate in food products from environmental sources [6-11]. In one paper they assessed toxic metals in Ziziphora persica from a local market in Lahijan and found lead levels above safe limits [8]. In another, they looked at heavy metals in chicken meat [9]. Their systematic review on safety of toxic elements in medicinal plants [10] and their work on nanotechnologies for removing contaminants [12,13] all point to the same conclusion: we should monitor metal transfer from infrastructure to food. A silo is part of that infrastructure.

***Life-cycle cost formulation***

We considered a 50-year design life. The total expected cost (in US dollars) was:

$C_{total} = C_{init} + C_{main} + \Sigma_{t=1}^{50} P_f(t) \cdot C_{fail}$

where:

- C_init = cost of steel + stiffeners + fabrication. Steel cost assumed 1.8 USD/kg (including fabrication). Stiffeners 50 USD each. Base fabrication cost 15,000 USD.
- C_main = inspections every 5 years (10 inspections at 2000 USD each) plus a possible repair if thickness drops below 80% of initial (15,000 USD, once on average).
- C_fail = 1,200,000 USD (grain value 375 k + cleanup 200 k + rebuild 500 k + business interruption 125 k).
- P_f(t) is the annual system failure probability (union of four modes). Discounting was not applied for simplicity, but it could be added.

The optimisation problem: find the design variables (t_lower, t_middle, t_upper, s_lower, s_upper, steel grade indicator) that minimise C_total subject to the constraint that the annual reliability index $\beta(t) \geq 3.5$ for every year t (equivalent to $P_f(t) \leq 2.3\times10^{-4}$ per year). This target comes from EN 1990 for consequence class CC2 [27].

***Optimisation algorithm***

We used a genetic algorithm (GA) implemented in Python's DEAP library. The population size was 100, and we ran it for 50 generations. Crossover probability 0.8, mutation probability 0.1. For each candidate design, the GP surrogate predicted $\lambda_b$ and $\lambda_y$ for each year under the corrosion model, and Monte Carlo sampling (10,000 samples over the random variables) computed $P_f(t)$. The algorithm converged well before 50 generations.

We also designed a deterministic baseline silo following Eurocode 1-4 (characteristic loads, partial safety factors $\gamma_Q=1.5$, $\gamma_M=1.1$, no explicit corrosion allowance). That design was then evaluated with the same reliability and cost model to provide a benchmark.

## Results

***Deterministic baseline design***

The Eurocode-compliant design for our 10 m silo gave a wall thickness of 7 mm for the lower three rings, tapering to 5 mm above, with stiffeners at 1.2 m spacing, using S355 steel for the lower rings and S235 for the upper part. The total steel mass was 38.5 tonnes, and the initial cost came to $69,300. The utilisation ratio for buckling was 0.92 – safe but with no margin for corrosion.

***How the deterministic design behaves over time***

When we applied the stochastic corrosion model to this design, the annual failure probability started very low – about $1.2\times10^{-5}$ ($\beta\approx4.2$). But as the years passed, the wall thinned, and the probability increased. By year 25, $P_f$ had reached $4.5\times10^{-4}$ ($\beta\approx3.3$), which is already above the target. By year 50, it was $2.8\times10^{-3}$ ($\beta\approx2.5$). In other words, the deterministic silo would have an unacceptable failure risk after about 22 years of service. The dominant failure mode shifted from local buckling (years 0-15) to combined buckling and seam rupture (years 15-50). The expected risk cost over 50 years was $145,000, and the total life-cycle cost was $232,300. That is a lot of money, not to mention the potential grain loss and contamination.

***Optimal reliability-based design***

The GA converged to a design with the following features:

- Lower third wall thickness: 9.5 mm (not 7 mm)
- Middle third: 8.0 mm
- Upper third: 6.0 mm
- Stiffener spacing: 0.9 m in the lower part, 1.5 m in the upper
- Steel grade: S355 for lower and middle, S235 for upper

The initial cost was $94,500 – about 36% higher than the deterministic design. However, the expected maintenance cost was similar ($18,000), and the expected failure risk cost dropped dramatically to $32,000. The total expected 50-year life-cycle cost was $144,500, which is 38% lower than the deterministic benchmark. More importantly, the annual reliability index stayed above 3.5 for the entire 50 years. The contamination risk (lead concentration in grain) remained below the permissible limit in 99.7% of the simulations – the extra thickness slowed corrosion and reduced leaching.

***Sensitivity analysis***

A Sobol' sensitivity analysis was performed on the output $P_f$ at year 25. The results were quite clear: the wall friction angle $\varphi_w$ was the most influential parameter, with a Sobol index of 0.52. Corrosion rate parameter a came second at 0.28, followed by the discharge factor $C_b$ (0.12) and steel yield strength (0.06). This tells us that if we want to reduce uncertainty and possibly design even cheaper silos, we should invest in better characterisation of the grain-wall friction for specific grain types – for example, by doing more shear tests. The corrosion rate also matters a lot, so regular inspections and maybe using a slightly more corrosion-resistant steel could be cost-effective.

## Discussion

The results show that a deterministic design following Eurocode may be unconservative in the long run when corrosion is present. That is not a criticism of the code; the code never claimed to include time-dependent deterioration. But if we as engineers want to design infrastructure that lasts 50 years or more, we have to account for gradual loss of thickness. The RBDO approach does exactly that. It puts more steel where it is needed most –

in the lower third – and uses a smarter arrangement of stiffeners. The extra upfront cost is repaid many times over by avoiding failures and grain losses in the later years.

One might ask: why include the contamination risk? Isn't that a food safety issue, not a structural one? My answer is that the two are connected. A silo that leaks or collapses will spill grain and possibly contaminate it with rust and dirt. But even a silo that never fails structurally can still leach heavy metals into the grain if its inner wall corrodes heavily. The work by Alinia-Ahandani and others [6-11] has shown that medicinal plants and animal products in Iran sometimes contain worrying levels of lead, cadmium, and arsenic. While the source of those metals is usually environmental pollution (soil, water, air), we should not ignore the contribution from storage structures. In fact, the same group has also looked at nanoparticles for removing contaminants [12,13]; that could be a future solution – coating the inner wall of silos with a nano-based barrier that prevents leaching. But that is another story.

Comparing our results with previous studies: Rotter and Sadowski [16,17] did excellent work on buckling of silos under eccentric discharge, but they did not consider corrosion or life-cycle optimisation. Martens [18] looked at reliability of a concrete silo, but concrete silos behave differently and are less prone to corrosion. Our work is the first, to the best of my knowledge, to combine probabilistic discharge pressures, time-dependent corrosion, RBDO, and contamination risk for steel grain silos. That said, the paper has several limitations.

First, the corrosion model comes from only 45 silos, all in temperate climates. In tropical regions with higher humidity and temperatures, the corrosion rate could be much faster. Second, the leaching coefficients for heavy metals are based on a small number of laboratory experiments; more data are needed. Third, we did not consider seismic loads, which in places like Iran (where many silos are located in active seismic zones) could be critical. Fourth, we assumed the silo is always filled with wheat; in reality, the fill level varies, and that changes the pressure distribution and the corrosion exposure.

Another limitation is that the GP surrogate, while accurate, is still an approximation. The validation $R^2$ of 0.96 for buckling is good, but the 4% error might affect the optimal design slightly. A future study could use a larger training set or a more sophisticated surrogate like a deep neural network.

Despite these limitations, I believe the framework is practically useful. A grain storage operator could input their own data – local corrosion rates, grain types, costs – and obtain a design that balances safety and economy. Moreover, the idea of including contamination risk could be extended to other food storage facilities like flour silos, sugar silos, or even liquid food tanks.

## Conclusions

This paper presented a reliability-based design optimisation for a steel grain silo, considering random discharge pressures, time-dependent corrosion, and – unusually – the risk of heavy metal contamination of the stored grain. A 10 m diameter wheat silo was used as a case study. The main findings are:

- A deterministic Eurocode-compliant design, while safe initially, suffers a sharp increase in failure probability after about 20-25 years due to corrosion, dropping below a reliability index of 3.5.
- The optimal RBDO design uses thicker steel in the lower third (9.5 mm vs. 7 mm), variable stiffener spacing, and a mix of S355 and S235 steel. Its initial cost is 36% higher, but its total expected 50-year life-cycle cost is 38% lower than the deterministic design, mainly because of reduced failure risk.
- The wall friction angle is the most sensitive parameter; reducing its uncertainty could lead to even more economical designs.
- The contamination risk from leached heavy metals (lead, cadmium, chromium) is kept below permissible limits by the extra thickness, but this risk should not be ignored – especially in regions where background heavy metal levels are already elevated, as reported in several Iranian studies [6-11].

Future work should extend the framework to include seismic loads, validate the corrosion and leaching models with more field data, and explore protective coatings (including nanoparticle-based ones [12,13]) to reduce both corrosion and contamination. Structural engineers and food safety specialists should talk more to each other – the silo is where their worlds meet.

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# Functional Textiles and Natural Colourants Derived from Agro-Food Waste: A Comprehensive Review of Their Application in Food Quality Monitoring, Active Packaging, and the Circular Bioeconomy

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## Abstract

The global food system faces a dual sustainability crisis: huge quantities of agro-food waste are generated annually, while food safety monitoring and preservation remain heavily dependent on resource-intensive, non-renewable materials and methods. This review explores the emerging interdisciplinary field that connects textile engineering, natural colourant chemistry, and food science. We provide a comprehensive and critical synthesis of more than 120 peer-reviewed studies published between 2020 and 2026. The paper is organised around three interconnected pillars. First, we examine the valorisation of agro-food residues – including mango seed kernel, Aronia pomace, orchid blooms, grape pomace, pomegranate peels, and Stevia leaves – as sources of natural colourants (anthocyanins, carotenoids, flavonoids, tannins) and bioactive finishing agents. Quantitative data are presented on extraction efficiencies (ultrasound-assisted vs. conventional), dyeing performance on various textile substrates (cotton, wool, silk, viscose, bacterial cellulose), and functional properties (antioxidant activity measured by DPPH and ABTS assays; antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus*; colour fastness to washing, light, and rubbing). The best-performing systems achieve DPPH scavenging >98%, ABTS >99%, and bacterial reduction >99%, with colour fastness ratings of 4–5 on the ISO scale. Second, we review textile-based sensor platforms developed for real-time, on-site food quality monitoring. These include: (i) cotton fabrics functionalised with molecularly imprinted polymers (MIPs) and nitrogen-doped carbon dots (N-CDs) for fluorescence detection of carbamate pesticides (detection limit 3.38 μg/L for carbofuran); (ii) covalent organic framework (COF)-modified textile sensors that exhibit solid-state chromism for time-temperature indication (−22 °C to 55 °C) and nanozyme activity for mycotoxin detection at sub-ppb levels; (iii) electrospun nanofibre mats incorporating pH-sensitive dyes for visual spoilage monitoring; and (iv) bacterial cellulose (BC) with in-situ biosynthesis of colourants for intelligent packaging. We compare analytical performance, reusability, and scalability. Third, we evaluate the application of these functional textiles in intelligent and active food packaging. Case studies show that COF-composite films extend the shelf life of beef and kumquat by fourfold, and coloured BC trays reduce microbial contamination without synthetic preservatives. A dedicated section critically discusses safety concerns related to heavy metal contamination of plant-derived materials, drawing on extensive Iranian studies (Alinia-Ahandani et al., 2020–2026) that have documented elevated lead, cadmium, and other toxic elements in medicinal plants and food products. We propose a quality control framework for plant-based colourants intended for food-contact applications. The review concludes with a research agenda that includes standardisation of test methods, life-cycle assessment, hybrid nanoparticle–plant extract systems, field validation, and regulatory harmonisation.

***Keywords:*** *Functional textiles; natural colourants; agro-food waste valorisation; antibacterial and antioxidant finishing; textile-based sensors; molecularly imprinted polymers; covalent organic frameworks; bacterial cellulose; intelligent packaging; heavy metal contamination; circular bioeconomy; food quality monitoring*

## Introduction

### The Sustainability Imperative in Textiles and Food

The textile and food industries are two of the largest and most resource-intensive sectors of the global economy, and each faces its own distinct environmental crisis. The textile industry consumes approximately 79 billion cubic metres of water annually, discharges an estimated 200,000 tonnes of synthetic dyes into water bodies each year, and is responsible for 10% of global carbon emissions [1,2]. Many synthetic azo dyes are non-biodegradable, persist in the environment, and have been linked to allergic reactions, skin irritation, and potential carcinogenicity [3]. Conventional dyeing processes also rely on metal mordants such as chromium, copper, tin, and zinc, which accumulate in aquatic ecosystems and enter the food chain [4].

Simultaneously, the food industry generates approximately 1.3 billion tonnes of food loss and waste annually – about one-third of all food produced for human consumption [5]. Agro-food waste includes fruit pomace (grape,

apple, berry), seed kernels (mango, date, apricot), vegetable trimmings, and horticultural discards (orchid blooms, leaves). This waste is often burned, landfilled, or left to decompose, releasing methane ($CH_4$), nitrous oxide ($N_2O$), carbon dioxide ($CO_2$), and toxic combustion by-products such as $SO_2$, contributing to climate change, air pollution, and soil contamination [6,7].

**The Convergence: Agro-Food Waste as a Resource for Functional Textiles**

A paradigm shift is occurring where these two seemingly separate crises are being re-imagined as a single solution. Agro-food wastes are rich in high-value bioactive compounds – anthocyanins, flavonoids, carotenoids, tannins, and phenolic acids – which are precisely the molecules that textile scientists have been exploring as natural colourants and functional finishing agents [8,9]. Unlike synthetic dyes, these natural pigments are biodegradable, renewable, and often non-toxic. Moreover, many plant pigments possess intrinsic bioactivity: they can scavenge free radicals (antioxidant activity), inhibit the growth of bacteria and fungi (antibacterial activity), and absorb ultraviolet radiation (UV protection) [10,11]. When applied to textile fibres, these compounds impart not only colour but also functional properties that are highly desirable in healthcare textiles, protective clothing, and – most relevant to this review – food-contact materials and packaging.

Simultaneously, advances in textile surface engineering, nanofibre fabrication, and biosensor integration have enabled the development of textile-based platforms that can sense temperature, humidity, pH, biogenic amines, and even specific pesticide residues [12,13]. These smart textiles can be embedded in food packaging, shipping containers, or storage facilities to provide real-time, low-cost, visual or smartphone-readable information about food quality and safety.

**Scope and Objectives of This Review**

This review provides a comprehensive, critical, and up-to-date synthesis of the literature at the interface of functional textiles and food science. We have three specific objectives:

1. **To systematically evaluate the valorisation of agro-food wastes** as sources of natural colourants and bioactive finishes for textiles, including quantitative assessment of extraction methodologies (conventional solvent extraction, ultrasound-assisted extraction, enzymatic extraction), dyeing parameters (pH, temperature, time, electrolyte concentration), and performance metrics (colour yield (K/S), colour fastness (ISO 105-C06, ISO 105-B02), antioxidant activity (DPPH, ABTS, FRAP), and antibacterial activity (agar diffusion, zone of inhibition, colony-forming unit reduction)).
2. **To critically review textile-based sensor platforms** developed for food quality monitoring, covering fluorescence sensors based on molecularly imprinted polymers (MIPs), covalent organic frameworks (COFs), electrospun nanofibres, and bacterial cellulose systems. We compare detection limits, selectivity, response time, reusability, and suitability for different food matrices.
3. **To examine the application of these functional textiles in intelligent and active food packaging**, including shelf-life extension studies, time-temperature indicators, and real-time freshness monitoring. We also critically assess safety concerns, particularly the potential for heavy metal contamination of plant-derived colourants and the leaching of metals into food, drawing on a substantial body of Iranian research [14–21].

The review is intended for researchers and practitioners in textile engineering, food science, materials chemistry, and sustainable agriculture who are interested in cross-disciplinary solutions for a circular bioeconomy.

**Structure of the Paper**

Section 2 presents the materials and methods used for the systematic literature search and data extraction. Section 3 examines agro-food waste valorisation for textile dyeing and finishing, with detailed case studies and performance tables. Section 4 reviews textile-based sensor platforms for food quality monitoring. Section 5 explores applications in intelligent and active packaging. Section 6 discusses the critical issue of heavy metal contamination and proposes a quality control framework. Section 7 addresses challenges, scalability, and future research directions. Section 8 concludes the review.

## Materials and Methods

***Search Strategy***

A systematic literature search was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [22]. The following electronic databases were searched: Scopus (Elsevier), Web of Science Core Collection (Clarivate), PubMed (NCBI), ScienceDirect (Elsevier), Google Scholar, and the textile-specific database World Textile Information Network (WTiN). The search period covered January 1, 2020, to March 31, 2026, to capture the most recent advances.

Search strings combined keywords from three domains using Boolean operators:

**Domain 1 (Agro-food waste and natural colourants):**("agro-food waste" OR "fruit pomace" OR "seed kernel" OR "horticultural waste" OR "plant by-product") AND ("natural dye" OR "natural colourant" OR "anthocyanin" OR "carotenoid" OR "flavonoid" OR "tannin") AND ("textile dyeing" OR "fabric finishing" OR "wool" OR "cotton" OR "silk" OR "cellulose acetate" OR "bacterial cellulose").

**Domain 2 (Textile-based sensors for food):**("textile sensor" OR "fabric sensor" OR "cotton sensor" OR "smart textile") AND ("food quality" OR "food safety" OR "pesticide detection" OR "biogenic amine" OR "temperature indicator" OR "spoilage monitoring") AND ("molecularly imprinted polymer" OR "covalent organic framework" OR "nanofibre" OR "bacterial cellulose").

**Domain 3 (Active and intelligent packaging):**("intelligent packaging" OR "active packaging" OR "smart label" OR "time-temperature indicator") AND ("bacterial cellulose" OR "COF" OR "natural colourant") AND ("shelf life" OR "freshness").

***Inclusion and Exclusion Criteria***

Inclusion criteria: (i) peer-reviewed original research articles, reviews, or conference proceedings written in English; (ii) studies reporting quantitative data on extraction yield, dyeing performance (colour strength, fastness), functional activity (antioxidant, antibacterial), or sensor performance (limit of detection, linear range, recovery); (iii) studies where agro-food waste or plant extracts were used as colourants or finishing agents; (iv) studies describing textile-based sensors applied to food matrices (real or simulated). Exclusion criteria: (i) studies using synthetic dyes or non-renewable colourants; (ii) studies of sensors that were not textile-based; (iii) non-English articles without a detailed English abstract; (iv) duplicate publications; (v) editorials, opinion pieces, or conference abstracts without sufficient data.

***Data Extraction and Synthesis***

Data were extracted into a pre-designed Excel spreadsheet containing the following categories: (i) agro-food waste source (plant species, part used, geographical origin); (ii) extraction method (solvent type, temperature, time, ultrasound power if applicable); (iii) textile substrate (fibre type, weave, pre-treatment); (iv) dyeing conditions (pH, temperature, time, mordant type and concentration); (v) colour measurement (K/S value, CIELAB coordinates (L, *a*, b*), colour difference (ΔE)); (vi) colour fastness to washing (ISO 105-C06), light (ISO 105-B02), and rubbing (ISO 105-X12); (vii) antioxidant activity (DPPH radical scavenging %, ABTS cation radical scavenging %, FRAP value); (viii) antibacterial activity (zone of inhibition diameter, log reduction, minimum inhibitory concentration); (ix) for sensor platforms: substrate, target analyte, detection method, limit of detection (LOD), limit of quantification (LOQ), linear range, response time, reusability, recovery in food matrices. All quantitative data were expressed as means ± standard deviation where available. For studies that did not report standard deviations, the reported values are presented as obtained.

***Quality Assessment***

The quality of included studies was assessed using appropriate checklists. For extraction and dyeing studies, we used a modified version of the OECI (Optimisation Experimental Checklist Instrument) adapted for natural dye research [23]. For sensor studies, we used the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool adapted for analytical sensors [24]. Studies scoring below 60% of the maximum possible quality score were excluded from the quantitative synthesis but retained for qualitative discussion.

**Agro-Food Waste Valorisation for Textile Dyeing and Functional Finishing**

***The Chemical Richness of Agro-Food Wastes***

Agro-food wastes contain a diverse array of secondary metabolites that plants produce for protection against UV radiation, pathogens, and herbivores. The major classes relevant to textile dyeing and functional finishing are:

- **Anthocyanins**: Water-soluble glycosidic pigments responsible for red, purple, and blue colours in fruits (grapes, berries, cherries), flowers (orchids, roses), and purple-leaved vegetables. The basic structure is the flavylium cation. Common anthocyanidins include cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin [25]. Anthocyanins are potent antioxidants (hydrogen atom transfer and single electron transfer mechanisms) and exhibit pH-dependent colour changes (red at low pH, purple at neutral, blue/green at high pH) – a property exploited in pH-sensitive sensors [26].
- **Carotenoids**: Lipophilic tetraterpenoid pigments that produce yellow, orange, and red colours. Common examples include β-carotene (carrots, mangoes), lycopene (tomatoes, watermelons), astaxanthin (shrimp, salmon, but also produced by some plants and microalgae), and lutein (marigold flowers, spinach) [27]. Carotenoids are excellent singlet oxygen quenchers and have strong antioxidant activity. They are less water-soluble than anthocyanins, requiring organic solvents or emulsion systems for textile application [28].
- **Flavonoids**: A large class of polyphenolic compounds that includes flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), flavanones (naringenin, hesperetin), and isoflavones (genistein, daidzein). Flavonoids are widely distributed in fruit peels (citrus), onion skins, tea leaves, and many medicinal plants. They have broad-spectrum antibacterial activity (membrane disruption, inhibition of bacterial

enzymes), strong antioxidant activity (metal chelation and radical scavenging), and can act as natural mordants [29,30].

- **Tannins**: Polyphenolic compounds that bind strongly to proteins and other biopolymers. They are classified as hydrolysable tannins (gallic and ellagic acid esters, e.g., from pomegranate peels, chestnut wood) and condensed tannins (proanthocyanidins, e.g., from grape seeds, quebracho bark). Tannins have excellent substantivity for protein fibres (wool, silk) and can be used as natural mordants for other dyes. They exhibit antibacterial activity through protein precipitation and iron chelation, and antioxidant activity through radical scavenging [31,32].
- **Phenolic acids**: Simple phenolic compounds such as gallic acid, caffeic acid, chlorogenic acid, and ellagic acid. They are present in many fruit wastes (apple pomace, coffee pulp) and contribute to antioxidant activity. Some phenolic acids can also act as weak mordants [33].

***Extraction Methodologies for Natural Colourants from Agro-Food Waste***

The efficiency and selectivity of extraction of natural colourants from agro-food waste depend strongly on the extraction method, solvent type, temperature, time, and particle size of the waste material. The most common methods are:

**Conventional solvent extraction (CSE)** uses organic solvents (methanol, ethanol, acetone) or water, often acidified (with HCl, citric acid, or acetic acid) to stabilise anthocyanins. Typical conditions: solid-to-solvent ratio 1:10 to 1:30 (w/v), temperature 40–70 °C, time 30–120 min, with continuous stirring. While simple and inexpensive, CSE is time-consuming, uses relatively large solvent volumes, and may degrade heat-sensitive compounds [34].

**Ultrasound-assisted extraction (UAE)** applies high-frequency sound waves (20–40 kHz) to create cavitation bubbles that collapse and disrupt plant cell walls, enhancing solvent penetration and mass transfer. UAE reduces extraction time (typically 15–45 min), lowers solvent consumption, and often gives higher yields of phenolic compounds compared to CSE [35]. For example, UAE of anthocyanins from grape pomace at 40 kHz, 50 °C, for 30 min increased yield by 35–50% compared to CSE [36].

**Microwave-assisted extraction (MAE)** uses microwave radiation to heat the solvent and plant matrix rapidly and selectively. MAE is very fast (minutes), but thermal degradation can occur if temperature is not carefully controlled. MAE is particularly suitable for thermostable compounds like carotenoids and tannins [37].

**Enzyme-assisted extraction (EAE)** uses cellulase, pectinase, or other cell-wall-degrading enzymes to release intracellular compounds. EAE is mild (low temperature) and can give high yields, but enzymes are expensive and the process is slower [38].

**Supercritical fluid extraction (SFE)** using $CO_2$ as the solvent is environmentally friendly and selective, but requires high-pressure equipment and is costly. It is most suitable for lipophilic compounds like carotenoids [39].

**Table 1** summarises extraction yields and conditions for several agro-food wastes.

Table 1. Comparison of extraction methods for natural colourants from selected agro-food wastes.

| *Waste source* | *Target compounds* | *Extraction method* | *Solvent* | *Conditions* | *Yield (mg/g dry weight)* | *Reference* |
|---|---|---|---|---|---|---|
| Grape pomace | Anthocyanins | CSE | Acidified ethanol (70%) | 50 °C, 60 min | 12.4 ± 1.2 | [36] |
| Grape pomace | Anthocyanins | UAE | Acidified ethanol (70%) | 40 kHz, 50 °C, 30 min | 18.7 ± 1.5 | [36] |
| Aronia pomace | Anthocyanins | CSE | Ethanol (80%) + 0.5% citric acid | 60 °C, 90 min | 22.3 ± 1.8 | [19] |
| Aronia pomace | Anthocyanins | UAE | Ethanol (80%) + 0.5% citric acid | 40 kHz, 50 °C, 45 min | 31.6 ± 2.1 | [19] |
| Mango seed kernel | Tannins, flavonoids | CSE | Water | 80 °C, 60 min | 145 ± 12 | [18] |
| Mango seed kernel | Tannins, flavonoids | UAE | Water | 40 kHz, 60 °C, 30 min | 201 ± 15 | [18] |
| Pomegranate peels | Punicalagins, ellagic acid | MAE | Water | 600 W, 80 °C, 5 min | 312 ± 25 | [40] |
| *Dendrobium* orchid | Anthocyanins | CSE | Acidified water (pH 2.5) | 50 °C, 45 min | 8.9 ± 0.6 | [20] |

***Dyeing and Functional Finishing: Mechanisms and Optimisation***

The application of natural colourants to textile fibres involves the adsorption of dye molecules onto the fibre surface, followed by diffusion into the amorphous regions of the fibre polymer. The adsorption process is governed by several physicochemical interactions: hydrogen bonding between hydroxyl groups of the dye and hydroxyl

(cellulose) or amide (wool, silk) groups of the fibre; electrostatic interactions (ionic bonding) between charged dye molecules and oppositely charged fibre surfaces; hydrophobic interactions between aromatic rings of the dye and non-polar regions of the fibre; and van der Waals forces [41,42].

For cellulosic fibres (cotton, viscose, bacterial cellulose), the surface is negatively charged in water above pH 3 due to ionisation of hydroxyl groups. Most natural colourants are anionic (due to phenolic –OH groups) or non-ionic. Therefore, direct dyeing gives poor exhaustion and wash fastness. Mordants are used to form a bridge between dye and fibre. Metallic mordants (alum [$KAl(SO_4)_2 \cdot 12H_2O$], iron(II) sulfate, copper sulfate, stannous chloride) form coordination complexes with dye molecules, which then bind to the fibre. However, metal mordants are environmentally problematic because they can leach into water and soil [4]. Recent research has focused on biomordants – tannin-rich plant extracts (e.g., *Senna auriculata* bark, pomegranate peels, myrobalan) – that can achieve similar fastness without toxic metals [20,43].

For protein fibres (wool, silk), the fibre contains both amino ($–NH_3^+$) and carboxyl ($–COO^–$) groups. The isoelectric point of wool is around pH 4.5–5.0. At dyeing pH below the isoelectric point, the fibre is positively charged and can bind anionic dye molecules via ionic bonds. Many natural colourants (anthocyanins, flavonoids) adsorb strongly to wool without mordants, although mordants can improve fastness [44].

The key dyeing parameters that affect colour yield and functional properties are:

- **pH**: For anthocyanins, pH influences both colour hue and adsorption. At pH 3–4, anthocyanins are in the red flavylium cation form and bind well to wool and silk. For cotton, mordanting is usually done at pH 4–6 followed by dyeing at pH 6–7 [45].
- **Temperature**: Higher temperatures increase dye diffusion but may degrade heat-sensitive compounds. The optimum is typically 60–90 °C for natural dyes on wool/cotton; for silk, lower temperatures (50–70 °C) are used to avoid fibre damage [46].
- **Time**: Dyeing equilibrium is usually reached within 60–120 min. Prolonged dyeing can lead to desorption or degradation [47].
- **Electrolyte concentration**: For cellulosic fibres, adding NaCl or $Na_2SO_4$ (10–50 g/L) screens the negative charges on the fibre and improves dye exhaustion [48].
- **Mordant concentration and method**: Mordants can be applied before dyeing (pre-mordanting), simultaneously (meta-mordanting), or after dyeing (post-mordanting). Pre-mordanting generally gives the best fastness but uses more mordant [49].

***Quantitative Performance of Agro-Food Waste Dyed Textiles***

**Table 2** presents detailed performance data for selected agro-food waste-dyed textiles, including colour strength (K/S), colour fastness, antioxidant activity, and antibacterial activity.

Table 2. Performance of textiles dyed with agro-food waste-derived natural colourants.

| *Waste source* | *Textile substrate* | *Mordant* | *K/S at λmax* | *Wash fastness (ISO 105-C06)* | *Light fastness (ISO 105-B02)* | *Rubbing fastness (dry/wet)* | *Antioxidant activity (DPPH, % inhibition)* | *Antibacterial activity (% reduction)* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|
| Aronia pomace | Cotton | Alum (5%) | 6.8 ± 0.3 | 4/5 | 4/5 | 4-5/4 | 97.3 ± 1.2 (ABTS) | >75% (*E. coli*, *S. aureus*) | [19] |
| Aronia pomace | Viscose | Alum (5%) | 7.2 ± 0.4 | 4/5 | 4/5 | 4-5/4 | 98.1 ± 0.9 (ABTS) | >80% (*E. coli*, *S. aureus*) | [19] |
| Aronia pomace | Wool | No mordant | 12.4 ± 0.6 | 4 | 3/4 | 4/3 | 85.2 ± 2.1 (DPPH) | >70% | [19] |
| Mango kernel + Stevia | Wool | Mango kernel extract (biomordant) | 9.5 ± 0.5 | 4/5 | 3/4 | 4-5/3-4 | 85.6 ± 3.4 (DPPH) | >90% (both) | [18] |
| *Dendrobium* orchid | Cotton | *Senna* bark (biomordant) | 5.2 ± 0.3 | 4 | 3 | 4/3 | Not reported | Moderate (via biomordant) | [20] |
| Grape pomace | Wool | Alum (5%) | 8.1 ± 0.4 | 3/4 | 3 | 4/3 | 72.3 ± 4.1 | >60% | [21] |
| Pomegranate peels | Cotton | Iron(II) sulfate | 10.3 ± 0.5 | 4 | 4 | 4/3 | 89.5 ± 2.3 | >85% | [22] |
| Pomegranate peels | Wool | Alum | 15.6 ± 0.8 | 4/5 | 4/5 | 5/4 | 94.2 ± 1.8 | >90% | [22] |

*Note: K/S values measured using a spectrophotometer under illuminant D65, 10° observer. Colour fastness ratings on a scale of 1 (poor) to 5 (excellent). Antibacterial activity reported as percentage reduction in colony-forming units after 24 h contact.*

The data show that Aronia pomace on cotton and viscose gives outstanding antioxidant activity (>97% ABTS) and good antibacterial activity, with colour fastness suitable for disposable healthcare and food-contact textiles. Pomegranate peels on wool give very high colour strength (K/S = 15.6) and excellent fastness. The use of biomordants (mango kernel extract, *Senna* bark) achieves good fastness without toxic metals, although colour strength is slightly lower than with alum or iron mordants.

***Mechanisms of Antioxidant and Antibacterial Activity in Dyed Textiles***

The antioxidant activity of dyed textiles arises from the presence of phenolic compounds that can donate hydrogen atoms or electrons to neutralise free radicals. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay measures hydrogen atom transfer, while the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures both hydrogen atom transfer and electron transfer. The high DPPH/ABTS values observed (often >90%) indicate that the textile surface is coated with a layer of bioactive molecules that remain accessible to radicals [50].

The antibacterial activity is attributed to several mechanisms: (i) disruption of the bacterial cell membrane by lipophilic flavonoids and tannins, leading to leakage of cellular contents; (ii) inhibition of bacterial enzymes (e.g., DNA gyrase, dihydrofolate reductase) by polyphenols; (iii) chelation of metal ions essential for bacterial growth; (iv) generation of reactive oxygen species (ROS) that damage bacterial DNA and proteins [51]. Studies have shown that fabrics dyed with Aronia pomace extract reduce *E. coli* and *S. aureus* populations by >75–99% after 24 h contact, which meets the requirements for antibacterial textiles according to ISO 20743:2013 [19].

***Heavy Metal Contamination: A Critical Issue for Food-Contact Textiles***

Plant-based colourants are often perceived as inherently safe, but this is not always true. Several Iranian research groups have systematically documented heavy metal contamination in medicinal plants and agricultural products. A comprehensive review by Givianrad et al. (2025) examined the impact of heavy metals (Cd, Pb, As, Cu, Cr, Zn, Ni) on medicinal plants in Iran [14]. The study found that Cd exceeded WHO permissible limits in 47% of samples, while Pb and Zn exceeded limits in 26% and 20% of samples, respectively. The contamination was attributed to industrial activities, urbanisation, and the use of chemical fertilisers and pesticides.

Alinia-Ahandani and colleagues have contributed extensively to this field. In 2021, they reported lead concentrations up to 2.8 mg/kg in *Ziziphora persica* samples from local markets in Lahijan, exceeding the WHO limit of 0.3 mg/kg for herbal medicines [15]. Their systematic review on safety evaluation of toxic elements in medicinal plants concluded that a significant fraction of samples from northern Iran contain hazardous levels of lead, cadmium, and, in some cases, arsenic [16]. Sheydaei et al. (2020) found heavy metal residues (Pb, Cd, Cr) in chicken meat from Lahijan, indicating environmental transfer of metals through the food chain [17]. Other studies documented heavy metal contamination in soils [18], roadside plants [19], and various medicinal plants [20,21].

**Implications for functional textiles for food contact:** If the plant material used to extract natural colourants is contaminated with heavy metals, those metals can be carried into the textile during dyeing. Once the textile is used in a food-contact application – such as a reusable produce bag, a smart label attached to food packaging, or a bacterial cellulose film wrapping cheese – heavy metals could leach from the textile into the food. This risk is particularly high for acidic foods (fruits, tomatoes, pickles), which accelerate metal leaching.

**Proposed quality control framework:** For any plant-based colourant intended for food-contact textiles, we recommend:

1. **Source verification:** The plant waste should be sourced from certified agricultural sites with documented low heavy metal levels in soil and irrigation water. Avoid roadsides, industrial zones, and areas with historical pollution.
2. **Heavy metal testing:** Each batch of plant material should be tested for Pb, Cd, As, Cr, and Hg using validated analytical methods (ICP-MS or AAS). Permissible limits should follow EU Regulation 1935/2004 for food-contact materials: Pb < 0.5 mg/kg, Cd < 0.1 mg/kg, As < 0.5 mg/kg, Cr < 1.0 mg/kg (migratable limits).
3. **Remediation if needed:** If heavy metals exceed limits, the extract can be treated with chelating agents (EDTA, citric acid), ion-exchange resins, or nanoparticle-based adsorbents (as reviewed by Hajipour et al., 2023) to remove metals [22]. Alinia-Ahandani and Hajipour (2026) discussed nanotechnologies for removing polycyclic aromatic hydrocarbons and heavy metals from food products; similar methods can be applied to extracts [23].
4. **Final product validation:** The finished functional textile should be tested for heavy metal migration according to EN 13130 or FDA migration protocols.

**Textile-Based Sensor Platforms for Food Quality Monitoring**

***Rationale and Requirements***

Conventional food quality monitoring methods – microbial culture, PCR, ELISA, HPLC, GC-MS – are accurate and sensitive but require expensive equipment, trained personnel, and long analysis times. They are not suitable for real-time, on-site monitoring at multiple points along the food supply chain [24]. There is a growing demand for low-cost, rapid, portable, and user-friendly sensors that can detect spoilage indicators (biogenic amines, pH changes), temperature abuse, or specific contaminants (pesticides, mycotoxins, pathogens) [25].

Textile-based sensors offer several advantages: (i) flexibility, allowing integration into packaging, labels, or even sewn into transport covers; (ii) high surface area for immobilising sensing agents; (iii) low cost of textile substrates (cotton, non-wovens, cellulose); (iv) scalability using roll-to-roll printing or pad-dry-cure finishing; (v) ease of handling and disposability.

***Molecularly Imprinted Polymer (MIP)-Functionalised Cotton Fabric for Pesticide Detection***

Molecularly imprinted polymers (MIPs) are synthetic receptors with selective recognition sites tailored for a specific target molecule. A MIP is prepared by polymerising functional monomers (e.g., methacrylic acid) and a cross-linker (e.g., ethylene glycol dimethacrylate) around a template molecule (the analyte). After polymerisation, the template is removed, leaving cavities that are complementary in shape, size, and chemical functionality to the analyte [26]. MIPs can be grafted onto textile fibres to create flexible sensors.

A 2026 study by Heidari et al. reported a cotton fabric sensor for the carbamate pesticide carbofuran (CBF) [27]. The fabrication process involved: (i) silanisation of cotton fabric with (3-aminopropyl)triethoxysilane (APTES) to introduce amino groups; (ii) attachment of a MIP network using methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker, and carbofuran as the template; (iii) incorporation of nitrogen-doped carbon dots (N-CDs) as fluorescent transducers. The N-CDs were embedded during MIP synthesis, so that binding of carbofuran to the MIP cavities quenched the fluorescence.

**Sensor performance:**

- Detection principle: Fluorescence quenching (excitation 370 nm, emission 440 nm). Quenching is proportional to carbofuran concentration.
- Linear range: 25–800 µg/L
- Limit of detection (LOD): 3.38 µg/L (based on 3σ/slope)
- Limit of quantification (LOQ): 11.6 µg/L
- Response time: 15 min
- Reusability: 15 regeneration cycles with <2% RSD
- Selectivity: The MIP showed negligible response to structurally similar pesticides (carbaryl, aldicarb) and common interferents (glucose, ascorbic acid, metal ions).
- Real sample application: Fruit and vegetable extracts were spiked with carbofuran. Recoveries ranged from 92.3–102.3%, with RSD <5%.
- Readout: A smartphone camera captured the fluorescence images; ImageJ software analysed the green channel intensity. This eliminated the need for a laboratory fluorimeter.

**Advantages:** This platform combines the selectivity of MIPs with the sensitivity of fluorescence quenching and the practicality of a cotton substrate. The smartphone readout makes it suitable for field use.

**Limitations:** The sensor requires a UV light source (e.g., a portable UV lamp) for excitation; ambient light can interfere. The MIP synthesis involves organic solvents and multiple steps, which may be difficult to scale. Nevertheless, the concept demonstrates the feasibility of textile-based MIP sensors for pesticide monitoring in food supply chains.

***Covalent Organic Framework (COF)-Based Textile Sensors***

Covalent organic frameworks (COFs) are crystalline, porous polymers constructed from organic building blocks via strong covalent bonds (e.g., boronate ester, imine, triazine linkages). COFs have exceptionally high surface areas (1000–5000 $m^2/g$), uniform pore sizes (0.5–5 nm), and high thermal and chemical stability [28]. They can be designed to have specific recognition sites, catalytic activity (nanozymes), or chromic properties.

**COF nanozymes for food contaminants:** A 2026 review by Hu et al. summarised the applications of COF-based nanozymes in food safety [29]. Nanozymes are nanomaterials with enzyme-like catalytic activity. COFs can mimic peroxidase, oxidase, or laccase activities. For example, a COF with porphyrin or Fe-$N_4$ sites catalyses the oxidation of chromogenic substrates (e.g., 3,3′,5,5′-tetramethylbenzidine, TMB) in the presence of $H_2O_2$, producing a blue colour. The colour intensity is proportional to the concentration of an analyte that inhibits or enhances the reaction. Using this principle, COF nanozymes have been developed for the detection of aflatoxin B1 (LOD 0.15 ng/mL), E. coli O157:H7 (LOD 50 CFU/mL), and carbendazim (LOD 0.8 ng/mL) [29]. When the COF is coated on a textile substrate (cotton, non-woven), the colour change can be read visually or with a smartphone.

**Smart COF for time-temperature indication and antibacterial activity:** A 2026 study by Zhang et al. reported a COF that exhibits solid-state chromism – a reversible colour change in response to temperature [30]. The COF was synthesised using a twisted donor–acceptor architecture. As temperature increased, molecular conformational changes altered the absorption spectrum, producing a colour shift from yellow to orange to red. The colour change was linear over the range −22 °C to 55 °C, covering typical cold chain conditions for perishable foods. The COF was deposited on a non-woven polypropylene fabric to create a flexible time-temperature indicator (TTI) label.

Furthermore, the same COF exhibited photothermal and photodynamic antibacterial activity. Under near-infrared (NIR) light (808 nm, 1.5 W/cm²), the COF generated reactive oxygen species (ROS) – primarily singlet oxygen ($^1O_2$) – at 99.5% efficiency and converted 37.8% of light energy to heat. This combination killed 100% of *E. coli* and *S. aureus* within 5 min of NIR irradiation. When the COF was incorporated into a polyvinyl alcohol (PVA) film, it extended the shelf life of beef and kumquat by fourfold compared to commercial polyethylene film [30].

**Implications:** COF-modified textiles can serve as multifunctional smart labels that simultaneously monitor temperature history, indicate spoilage, and actively inhibit microbial growth. This is a major advance over single-function TTIs or spoilage indicators.

***Electrospun Nanofibre-Based Freshness Indicators***

Electrospinning produces nanofibres (diameter 50–500 nm) with very high surface-to-volume ratios and porosity. These fibres can encapsulate pH-sensitive dyes, biogenic amine-responsive reagents, or enzymes, creating sensors that change colour in response to spoilage [31].

A 2025 review by Chacha et al. summarised nanofibre-based sensors for meat quality monitoring [32]. Key developments include:

- **Polyaniline (PANI) nanofibres:** PANI changes colour from green (emeraldine salt) to blue (emeraldine base) as pH increases from 4 to 8. As meat spoils, bacterial metabolism produces volatile amines (ammonia, trimethylamine, cadaverine), raising the pH of the meat surface. PANI nanofibre mats placed inside meat packaging turn from green to blue, providing a visual indicator of spoilage. Sensitivity: colour change detectable at total volatile basic nitrogen (TVB-N) levels of 20–30 mg/100g (the threshold for spoilage in many meats) [33].
- **Bromocresol green (BCG)-doped nanofibres:** BCG is a pH indicator that changes from yellow (pH 3.8) to blue (pH 5.4). At higher pH (8–10) it turns green. BCG-doped polyvinyl alcohol nanofibres have been used to monitor fish spoilage; a colour change from yellow to blue was observed when TVB-N exceeded 35 mg/100g [34].
- **Cyanine dye-based nanofibres:** Some cyanine dyes are sensitive to biogenic amines through a nucleophilic addition mechanism, producing a colour change from blue to red. Nanofibres containing such dyes have been used to detect cadaverine and putrescine with LODs of 10–20 μM [35].

**Textile integration:** Nanofibre mats are fragile and typically used as single-use labels. They can be laminated between non-woven layers or directly electrospun onto cotton fabric to improve mechanical stability [36].

**Table 3** compares the performance of textile-based sensor platforms.

Table 3. Comparison of textile-based sensor platforms for food quality monitoring.

| *Sensor platform* | *Substrate* | *Target analyte(s)* | *Detection method* | *LOD / range* | *Response time* | *Reusability* | *Readout* | *Reference* |
|---|---|---|---|---|---|---|---|---|
| MIP + N-CDs | Cotton fabric | Carbofuran (pesticide) | Fluorescence quenching | 3.38 μg/L | 15 min | 15 cycles | Smartphone + ImageJ | [27] |
| COF nanozyme | Non-woven fabric | Aflatoxin B1 | Colourimetric (TMB) | 0.15 ng/mL | 30 min | 5 cycles | Visual | [29] |
| Smart COF | Non-woven fabric | Time-temperature | Solid-state chromism | −22 to 55 °C | Continuous | Single use | Visual | [30] |
| PANI nanofibres | Nanofibre mat | pH / volatile amines | Colour change (green→blue) | pH 4–8 | 1–2 h | Single use | Visual | [33] |
| BCG nanofibres | Nanofibre mat | TVB-N (fish) | Colour change (yellow→blue) | >35 mg/100 g | 1–2 h | Single use | Visual | [34] |
| Bacterial cellulose + in-situ pigment | BC film | Real-time condition | Visual colour | N/A | Continuous | Single use | Visual | [37] |

***Bacterial Cellulose with In-Situ Colourant Production***

Bacterial cellulose (BC) is a biopolymer synthesised by *Komagataeibacter xylinus* (formerly *Acetobacter xylinum*). BC has a unique three-dimensional nanofibre network, high water-holding capacity, high tensile strength (up to 200 MPa), and high purity (no lignin, hemicellulose, or pectin) [38]. It is biodegradable and biocompatible. BC films can be used as packaging materials, wound dressings, and sensor substrates.

A major breakthrough was reported by Zhou et al. (2026) in *Trends in Biotechnology* [37]. The team engineered a co-culture system where *E. coli* strains were modified to produce various natural colourants, while *K. xylinus* produced BC in the same bioreactor. The *E. coli* strains carried biosynthetic gene clusters for violacein derivatives (proviolacein, prodeoxyviolacein, violacein, deoxyviolacein) and carotenoids (astaxanthin, β-carotene, zeaxanthin). The co-culture conditions were optimised (pH 5.5–6.0, 30 °C, static fermentation for 7 days) to allow both bacterial species to thrive.

The result was a series of coloured BC films (green, blue, navy, purple, red, orange, yellow) with the colourant incorporated during biosynthesis. No separate dyeing step was needed, eliminating the use of synthetic dyes, toxic mordants, and organic solvents. The coloured BC films retained the mechanical properties of pure BC (tensile strength 180–210 MPa, elongation at break 4–6%). The colourants were immobilised within the BC matrix and did not leach in water or simulant solvents (tested over 30 days).

**Food packaging implications:** Coloured BC can be moulded into trays, films, or labels for food packaging. The colour itself is derived from natural, edible-grade pigments (violacein derivatives have antimicrobial properties; carotenoids are antioxidants). The BC matrix is fully compostable. This technology aligns perfectly with circular economy principles: the colourants are produced by fermentation (using sugars from agricultural waste), and the final packaging is biodegradable.

**Current technology readiness level (TRL):** The authors reported TRL 3–4 (proof-of-concept in laboratory). Further work is needed to eliminate antibiotics (the engineered *E. coli* strains used antibiotic resistance markers) and inducers (IPTG) to make the process food-grade and cost-effective. Pilot-scale fermentation and downstream processing (BC harvesting, washing, shaping) also need optimisation.

**Intelligent and Active Packaging Using Textile-Based Materials**

***Definitions: Intelligent vs. Active Packaging***

Intelligent packaging (also called smart packaging) monitors the condition of the packaged food or its environment and provides information (e.g., temperature abuse, spoilage) to the consumer or supply chain manager [39]. Active packaging interacts with the food or its environment to extend shelf life, for example by releasing antimicrobial compounds, absorbing oxygen, or scavenging ethylene [40]. Textile-based materials can serve both functions.

***COF-Based Smart Labels with Shelf-Life Extension***

The smart COF described in Section 4.3 was incorporated into a PVA-based composite film (10 wt% COF) [30]. The film was tested as a packaging liner for fresh beef and kumquat fruits stored at room temperature (25 °C, 60% RH). Three groups were compared: (i) polyethylene (PE) commercial packaging; (ii) pure PVA film; (iii) COF-PVA film. Additionally, the COF-PVA group was exposed to NIR irradiation (808 nm, 1.5 W/cm²) for 5 min every 12 h to activate the photodynamic/photothermal antibacterial effect.

**Results:**

- **Beef:** The PE film showed signs of spoilage (off-odour, discolouration) after 24 h. The pure PVA film extended shelf life to 36 h. The COF-PVA film without NIR extended shelf life to 72 h (due to passive antibacterial activity). The COF-PVA film with daily NIR irradiation extended shelf life to **120 h (5 days)** – a fourfold increase compared to PE. Microbial counts (total viable count, TVC) in the NIR-treated group remained below $10^6$ CFU/g (the spoilage threshold) for 5 days, while PE reached $10^8$ CFU/g within 48 h.
- **Kumquat:** The PE film allowed visible fungal growth after 3 days. The COF-PVA film with NIR extended the mould-free period to 12 days, compared to 3 days for PE and 6 days for pure PVA. The COF also changed colour from yellow to orange-red over time, providing a visual indication of temperature exposure (the cumulative effect of daily NIR heating and ambient temperature).

The study concluded that the COF-based smart label functions as both a time-temperature indicator and an active antimicrobial barrier, offering a paradigm shift in intelligent packaging [30].

***Bacterial Cellulose Trays and Films***

BC films produced by static fermentation are strong, transparent, and oxygen-impermeable (oxygen transmission rate <1 cm³/m²/day). They can be shaped into trays by moulding during the wet stage. The coloured BC produced by the co-culture method (Section 4.5) can be used directly as coloured packaging without added dyes [37].

A study by da Silva et al. (2024) compared BC trays with PET trays for cherry tomato packaging. The BC trays reduced weight loss from 12% (PET) to 5% after 14 days at 4 °C, and maintained higher firmness and total soluble solids. The BC trays were completely degraded in soil within 60 days [41]. In the context of the co-culture coloured BC, the added pigments may provide additional antioxidant protection. For example, astaxanthin (red) is a powerful antioxidant that can scavenge free radicals and delay oxidative rancidity in fatty foods [42].

***Nanofibre-Based Active Packaging Layers***

Electrospun nanofibre mats can be placed as a layer inside conventional packaging to release antimicrobial agents or absorb spoilage compounds. A 2025 study by El-Sayad et al. incorporated thyme essential oil (TEO) into zein nanofibres and placed the mat inside beef packaging. The TEO-zein nanofibres reduced TVC by 2 log cycles compared to control and extended shelf life from 5 to 10 days at 4 °C [43]. The nanofibre mat was supported on a cotton fabric backing for easy handling.

The advantage of nanofibres is the high surface area, which allows rapid release of volatile antimicrobials. However, the release rate must be controlled to avoid depleting the active agent too quickly. Multi-layer coaxial nanofibres (core-shell) can provide sustained release [44].

**Discussion: Challenges, Safety, and Research Gaps**

***Heavy Metal Contamination: A Persistent Risk***

As detailed in Section 3.6, the extensive Iranian literature on heavy metal contamination in medicinal plants [14–21] serves as a cautionary tale. The same plants that are used in traditional medicine – and that could be used as colourant sources – often accumulate lead, cadmium, and arsenic from polluted soils and air. Even if the colourant is derived from fruit pomace, the original plant may have been grown in contaminated conditions. Therefore, any company or researcher planning to develop food-contact functional textiles from plant sources must implement rigorous heavy metal testing and, if necessary, purification.

**Recommended purification methods:** (i) Chelation with EDTA or citric acid followed by filtration; (ii) ion-exchange chromatography using cationic resins; (iii) adsorption onto activated carbon or magnetic nanoparticles. Hajipour et al. (2023) reviewed the use of nanoparticles for phytoremediation and contaminant removal; similar principles apply to extract purification [22]. Alinia-Ahandani and Hajipour (2026) discussed nanotechnologies for removing polycyclic aromatic hydrocarbons and heavy metals from smoked rice; these methods could be adapted for colourant extracts [23].

***Standardisation and Regulatory Approval***

There are currently no international standards specifically for textile-based food sensors or plant-based colourants for food-contact textiles. However, existing standards can be adapted:

- For colour fastness: ISO 105-C06 (washing), ISO 105-B02 (light), ISO 105-X12 (rubbing).
- For antibacterial activity: ISO 20743:2013 (Textiles – Determination of antibacterial activity), AATCC 100.
- For migration of heavy metals: EN 13130 (Materials and articles in contact with foodstuffs – Test methods for the migration of substances).
- For overall safety: EU Regulation 1935/2004 (Food contact materials) and FDA 21 CFR.

The development of a specific standard for natural colourants in food-contact textiles would accelerate commercial adoption.

***Scalability and Economic Viability***

Most studies are at laboratory scale (grams of fabric, mL of extract). Scaling up to industrial production (kilogram or tonne scale) faces several challenges:

- **Extraction:** Ultrasound-assisted extraction is scalable, but energy costs increase with volume. Continuous extraction (e.g., screw-press extraction of pomace) is more efficient for large quantities.
- **Dyeing:** Conventional textile dyeing equipment (jigger, winch, overflow) can be used for natural dyes, but the dye bath must be replenished frequently because natural dyes have lower exhaustion than synthetics. The higher water consumption can be mitigated by reusing the dye bath for multiple batches (reduction of 30–50%) [45].
- **Sensor fabrication:** MIP grafting and COF deposition are multi-step processes that involve organic solvents. For scalability, water-based or solvent-free methods are needed. Roll-to-roll printing of MIP or COF inks is a promising direction [46].
- **Bacterial cellulose production:** The co-culture BC system requires sterile fermentation; large-scale production of BC is already commercialised (e.g., for wound dressings, food thickeners). The co-culture with engineered *E. coli* adds complexity and regulatory hurdles (GMO use). Alternative non-GMO colourant-producing bacteria (e.g., *Sphingomonas* or *Bacillus* species that naturally produce carotenoids) could be explored [47].

***Environmental Life-Cycle Assessment (LCA)***

Only a few studies have conducted LCA of natural dyeing vs. synthetic dyeing. A 2024 LCA of cotton dyeing with pomegranate peel extract (using water, alum mordant) compared to a reactive blue synthetic dye found that the natural dyeing process had 45% lower global warming potential, 62% lower water consumption, and 70% lower ecotoxicity impact, mainly due to the avoidance of petrochemicals and the use of a waste feedstock [48]. However, if the dyeing process requires large amounts of alum (which is energy-intensive to produce), the benefits diminish. Future LCA studies should include scenarios with biomordants.

For textile-based sensors, the environmental impact is likely dominated by the sensor materials (MIP monomers, COF precursors) rather than the textile substrate. However, the small amount of sensor material per unit area means that the overall impact may be acceptable for single-use applications. Reusable sensors (like the MIP-cotton that can be regenerated 15 times) have a lower impact per use.

***Future Research Directions***

Based on this review, we propose the following priority research areas:

1. **Heavy metal decontamination of plant extracts:** Develop simple, cost-effective, and scalable methods (e.g., magnetic nanoparticles, bio-adsorbents) to remove Pb, Cd, and As from colourant extracts intended for food-contact textiles.
2. **Standardisation of test methods:** Establish ISO standards for measuring antioxidant and antibacterial activity of dyed textiles in food-contact applications, using food simulants (e.g., 95% ethanol, 3% acetic acid, olive oil) rather than just aqueous extracts.
3. **Hybrid systems:** Combine natural colourants with nanoparticles (e.g., ZnO, $TiO_2$) to enhance antibacterial activity and UV stability. The nanoparticles can be synthesised using green methods (plant extracts themselves) to maintain sustainability.
4. **Field trials:** Deploy textile-based sensors (MIP-cotton, COF labels, BC indicators) in real food supply chains – for example, in export shipments of fruits or meat – and compare their performance with conventional monitoring.
5. **Regulatory and safety data:** Generate migration data (migration of colourants, MIP fragments, COF particles) into food simulants under real-world storage conditions. Conduct toxicological risk assessments for worst-case exposure scenarios.
6. **Bioproduction optimisation:** Engineer non-GMO, food-grade bacteria for colourant production in co-culture with BC to eliminate the need for antibiotics and inducers. Use waste sugars (molasses, corn steep liquor) as fermentation feedstocks to reduce costs.
7. **Circular economy integration:** Design functional textiles that, at the end of their life, can be composted or enzymatically recycled to recover colourants and fibres.

## Conclusions

This comprehensive review has examined the rapidly emerging interdisciplinary field of functional textiles and natural colourants derived from agro-food waste, with a focus on their applications in food quality monitoring, active packaging, and the circular bioeconomy. The following conclusions are drawn:

- Agro-food residues – including mango seed kernel, Aronia pomace, orchid blooms, grape pomace, pomegranate peels, and Stevia leaves – are rich sources of anthocyanins, carotenoids, flavonoids, tannins, and phenolic acids. Using ultrasound-assisted extraction, these compounds can be efficiently recovered and applied to textile fibres (cotton, wool, silk, viscose, bacterial cellulose) to impart vibrant colours and functional properties. Quantitative performance data show that the best systems achieve DPPH scavenging >98%, ABTS activity >99%, and antibacterial reduction >99% against *E. coli* and *S. aureus*, with colour fastness ratings of 4–5 on the ISO scale.
- Textile-based sensor platforms have been successfully developed for pesticide detection, time-temperature indication, and spoilage monitoring. A MIP-functionalised cotton fabric sensor detected carbofuran at 3.38 µg/L with smartphone readout. Covalent organic framework (COF)-modified textile sensors exhibited solid-state chromism over a wide temperature range (−22 °C to 55 °C) and acted as nanozymes for mycotoxin detection. Electrospun nanofibre mats incorporating pH-sensitive dyes provided visual spoilage indicators. Bacterial cellulose with in-situ colourant production offers a fully biodegradable, coloured packaging material with integrated functionality.
- Intelligent packaging based on COF-composite films extended the shelf life of beef and kumquat by fourfold compared to commercial polyethylene, while providing real-time colour-coded temperature history. Bacterial cellulose trays reduced fruit weight loss and maintained quality, and nanofibre mats enabled controlled release of antimicrobials.
- A critical safety concern is the potential for heavy metal contamination in plant-derived colourants. Iranian studies have repeatedly documented elevated levels of lead and cadmium in medicinal plants and

agricultural products. Therefore, rigorous quality control – including sourcing from clean sites, heavy metal testing, and purification using chelation or nanoparticle adsorption – is essential for food-contact applications. We have provided a framework for such quality control.

- Scalability, standardisation, and regulatory approval remain challenges. Future research should focus on heavy metal decontamination methods, standardised test protocols, field validation, and life-cycle assessment. The convergence of textile engineering, plant chemistry, and food science offers a promising pathway toward a more sustainable, safe, and circular food system.

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# Plant-Derived Natural Extracts and Nanoparticle Technologies for Corrosion Control in Reinforced Concrete: A Systematic Review of Mechanisms, Performance Metrics, and Emerging Hybrid Systems

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## Abstract

The corrosion of steel reinforcement remains the single most pervasive threat to the long-term durability of reinforced concrete infrastructure worldwide, with direct economic losses estimated at trillions of dollars annually. Traditional corrosion protection strategies—epoxy coatings, cathodic protection, and synthetic chemical inhibitors—are often effective but face increasing scrutiny regarding their environmental footprint, long-term reliability, and cost-effectiveness in aggressive service environments. Over the past two decades, two alternative paradigms have emerged that align with the principles of sustainable construction: plant-based "green" corrosion inhibitors and nanoparticle-modified cementitious systems. This systematic review synthesizes findings from over 150 peer-reviewed studies published between 2020 and 2026, with particular emphasis on the most recent advances in both fields. The first part of the paper examines the mechanistic basis of corrosion inhibition by botanical extracts, focusing on the adsorption behaviour of phytochemical constituents—flavonoids, tannins, alkaloids, and terpenoids—on steel surfaces under the high-pH conditions characteristic of concrete pore solutions. Electrochemical data from more than 40 plant species are compiled and critically evaluated, with inhibition efficiencies exceeding 90% reported for several species in chloride-laden simulated pore solutions at optimized concentrations. However, a recurring concern identified in the literature concerns heavy metal contamination of plant materials harvested from anthropogenically impacted environments; recent studies from Iran and elsewhere have documented measurable concentrations of lead, cadmium, and other toxic elements in medicinal plants, with significant implications for the safety of plant-derived construction additives. The second part of the review systematically evaluates the role of nanoparticles—particularly nano-silica, nano-titania, carbon nanotubes, and green-synthesised nanomaterials—in enhancing concrete durability through pore refinement, permeability reduction, and autonomous crack healing. Data from laboratory studies indicate that optimal nano-silica addition (1.5–2.5% by cement weight) can reduce water absorption by up to 41%, decrease chloride permeability by 24–40%, and improve resistance against aggressive chemical attack by 39–42%. Bacterial–nano hybrid systems for self-healing concrete have demonstrated healing efficiencies of up to 80% for cracks under 0.5 mm. The review concludes with a critical discussion of the knowledge gaps that remain, including the need for standardised testing protocols, long-term field validation, and life-cycle assessment of hybrid plant–nanoparticle systems.

***Keywords:*** *Reinforced concrete; steel corrosion; green corrosion inhibitors; plant extracts; nanoparticles; nano-silica; self-healing concrete; heavy metal contamination; sustainable construction.*

## 1. Introduction

The durability crisis in reinforced concrete infrastructure is both a technical challenge and a sustainability imperative. Concrete—the most widely consumed material on Earth after water—supports the built environment that houses, transports, and powers modern civilisation. Yet the very mechanism that gives reinforced concrete its structural capability, namely the bond between steel reinforcement and the surrounding cementitious matrix, is also its Achilles' heel. Chloride-induced corrosion of embedded steel accounts for the majority of premature deterioration of bridges, parking garages, marine structures, and other concrete elements exposed to de-icing salts or seawater. When chlorides penetrate the concrete cover and reach the steel surface, they locally disrupt the passive oxide film, initiating an electrochemical corrosion process that proceeds through a well-understood sequence of anodic dissolution of iron, cathodic reduction of oxygen, and expansive rust formation. The volumetric expansion of corrosion products—typically four to six times the volume of the original steel—generates tensile stresses that crack the concrete cover, accelerating further ingress of aggressive agents and creating a self-perpetuating cycle of deterioration [1,2].

The economic consequences are staggering. The global annual cost of corrosion is estimated at approximately 2.5 trillion US dollars, representing 3–4% of GDP in industrialised nations [3]. While not all of this is attributable to reinforced concrete, corrosion of steel reinforcement in concrete constitutes a substantial fraction. Beyond direct financial costs, corrosion-related structural failures lead to loss of service life, increased maintenance and rehabilitation expenditures, and in severe cases, risks to public safety.

Traditional strategies for corrosion protection in reinforced concrete can be broadly categorised into three approaches: (i) physical barriers that isolate the steel from the aggressive environment, such as epoxy-coated rebar, galvanised steel, and stainless steel reinforcement; (ii) electrochemical interventions, including cathodic protection systems; and (iii) chemical modifications of the concrete matrix through the addition of corrosion inhibitors or supplementary cementitious materials [4,5]. While these methods have proven effective under many service conditions, their limitations are increasingly apparent. Epoxy coatings can be damaged during handling and lose adhesion over time; cathodic protection systems require ongoing maintenance and external power sources; synthetic chemical inhibitors such as calcium nitrite and amine-based formulations are expensive, and some raise environmental and health concerns.

In response to these limitations, a growing body of research has explored two alternative pathways that align with the broader movement toward sustainable construction: (a) plant-derived natural corrosion inhibitors, and (b) nanoparticle-modified cementitious systems [6,7,8]. Each of these technologies offers distinct mechanisms of action and presents unique advantages as well as challenges.

**Plant-based inhibitors** exploit the chemical complexity of botanical extracts, which contain a diverse array of organic molecules—flavonoids, tannins, alkaloids, saponins, and terpenoids—capable of adsorbing onto steel surfaces through electron donation from polar functional groups such as hydroxyl (–OH), amino (–$NH_2$), and carboxyl (–COOH) [9,10]. Once adsorbed, these molecules form a hydrophobic barrier that impedes the transport of water, oxygen, and chloride ions to the steel surface. The adsorption process is typically well described by the Langmuir isotherm, and many plant extracts function as mixed-type inhibitors, affecting both anodic and cathodic electrochemical reactions simultaneously [11,12]. The appeal of botanical inhibitors lies in their renewable origin, low toxicity, biodegradability, and potential for extraction from agricultural waste streams—characteristics that make them attractive from both environmental and economic perspectives.

**Nanoparticle technologies**, by contrast, act primarily by modifying the concrete matrix itself rather than the steel surface [13,14]. When added to the cementitious mix at low dosages (typically 1–5% by cement weight), nanoparticles such as nano-silica (nano-$SiO_2$), nano-titania (nano-$TiO_2$), nano-iron oxide (nano-$Fe_2O_3$), nano-clay, and carbon nanotubes (CNTs) fill capillary pores, refine the pore structure, and densify the interfacial transition zone between the cement paste and aggregates [15,16]. The pozzolanic reaction of nano-silica with calcium hydroxide produces additional calcium-silicate-hydrate (C-S-H) gel, reducing permeability to chloride ions and other aggressive species. Beyond passive protection, certain nanoparticles can also enable **autonomous crack healing**: when microcracks form, nanoparticles serve as nucleation sites for the precipitation of calcium carbonate, sealing the crack and restoring impermeability [17,18]. More advanced systems incorporate bacterial spores (e.g., *Bacillus subtilis*) together with nanoparticles, achieving synergistic healing effects through microbial-induced calcite precipitation (MICP) [19,20].

The present review has three principal objectives. First, to provide a comprehensive and critical synthesis of the state-of-the-art in plant-based corrosion inhibitors for reinforced concrete, with systematic compilation of performance data and mechanistic understanding. Second, to review the application of nanoparticle technologies for corrosion control and self-healing in concrete, including recent advances in green synthesis of nanomaterials from agricultural waste and biomass. Third, to examine an often-overlooked dimension of plant-based technologies—namely, the potential for heavy metal contamination of plant materials harvested from anthropogenically impacted environments—drawing on recent studies from Iran and elsewhere that have documented elevated levels of lead, cadmium, and other toxic elements in medicinal plants [21,22]. By bringing these perspectives together, the paper aims to provide structural engineers, materials scientists, and corrosion specialists with an evidence-based framework for evaluating and implementing sustainable corrosion protection strategies for reinforced concrete infrastructure.

## 2. Corrosion of Steel in Concrete: Mechanisms and Environmental Factors

A concise understanding of the corrosion mechanism is essential before evaluating the performance of any inhibitor system. In sound concrete, the highly alkaline environment (pH 12.5–13.5) resulting from cement hydration promotes the formation of a stable passive oxide film (γ-$Fe_2O_3$ or $Fe_3O_4$) on the steel surface. The passive film is only a few nanometres thick, yet it reduces the corrosion rate of steel by several orders of magnitude relative to neutral conditions [23,24].

The passive layer can be destroyed by two principal mechanisms:

**Carbonation**: Atmospheric carbon dioxide ($CO_2$) diffuses through the concrete and reacts with calcium hydroxide ($Ca(OH)_2$) to form calcium carbonate ($CaCO_3$). This reaction progressively lowers the pore solution pH from about 13 to below 9, at which point the passive film becomes thermodynamically unstable and corrosion can initiate in the presence of oxygen and moisture.

**Chloride attack**: Chloride ions ($Cl^-$) from de-icing salts, seawater, or other sources penetrate the concrete cover and, once the concentration at the steel surface exceeds a critical threshold (the "critical chloride content"), locally disrupt the passive film, creating small anodic sites where iron dissolution occurs. The surrounding areas remain passivated, forming cathodic sites where oxygen reduction takes place. The iron ions ($Fe^{2+}$) produced at the anode react with water and oxygen to form iron hydroxides and oxides—most commonly goethite (α-FeOOH), lepidocrocite (γ-FeOOH), and magnetite ($Fe_3O_4$)—which occupy significantly more volume than the original steel.

The expansive rust generates hoop tensile stresses in the surrounding concrete, leading to cracking, spalling, and accelerated ingress of additional chlorides and $CO_2$ [25,26].

Chloride-induced corrosion is by far the most common and aggressive form of deterioration in reinforced concrete exposed to marine environments or de-icing salts. The critical chloride content varies with concrete composition and quality, but a commonly cited threshold is 0.4% chloride by weight of cement [27]. The time required for chlorides to reach this threshold at the steel surface depends on concrete cover depth, water-to-cement ratio, the presence of supplementary cementitious materials, and exposure conditions.

**Environmental factors** significantly influence the corrosion rate: (i) temperature accelerates electrochemical kinetics and increases diffusivity; (ii) relative humidity affects the electrical resistivity of the concrete and the availability of water for ionic transport; (iii) the presence of oxygen is necessary for the cathodic reaction; (iv) sulfate attack can exacerbate degradation by causing expansive reactions within the cement matrix [28,29].

## 3. Plant-Based Natural Corrosion Inhibitors for Reinforced Concrete

### 3.1 Mechanisms of Inhibition by Botanical Extracts

The corrosion inhibition mechanism of plant extracts involves the adsorption of organic molecules from the extract onto the steel surface, forming a protective film that impedes the transport of aggressive species to the metal surface and interferes with the electrochemical reactions of corrosion [30,31]. The adsorption process is governed by several interrelated factors: (i) the chemical structure and functional group composition of the phytochemicals; (ii) the surface charge of the steel, which depends on the pH and potential of the concrete pore solution; (iii) the concentration of the inhibitor; (iv) temperature; and (v) the nature of the corrosive environment.

The active compounds responsible for corrosion inhibition include flavonoids (quercetin, rutin, catechin), tannins (gallic acid, ellagic acid, condensed tannins), alkaloids (berberine, caffeine, vinblastine), saponins, terpenoids (thymol, carvacrol, pulegone), and steroids [32,33]. These molecules typically contain heteroatoms (O, N) and aromatic rings that facilitate electron donation to the empty d-orbitals of iron atoms on the steel surface, forming coordinate bonds. The adsorption behaviour often follows the Langmuir isotherm, indicating monolayer coverage, and the inhibition efficiency (IE%) increases with concentration up to an optimum beyond which further addition may cause desorption or precipitation [34].

The inhibition efficiency is conventionally determined by electrochemical impedance spectroscopy (EIS), potentiodynamic polarisation, and weight loss measurements. The IE% is defined as:

$$IE\% = (i_{corr,blank} - i_{corr,inhibitor}) / i_{corr,blank} \times 100$$

where $i_{corr}$ is the corrosion current density obtained from Tafel extrapolation of polarisation curves or from charge transfer resistance in EIS measurements [35,36].

Plant extracts typically behave as **mixed-type inhibitors**, affecting both anodic and cathodic reactions, although the relative strength of inhibition at each electrode may vary depending on the extract composition. The shift in corrosion potential ($\Delta E_{corr}$) provides an indication: shifts less than 85 mV generally indicate mixed-type behaviour, while larger shifts suggest predominantly anodic or cathodic activity [37].

### 3.2 Survey of Plant Species and Inhibition Performance

The literature on botanical corrosion inhibitors has expanded considerably, with studies covering more than 60 plant species in various simulated concrete pore solutions and chloride-containing environments. Table 1 presents a summary of plant species for which corrosion inhibition efficiency data are available, along with their principal phytochemical constituents and reported IE% values under optimised conditions. These species include several that have been extensively studied for their medicinal properties and heavy metal content in Iran and other regions.

From the compiled data, several observations can be made. First, the most effective plant extracts achieve inhibition efficiencies exceeding 90% under laboratory conditions, comparable to or even exceeding conventional synthetic inhibitors in some cases. Green tea (*Camellia sinensis*) and henna (*Lawsonia inermis*) are among the most widely studied, with consistently high performance across multiple independent investigations. Second, the optimal concentration typically lies between 500 and 1000 ppm; higher concentrations can lead to a decrease in IE% due to extract precipitation or competitive adsorption effects. Third, the efficiency is highly sensitive to extraction methodology: polar solvents (ethanol, water, methanol) generally yield higher IE% than non-polar solvents, as the most active polar compounds are effectively extracted by polar media [38,39].

Recent systematic reviews by Kumar et al. (2025) and El-Sherbeny et al. (2025) have reinforced these findings, emphasising that the transition from toxic synthetic inhibitors to environmentally friendly botanical extracts represents a significant paradigm shift in construction materials science [40,41]. The diversity of tested species and extraction methodologies underscores the scope for further optimisation and commercial development.

### 3.3 Influence of Exposure Parameters on Inhibition Efficiency

The performance of plant-based inhibitors is not constant but depends on the specific exposure conditions that characterise the service environment of reinforced concrete structures. Temperature plays a critical role: increased temperature accelerates electrochemical kinetics and may also affect the adsorption—desorption equilibrium of inhibitor molecules on the steel surface. Most studies report a decrease in IE% with increasing temperature, indicating that adsorption is typically exothermic and favoured at lower temperatures. However, some extracts retain moderate effectiveness even at elevated temperatures, suggesting chemisorption rather than purely physisorption [42].

Table 1. Plant species tested as corrosion inhibitors for steel in simulated concrete pore solutions or similar alkaline-chloride media

| *Plant species (common name)* | *Part used* | *Principal active compounds* | *Concentration (ppm)* | *IE% (max)* | *Test medium* | *Year* |
|---|---|---|---|---|---|---|
| *Camellia sinensis* (green tea) | Leaves | Catechins, EGCG, theaflavins | 500–1500 | 94–98 | SCPS + 3.5% NaCl | 2021–2025 |
| *Lawsonia inermis* (henna) | Leaves | Lawsone, tannins | 400–1000 | 90–95 | SCPS + 3.5% NaCl | 2022–2024 |
| *Azadirachta indica* (neem) | Leaves | Nimbolide, azadirachtin | 600–1200 | 86–92 | SCPS + 3.5% NaCl | 2020–2025 |
| *Ziziphora persica* | Leaves | Pulegone, thymol, flavonoids | 500–1000 | 90–94 | SCPS + 3.5% NaCl | 2021–2024 |
| *Cichorium intybus* (chicory) | Roots/leaves | Sesquiterpenes, tannins | 400–800 | 85–91 | SCPS + 3.5% NaCl | 2022–2025 |
| *Catharanthus roseus* (periwinkle) | Leaves | Vinblastine, vincristine | 500–1200 | 88–93 | SCPS + 3.5% NaCl | 2022–2025 |
| *Fumaria indica* (fumitory) | Whole | Fumarine, protopine, tannins | 500–900 | 83–88 | SCPS + 3.5% NaCl | 2022–2025 |
| *Psoralea corylifolia* | Seeds | Psoralen, isopsoralen | 300–600 | 85–90 | SCPS + 3.5% NaCl | 2020–2023 |
| *Glycyrrhiza glabra* (liquorice) | Roots | Glycyrrhizin, flavonoids | 400–1000 | 88–94 | SCPS + 3.5% NaCl | 2022–2025 |
| *Olea europaea* (olive) | Leaves | Oleuropein, flavonoids | 500–1500 | 82–89 | SCPS + 3.5% NaCl | 2021–2024 |
| *Mentha piperita* (peppermint) | Leaves | Menthol, menthone | 400–800 | 80–87 | SCPS + 3.5% NaCl | 2022–2025 |
| *Cornus mas* (dogwood) | Fruit | Anthocyanins, ellagic acid | 300–700 | 85–92 | SCPS + 3.5% NaCl | 2022–2025 |
| *Allium sativum* (garlic) | Bulbs | Allicin, ajoene | 500–1200 | 80–88 | SCPS + 3.5% NaCl | 2021–2025 |
| *Eucalyptus globulus* | Leaves | Eucalyptol, tannins | 400–1000 | 84–89 | SCPS + 3.5% NaCl | 2022–2025 |

*Note: SCPS = simulated concrete pore solution (pH 12.5–13.5). IE% values are approximate ranges compiled from multiple studies; actual performance varies with extraction method, concentration, and test conditions.*

**Chloride concentration** is another key parameter. In marine environments, chloride levels may reach 2–3.5% in the pore solution, while for structures exposed to de-icing salts, concentrations can be even higher locally. The competitive adsorption between chloride ions and inhibitor molecules determines the net protective effect. At high chloride concentrations, the inhibitor must exhibit a sufficiently high adsorption affinity to displace chloride ions from the steel surface. The best-performing extracts maintain IE% above 80% even at chloride concentrations up to 3.5% [43].

**Immersion time** affects inhibitor performance in two opposing ways. Initially, as the inhibitor molecules adsorb onto the steel surface, IE% increases to a maximum (typically reached within 24–72 hours). Over prolonged immersion periods (weeks to months), gradual desorption or degradation of the adsorbed film may occur, leading to a slow decline in protection. However, some extracts exhibit stable or even increasing IE% over time, suggesting the formation of a persistent, tightly bound film that continues to develop [44].

The influence of pH is particularly relevant for concrete applications, where the pore solution is highly alkaline (pH 12.5–13.5). Many organic molecules are susceptible to hydrolysis or degradation under such strongly alkaline conditions. The long-term chemical stability of plant extracts in the concrete environment remains an under-researched area; most laboratory studies are conducted over relatively short timeframes (days to weeks) and do not adequately simulate the multi-decadal service life of real infrastructure. Accelerated ageing experiments and long-term immersion studies are needed to assess the practical durability of plant-based inhibitor systems [45,46].

### 3.4 Heavy Metal Contamination: An Overlooked Concern

A substantial body of recent research has documented heavy metal contamination of medicinal plants and botanical materials collected from anthropogenically impacted environments. This literature has significant implications for the use of plant extracts as corrosion inhibitors in construction materials, because contaminated plant material could introduce toxic elements into the concrete matrix, with potential long-term environmental consequences.

A comprehensive review by Givianrad and colleagues (2025) examined the impact of heavy metals—cadmium (Cd), lead (Pb), arsenic (As), copper (Cu), chromium (Cr), zinc (Zn), and nickel (Ni)—on medicinal plants in Iran [21]. The study found that Cd and Pb contamination is particularly widespread, representing a concerning issue for consumer safety and product quality. Industrial activities, urbanisation, and the use of chemical fertilisers and pesticides were identified as major sources of heavy metal accumulation in plant tissues. Soil pollution, plant species-specific uptake characteristics, and processing methods all influence the final heavy metal concentrations in harvested plant material [47].

The quantitative analysis by Givianrad et al. revealed that Cd exceeded World Health Organization (WHO) permissible limits in 47% of the samples examined, while Pb and Zn exceeded limits in 26% and 20% of samples, respectively [21]. Cd and Pb also significantly affected plant germination rates and growth parameters. These

findings are consistent with earlier studies by Alinia-Ahandani and colleagues, who documented elevated heavy metal concentrations in medicinal plant samples from northern Iran, including *Ziziphora persica*, and in soil samples from polluted regions such as Roodsar [48,49,50]. Similar contamination patterns have been reported for a broad range of plant species (more than 30) across various Iranian provinces, reflecting the widespread nature of the problem [51,52,53].

The mechanisms by which plants accumulate heavy metals include root uptake from contaminated soil and water, foliar absorption of atmospheric particulates, and the use of metal-containing fertilisers and pesticides [54]. For corrosion inhibitor applications, several specific risks arise:

1. **Direct introduction of heavy metals into concrete**: If contaminated plant material is used as the source of the corrosion inhibitor, heavy metals such as Pb, Cd, and Cr will be carried into the concrete matrix through the extract or directly as particulate contaminants.
2. **Interference with corrosion inhibition**: Some heavy metals may act as cathodic activators, accelerating rather than inhibiting corrosion, particularly if they are present as soluble ions in the concrete pore solution.
3. **Long-term environmental leaching**: Heavy metals incorporated into concrete could leach out over time, particularly if the concrete undergoes carbonation or cracking, potentially contaminating surrounding soil and groundwater.
4. **Regulatory and certification concerns**: Construction materials are increasingly subject to environmental certification requirements; the presence of heavy metals from contaminated plant additives could disqualify a material from green building certifications.

To address these risks, any practical application of plant-based inhibitors must incorporate adequate quality control measures, including (i) sourcing plant material from clean, certified agricultural sites rather than from roadsides or industrially impacted areas; (ii) pre-treatment of plant material or extracts to remove heavy metals (e.g., using chelation, ion exchange, or nanoparticle-based adsorption); and (iii) routine testing of final inhibitor products for heavy metal content prior to use in construction applications [55,56].

## 4. Nanoparticle Technologies for Corrosion Control and Self-Healing Concrete

### 4.1 Mechanisms of Nanoparticle Action in Cementitious Systems

The addition of nanoparticles to cementitious materials influences concrete performance through multiple interconnected mechanisms operating at the nanometre-to-micrometre scale. The most important pathways for corrosion protection include:

**Pore refinement and permeability reduction**: The smallest pores in cement paste are of nanometre dimensions. When nanoparticles such as nano-silica (nano-$SiO_2$) are added to the mix, they fill these pores, reducing the pore connectivity and dramatically decreasing permeability to water, chloride ions, and other aggressive species. Additionally, nano-silica acts as a highly effective pozzolan: it reacts with calcium hydroxide ($Ca(OH)_2$)—a byproduct of cement hydration—to form additional calcium-silicate-hydrate (C-S-H) gel, the primary binding phase in concrete. This secondary C-S-H gel further densifies the microstructure and refines the pore network [57,58,59]. Studies have demonstrated that chloride diffusion coefficients can be reduced by 40–60% with optimal nano-silica addition [60].

**Interfacial transition zone (ITZ) densification**: The ITZ between cement paste and aggregates is the weakest and most porous region in concrete, forming a preferential pathway for the ingress of aggressive ions. Nanoparticles concentrate in the ITZ due to their high surface area and reactivity, making this region denser and more resistant to chloride penetration [61].

**Corrosion inhibition by nanoparticles**: Some nanoparticles can directly affect the corrosion electrochemistry on the steel surface. Nano-titania (nano-$TiO_2$), for example, has been shown to form a barrier layer on steel, while nano-zinc oxide (nano-ZnO) exhibits antibacterial properties that can prevent microbially induced corrosion in certain environments [62].

**Self-healing crack repair**: Perhaps the most innovative application of nanoparticles in concrete is the development of autonomous self-healing systems. When microcracks form in concrete—as they inevitably do due to shrinkage, thermal stresses, or mechanical loading—nanoparticles can act as nucleation sites for the precipitation of calcium carbonate ($CaCO_3$) from the surrounding cement matrix or from deliberately incorporated healing agents. Crystals of $CaCO_3$ form within the crack volume, progressively filling the void and restoring impermeability and, to some extent, mechanical strength [63,64].

### 4.2 Types of Nanoparticles and Their Performance

#### 4.2.1 Nano-silica (nano-$SiO_2$)

Nano-silica is the most extensively studied and commercially advanced nanoparticle for concrete durability enhancement. The addition of nano-silica is particularly effective because it simultaneously provides filler, pozzolanic, and nucleation effects. Experimental data from multiple studies (Table 2) indicate that optimal nano-silica contents typically fall between 1.5% and 2.5% by cement weight, with higher dosages potentially causing agglomeration and loss of workability.

Table 2. Reported performance improvements for nano-silica-modified concrete

| *Property* | *Improvement relative to control* | *Nano-$SiO_2$ dosage (% cement)* | *Reference year* |
|---|---|---|---|
| Compressive strength (28 days) | +15 % to +30 % | 1.5–3.0% | 2023–2025 |
| Water absorption reduction | –31 % to –41 % | 1.5–2.5% | 2024–2025 |
| Chloride permeability reduction | –24 % to –40 % | 1.5–2.5% | 2023–2025 |
| Contact angle increase | +66 % | 2.0% | 2024 |
| Durability improvement (sulfate) | +39 % | 2.0% | 2024 |
| Durability improvement (chloride) | +42 % | 2.0% | 2024 |

*Note: Data compiled from [9,10,11,57,58,59].*

Recent research has extended nano-silica applications to alkali-activated concrete systems incorporating multiple solid wastes. A study by Wang et al. (2025) examined nano-silica-modified alkali-activated concrete using fly ash and steel slag as precursors [65]. The results were striking: the concrete without nano-silica experienced a 28.6% strength decrease after 90 days of immersion in high-concentration seawater, whereas the nano-silica-modified group showed only a 2.1% strength decrease, corresponding to a corrosion resistance coefficient of 97%. Furthermore, the chloride ion concentration in the surface layer (5 mm depth) of the nano-silica-modified concrete was only 10% of that in the control after 90 days of seawater exposure. The concentrations of dissolved Al and Fe in seawater were measured at 2.6 µg/L and 1.2 µg/L respectively for the nano-silica-modified material, indicating extremely low risk of metal dissolution in marine environments [65].

A study published in *Defect and Diffusion Forum* (January 2026) investigated the effect of nano-silica addition on concrete durability at dosage levels of 0, 1, 1.5, 2, and 2.5% by cement weight [9]. The experimental results demonstrated that concrete modified with 2% nano-silica exhibited water absorption decreased by 41% and contact angle increased by 66% compared to the control, while chloride permeability decreased by 24%. Moreover, the durability of the 2% nano-silica specimen increased by 39% against sulfate attack and by 42% against chloride attack. Electrochemical impedance spectroscopy confirmed significantly improved corrosion resistance of the reinforced concrete [9].

**4.2.2 Nano-titania (nano-$TiO_2$)**

Nano-titania offers additional functionality beyond traditional corrosion protection: its photocatalytic properties can degrade organic pollutants on the concrete surface, making it attractive for urban and environmentally exposed structures. Typical dosages range from 1% to 5% by cement weight. The primary limitations of nano-titania are its relatively high cost and lower pozzolanic activity compared to nano-silica [66,67].

**4.2.3 Carbon nanotubes (CNTs)**

Carbon nanotubes can dramatically improve the mechanical properties of concrete at extremely low dosages (0.05–0.2% by cement weight). CNTs bridge microcracks and enhance tensile strength and fracture toughness, indirectly improving corrosion resistance by reducing crack formation. However, CNT dispersion remains a significant challenge; agglomeration is common without adequate sonication or the use of surfactants. High material cost also limits widespread adoption [68,69].

**4.2.4 Green-synthesised nanoparticles from biomass waste**

A rapidly expanding area of research concerns the production of nanoparticles from agricultural and biomass waste materials using green synthesis methods. This approach addresses two sustainability challenges simultaneously: (i) value-added utilisation of waste streams, and (ii) production of nanomaterials without the toxic precursors (e.g., tetraethyl orthosilicate, TEOS) used in conventional synthesis.

Hilal et al. (2025) compared the effect of nano date pits and nano sunflower seed shells on the properties of green cement mortar, demonstrating that agricultural waste can be effectively converted into functional nanomaterials for construction applications [70]. The study reported that nanoscale processing of these waste materials significantly enhances their performance as supplementary cementitious materials [71].

Ainomugisha Safiki (2025) systematically developed optimised protocols for engineering eco-friendly nanoparticles from sugarcane bagasse ash (SCBA) using mechanical milling and sol-gel synthesis methods [72]. The study successfully engineered small-sized SCBA nanoparticles (5–20 nm) with disfigured crystalline $SiO_2$ networks. Engineered mesoporous silica with pore diameters below 50 nm, a high surface area of approximately 700 $m^2/g$, and silica purity up to 97% was produced. A comparison of nano-additive and clinker-replacement approaches revealed that each 1.5% nano-SCBA can effectively substitute up to 15% of cement clinker, with an 18% improvement in early cement strength. Mortars containing low-medium surface area n-$SiO_2$ (263–579 $m^2/g$) outperformed high-SSA materials at water-to-binder ratio 0.5, with 3% n-$SiO_2$ of SSA 579 $m^2/g$ attaining compressive strength improvement of 41% [72].

**4.3 Bacterial–Nanoparticle Hybrid Self-Healing Systems**

A particularly innovative development in concrete durability is the combination of bacterial spores with nanoparticles to create autonomous self-healing systems. The microorganisms—typically spore-forming bacteria such as *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus megaterium*—remain dormant within the concrete matrix until cracks form and water ingress activates them [73,74]. The bacteria metabolise a calcium-containing

nutrient (e.g., calcium lactate or urea), converting it to calcium carbonate that precipitates within the crack, progressively sealing it.

Recent research has demonstrated that nanoparticles play a crucial facilitative role in these hybrid systems. A study published in 2025 investigated a synergistic approach combining bacterial spores of *Bacillus subtilis* with nano-silica and nano-calcium carbonate as healing agents [19]. The results showed that bacterial–nano hybrid systems achieved up to 80% healing efficiency for cracks under 0.5 mm, with significant improvements in durability indices compared to conventional self-healing approaches [19,20].

A review by the journal *Journal of Building Engineering* (Elsevier, 2025) examined the facilitative role of nanotechnology in advancing bacterial-based self-healing concrete [75]. Nanomaterials play a crucial role in protecting bacterial spores, increasing their viability and activity, promoting biomineralisation, and consequently improving the overall efficiency of microbial self-healing processes. The review also documented that bacterial self-healing has been shown to facilitate up to 22% recovery in compressive strength and 16% recovery in split tensile strength under curing conditions, with another report showing up to 74% strength recovery for concrete incorporating bacteria plus steel slag aggregates [75].

### 4.4 Long-Term Durability and Environmental Considerations

Despite the substantial laboratory evidence supporting the effectiveness of nanoparticle-modified concrete and bacterial–nano hybrid systems, several important knowledge gaps remain.

**Dispersion challenges**: The tendency of nanoparticles to agglomerate is well documented. Without adequate dispersion—typically achieved through ultrasonication, the use of superplasticisers, or surface functionalisation—the beneficial effects of nanoparticles are significantly reduced. Developing reliable, scalable dispersion methods for commercial concrete production remains an ongoing challenge [76,77].

**Long-term performance**: Most published studies report results from laboratory specimens tested over weeks or months. Few field studies have examined nano-modified concrete performance over multi-year service periods. The long-term stability of the nanoparticle–cement matrix interface and the durability of bacterial spores within the high-pH environment of concrete over decades of service are not yet fully understood [78].

**Health and safety**: The inhalation of airborne nanoparticles during concrete mixing poses potential respiratory health risks to construction workers. Adequate dust control measures and personal protective equipment are essential. Standardised occupational exposure limits for nanomaterials in construction environments are still under development [79].

**Environmental leaching**: The long-term fate of nanoparticles in aged concrete structures is uncertain. If nanoparticles are not permanently bound within the C-S-H matrix, they could leach out over time, entering soil and water systems. Preliminary studies suggest that nano-silica becomes well incorporated into the gel structure and exhibits low leachability, but more systematic environmental fate studies are needed for a broader range of nanomaterials [80].

## 5. Discussion: Synergies, Limitations, and Research Gaps

### 5.1 Comparative Assessment of Plant-Based and Nanoparticle Approaches

The two technologies examined in this review operate on fundamentally different principles and offer distinct advantages and limitations.

Plant-based inhibitors are attractive for their low cost, renewable origin, and low toxicity. They can be derived from abundant agricultural waste streams, potentially contributing to a circular economy. However, the current limitations are substantial: (i) the long-term chemical stability of organic molecules in the aggressive alkaline environment of concrete is poorly characterised; (ii) the potential for heavy metal contamination of plant materials (as documented extensively in the Iranian medicinal plant literature) must be addressed through quality control and purification; (iii) the performance of plant extracts in real concrete (as distinct from simulated pore solutions) has been minimally evaluated; (iv) the effect of plant extracts on concrete workability, setting time, and long-term mechanical properties requires systematic investigation.

Nanoparticle technologies, by contrast, offer robust, proven improvements in concrete microstructure and permeability, with a strong mechanistic understanding of their action. Nano-silica-modified concrete is already commercially available and has been used in high-performance marine structures. However, the drawbacks include higher material costs, technical challenges in nanoparticle dispersion, and potential health and environmental risks during production and construction.

### 5.2 Hybrid Strategies: Combining Plant Extracts and Nanoparticles

Emerging research suggests that combining plant extracts with nanoparticles may produce synergistic effects not achievable with either technology alone. Plant extracts can act as green dispersants for nanoparticles, preventing agglomeration through their surface-active properties. The organic molecules from the extract may additionally provide direct corrosion inhibition on the steel surface, while the nanoparticles densify the concrete matrix. A few studies have reported that such hybrid systems outperform single-component treatments [81,82], but the mechanisms remain incompletely understood, and standardised protocols for hybrid system testing are lacking.

A particularly promising direction is the green synthesis of nanoparticles using plant extracts—a method that simultaneously produces nanoparticles and coats them with organic molecules that may themselves be corrosion-inhibiting. This approach avoids the use of toxic chemical precursors and can potentially reduce the cost of nanoparticle production. The body of literature on green synthesis of nanoparticles for medical and agricultural applications (e.g., the work of Fatima et al., 2025, and Mehregan et al., 2026) [83,84] provides a foundation that could be adapted for construction material applications. However, the heavy metal contamination risk applies equally to plant extracts used for green synthesis, necessitating the same quality control measures.

### 5.3 Knowledge Gaps and Future Research Priorities

Based on this review, the following research priorities are identified:

1. **Long-term field validation**: Multi-year exposure studies of concrete specimens incorporating plant-based inhibitors and/or nanoparticles under realistic environmental conditions (marine, de-icing salt, industrial) are needed to establish practical durability.
2. **Standardised testing protocols**: The corrosion inhibition literature suffers from considerable heterogeneity in test conditions, measurement techniques, and reporting standards. The development of industry-recognised standard test methods—analogous to ASTM G109 for traditional corrosion inhibitors—would enable reliable comparison across studies and support regulatory approval.
3. **Heavy metal control and purification**: Research into cost-effective methods for removing heavy metals from plant extracts intended for construction applications is essential. Chelation, ion exchange, and nanoparticle-based adsorption (as reviewed by Hajipour et al., 2023) represent promising approaches [85,86].
4. **Alkaline stability of phytochemicals**: Systematic studies of the hydrolytic stability of flavonoids, tannins, and other active compounds under concrete-like alkaline conditions (pH 12.5–13.5, elevated temperatures) over extended timeframes (months to years) are needed.
5. **Life-cycle assessment (LCA)**: Quantitative LCA of plant-based and nanoparticle systems—including extraction, processing, transport, application, and end-of-life phases—is required to establish the net environmental benefits relative to traditional protection strategies.
6. **Scale-up and commercialisation pathways**: Research that bridges the gap between laboratory findings and industrial practice—including large-scale extraction methods, mix design optimisation, and economic analysis—is needed to support market adoption.

## 6. Conclusions

This systematic review has examined two emerging paradigms for corrosion protection in reinforced concrete: plant-based natural inhibitors and nanoparticle-modified cementitious systems. The following conclusions can be drawn from the synthesis of the available literature.

- Plant extracts from a wide range of species (more than 40) have demonstrated corrosion inhibition efficiencies exceeding 85% in simulated concrete pore solutions containing chlorides, with the best-performing extracts (green tea, henna, *Ziziphora persica*) achieving IE% above 90% under optimised conditions. The active phytochemicals—flavonoids, tannins, alkaloids—adsorb onto steel surfaces following Langmuir behaviour and function as mixed-type inhibitors.
- A substantial body of research from Iran and elsewhere has documented heavy metal contamination of medicinal plants, with Cd exceeding WHO limits in 47% of samples and Pb in 26%. These findings have direct implications for plant-based construction additives, as contaminated plant material could introduce toxic elements into concrete. Therefore, stringent quality control of raw plant material is not optional but essential for safe application.
- Nanoparticle technologies, particularly nano-silica at dosages of 1.5–2.5% by cement weight, provide robust and well-characterised improvements in concrete durability. Experimental data show water absorption reductions of up to 41%, chloride permeability decreases of 24–40%, and corrosion resistance improvements of 39–42% under aggressive chemical attack. Nano-silica-modified alkali-activated concrete has demonstrated a corrosion resistance coefficient of 97% after 90 days of seawater immersion, with chloride surface concentrations only 10% of control values.
- Bacterial–nanoparticle hybrid self-healing systems achieve up to 80% healing efficiency for cracks under 0.5 mm, with nanoparticles playing crucial roles in bacterial spore protection, nucleation site provision, and acceleration of the biomineralisation process.
- Green synthesis of nanoparticles from agricultural waste (sugarcane bagasse ash, date pits, sunflower seed shells) offers a sustainable pathway to produce nanomaterials for construction, with demonstrated compressive strength improvements of 41% at 3% n-$SiO_2$ addition. Such approaches also align with circular economy principles.
- Critical knowledge gaps remain, including the lack of long-term field validation, the absence of standardised testing protocols, and insufficient understanding of the alkaline stability of phytochemicals. Future research should prioritise these areas and integrate life-cycle assessment to guide sustainable implementation decisions.

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# Artificial Intelligence and Internet of Things in Food Supply Chain Sustainability: A Data-Driven Approach to Waste Reduction, Predictive Quality Management, and Circular Economy Integration

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## Abstract

Food loss and waste (FLW) represent one of the most paradoxical failures of the modern food system: roughly one-third of all food produced for human consumption – approximately 1.3 billion tonnes annually – is lost or wasted, while 828 million people face chronic hunger. This review provides a comprehensive and critical synthesis of how Artificial Intelligence (AI) and Internet of Things (IoT) technologies are being deployed across the food supply chain (FSC) to monitor, predict, and reduce FLW. We systematically evaluate 85 peer-reviewed studies (2020–2026) covering the entire chain from primary production to retail and consumer behaviour. Specific applications include: (i) AI-powered yield prediction and precision harvesting in agriculture; (ii) IoT-based cold chain monitoring with real-time quality degradation modelling; (iii) computer vision for dynamic expiry date prediction and automated sorting in food processing; (iv) AI-driven demand forecasting and inventory optimisation in retail; and (v) consumer-facing smart storage and digital nudging. We critically examine the technical barriers – sensor cost, data interoperability, model generalisation across food types – as well as organisational and behavioural challenges. Integration of nanotechnology-based sensors (e.g., for ethylene or volatile amines) with IoT platforms is highlighted as an emerging frontier. Life cycle assessment (LCA) of AI/IoT interventions shows that FLW reduction of 25–40% is achievable, with corresponding decreases in GHG emissions, water use, and land pressure. Finally, we discuss the role of circular economy principles and the need for open data standards. Our conclusion is that AI and IoT are no longer optional but essential for a resilient, low-waste food system, provided that implementation costs are addressed and data governance frameworks are established.

***Keywords:*** *Artificial intelligence, Internet of Things, food waste reduction, cold chain monitoring, predictive quality, circular economy, nanotechnology sensors, smart agriculture, demand forecasting*

## 1. Introduction

The scale of global food loss and waste is staggering, yet it has become so routine that most supply chain actors accept it as inevitable. According to the Food and Agriculture Organization (FAO), approximately 14% of food produced globally is lost between harvest and retail, and an additional 17% is wasted at the retail, food service, and household levels [1]. Combined, this represents about 1.3 billion tonnes per year, with an estimated economic value of nearly one trillion US dollars. The environmental cost is equally severe: FLW accounts for 8–10% of total anthropogenic greenhouse gas (GHG) emissions – roughly three times the emissions from global aviation [2]. Moreover, wasted food consumes about 250 $km^3$ of fresh water annually (equivalent to the annual flow of the Volga River) and occupies 1.4 billion hectares of land, nearly 30% of the world's agricultural land area [3].

Paradoxically, FLW occurs unevenly across the supply chain. In low-income countries, losses are concentrated at the early stages – harvest, drying, storage, and transport – due to inadequate infrastructure, lack of refrigeration, and pest infestation. In high-income countries, waste dominates at the consumer level (households and food service), driven by aesthetic standards, oversized portions, confusion about date labels, and suboptimal storage behaviour [4]. In middle-income nations, both upstream losses and downstream waste are significant, creating a complex challenge that cannot be solved by any single intervention.

Traditional approaches to FLW reduction have focused on logistics improvements (better roads, cold storage), packaging innovations (modified atmosphere), and consumer education campaigns. While valuable, these strategies are often reactive and static – they do not adapt to real-time variations in product condition, demand fluctuations, or environmental factors. Over the past five years, a new paradigm has emerged: the integration

of **Artificial Intelligence (AI)** and **Internet of Things (IoT)** to create a dynamic, data-driven food supply chain (FSC) [5]. In this paradigm, low-cost sensors (temperature, humidity, ethylene, vibration) generate continuous streams of data about food condition; these data are transmitted via wireless networks to cloud or edge platforms; and AI models – including machine learning, deep learning, and reinforcement learning – analyse the data to predict spoilage kinetics, optimise routing, adjust storage parameters, and even recommend dynamic pricing for soon-to-expire products.

The potential impact is substantial. A systematic review by the World Resources Institute (WRI) estimated that widespread adoption of AI/IoT solutions could reduce FLW by 25–40% across high-value supply chains (perishables, dairy, meat, seafood) [6]. Early case studies show impressive results: a large European retailer used a recurrent neural network (RNN) to forecast store-level demand for fresh produce, reducing waste by 30% while maintaining 98% product availability [7]. A cold-chain logistics company integrated IoT sensors with a digital twin model, enabling dynamic rerouting of shipments to avoid delays; this reduced spoilage of fresh berries by 22% [8].

Nevertheless, implementation is not trivial. Sensor hardware must be affordable, robust, and food-contact safe. Data interoperability across different supply chain actors (farmers, logistics providers, retailers) remains poor. AI models trained on one food product (e.g., strawberries) often fail to generalise to another (e.g., leafy greens) because spoilage mechanisms differ. Furthermore, the energy consumption of continuous IoT monitoring and cloud processing must itself be considered in life cycle assessments – a point often overlooked.

This review has four main objectives. First, to systematically map the application of AI and IoT across each stage of the FSC, from primary production to consumer behaviour. Second, to critically assess the technical and economic performance of these systems based on published data. Third, to highlight emerging technologies – particularly nanotechnology-based gas sensors that can detect spoilage volatiles (ethylene, ammonia, biogenic amines) at parts-per-billion levels – that can be integrated with IoT platforms [9–11]. Fourth, to discuss the barriers to adoption and propose a research agenda. Throughout, we also draw attention to the quality and safety of raw agricultural materials – including contamination by heavy metals, pesticide residues, and mycotoxins – because sensor systems designed for waste reduction can simultaneously monitor for these hazards, as shown in recent studies from Iran and elsewhere [12–18]. By integrating food safety and waste reduction, the value proposition of AI/IoT becomes even stronger.

## 2. Materials and Methods

### 2.1 Search Strategy

A systematic search was conducted following PRISMA guidelines [19]. Databases: Scopus, Web of Science, IEEE Xplore, PubMed, and Google Scholar. Search period: January 2020 to March 2026. Search strings combined terms:

("artificial intelligence" OR "machine learning" OR "deep learning" OR "neural network" OR "predictive analytics") AND
("Internet of Things" OR "IoT" OR "wireless sensor network" OR "RFID" OR "smart sensor") AND
("food waste" OR "food loss" OR "perishable" OR "shelf life" OR "spoilage" OR "cold chain" OR "supply chain") AND
("agriculture" OR "harvest" OR "logistics" OR "retail" OR "consumer").

For nanotechnology-sensor integration, we added: ("nanosensor" OR "biosensor" OR "ethylene sensor" OR "volatile organic compound" OR "biogenic amine") AND ("IoT" OR "wireless").

### 2.2 Inclusion and Exclusion Criteria

Inclusion: (i) peer-reviewed original research or reviews in English; (ii) reporting quantitative outcomes (e.g., % waste reduction, prediction accuracy, cost savings); (iii) field-tested or validated with real data (not purely simulation); (iv) for sensor papers: detection limit and response time reported. Exclusion: purely theoretical studies without data; studies on non-food waste (e.g., municipal solid waste); conference abstracts without sufficient detail.

### 2.3 Data Extraction

Extracted parameters: supply chain stage, food commodity, sensor type (temperature, humidity, gas, motion), communication protocol (Wi-Fi, LoRaWAN, NB-IoT, 5G), AI/ML algorithm (random forest, XGBoost, LSTM, CNN, etc.), sample size, prediction accuracy ($R^2$, RMSE, F1-score), reported waste reduction (%), implementation cost (USD), and payback period (months). For nano-sensors: target analyte, detection limit (ppb/ppm), response time (seconds), and integration method.

### 2.4 Quality Assessment

Studies were assessed using an adapted version of the QUADAS-2 tool for predictive models and a custom scoring system for IoT field deployments. LCA studies were evaluated against ISO 14040/14044 criteria.

## 3. Results

### 3.1 AI/IoT Applications Across the Food Supply Chain

We identified 85 studies that met inclusion criteria. The distribution across supply chain stages is shown in **Table 1** .

| *Stage* | *Number of studies* | *Typical technologies* | *Reported waste reduction* | *Key challenges* |
|---|---|---|---|---|
| Primary production (pre-harvest & harvest) | 22 | Satellite/Drone imagery + CNNs, soil IoT sensors, yield prediction models | 15–25% (reduced premature harvest losses) | Model transferability across climates |
| Post-harvest storage & transport (cold chain) | 30 | Wireless T/H sensors, ethylene nanosensors, LSTM spoilage prediction, digital twins | 20–35% | Sensor cost; power supply; data gaps |
| Food processing & packaging | 12 | Computer vision for dynamic expiry labelling, IoT-enabled smart packaging (TTI) | 10–20% | Integration with existing lines |
| Retail (supermarkets, food service) | 15 | AI demand forecasting (XGBoost, RNN), dynamic pricing, smart shelves | 25–40% | Data silos between suppliers and retailers |
| Household consumption | 6 | Smart fridges with inventory tracking, camera-based waste logging, digital nudging apps | 10–30% | User engagement; privacy |

### 3.2 Cold Chain Monitoring: The Largest Opportunity

The cold chain – from farm to retail refrigerated display – is where most perishable food waste occurs (estimated 25% of fresh produce, 20% of dairy, 35% of seafood) [20]. Thirty studies focused on IoT-enabled cold chain monitoring. The most robust findings are:

- **Wireless sensor networks (WSN)**: Low-power temperature/humidity sensors (e.g., DHT22, SHT30) combined with LoRaWAN or NB-IoT provide continuous monitoring with battery life >6 months. In a field test across 12 refrigerated trucks carrying strawberries, real-time alerts for temperature excursions (>4°C for >2 hours) reduced spoilage from 18% to 6% [21].
- **Predictive quality models**: Using historical time-series of temperature and vibration, Long Short-Term Memory (LSTM) networks predict remaining shelf life with an error of ±0.8 days (compared to traditional kinetic models which have ±2.3 days error) [22]. A study on fresh Atlantic salmon used an LSTM with IoT inputs (T, humidity, gas – trimethylamine) achieved $R^2 = 0.93$ for spoilage prediction [23].
- **Digital twins**: A digital twin of a cold storage facility – which mirrors the real-time physical state – allows operators to simulate "what if" scenarios (e.g., door opening frequency, compressor failure). One cold storage operator reduced energy use by 18% while reducing spoilage by 12% using this approach [24].

### 3.3 AI for Demand Forecasting and Retail Waste Reduction

Retail waste is driven primarily by mismatches between supply (often based on historical averages) and variable demand (weather, promotions, holidays, local events). Machine learning models that incorporate multiple data streams – point-of-sale (POS) data, weather forecasts, calendar features, even social media sentiment – have become highly accurate. In a meta-analysis of 15 retail studies, the weighted average waste reduction was **32%** for fresh produce, 28% for bakery, and 19% for dairy [25].

The best performing algorithm was a gradient-boosted ensemble (XGBoost + LightGBM) that achieved a mean absolute percentage error (MAPE) of 12% for daily store-item demand, compared to 28% for traditional moving averages [26]. A large UK supermarket chain implemented such a system across 500 stores: waste fell by 25% within one year, and out-of-stock events decreased by 15% [27]. Importantly, the system also enabled **dynamic pricing** – automatically discounting items approaching their use-by date – which shifted 35% of otherwise wasted products to sold at reduced margin but positive contribution.

### 3.4 Emerging Sensor Technologies: Nanosensors and Biosensors

Traditional temperature and humidity sensors are insufficient for many fresh foods because spoilage is driven by biochemical changes (e.g., ethylene production in climacteric fruits, biogenic amine accumulation in fish). Nanotechnology has enabled a new generation of highly sensitive, low-cost gas sensors that can be integrated directly into IoT platforms [9,10]. Key examples:

- **Ethylene nanosensors** for fruit ripening monitoring: Carbon nanotube-based chemiresistors detect ethylene down to 10 ppb. When integrated with a wireless transmitter and placed inside a fruit container, the system alerts the operator when ethylene exceeds a threshold (e.g., 50 ppb for bananas), triggering ventilation or removal of ripening fruit [28]. Field tests on banana shipments reduced over-ripening waste by 40% [29].
- **Ammonia and biogenic amine sensors** for fish and meat: Polyaniline- or metal oxide-based nanosensors respond to trimethylamine (TMA) and cadaverine – key spoilage markers. A recent study demonstrated a flexible, printed nanosensor array that attaches to the inner surface of packaged fish fillets. Data transmitted via NFC to a smartphone can calculate remaining shelf life with 89% accuracy [30].
- **Heavy metal and pesticide detection**: While not directly for waste reduction, the same IoT infrastructure can be used to monitor contaminants. As reported in multiple studies from Iran and other regions, raw agricultural materials (medicinal plants, rice, vegetables) sometimes contain elevated levels of lead, cadmium, or arsenic due to soil pollution [12–18]. Nanosensors functionalised with specific chelators can detect these metals at ppb levels. Integrating such sensors into receiving docks could enable real-time

rejection of contaminated batches, preventing both waste (if rejected early) and health risks. Hajipour et al. (2023) reviewed the application of nanoparticles in phytoremediation and sensing, highlighting the dual benefit of remediation and detection [17]. Alinia-Ahandani & Hajipour (2026) provided a broader perspective on nanotechnologies for removal of polycyclic aromatic hydrocarbons (PAHs) from smoked rice, which can be adapted for sensor development [18].

**3.5 Life Cycle Assessment and Economic Viability**

Five LCA studies examined the net environmental impact of deploying AI/IoT for FLW reduction [31–35]. The key finding is that the **embodied emissions** of sensors, communication infrastructure, and cloud computing are small (typically <2% of the emissions from the wasted food that is saved). For example, a system of 1,000 IoT temperature loggers distributed across a cold chain (each with a 5-year lifespan) has an embedded carbon footprint of approximately 0.5 tonnes $CO_2$-eq, while the food waste it prevents over the same period avoids 50–100 tonnes $CO_2$-eq [31]. Thus, even with conservative assumptions, the net benefit is strongly positive.

Economic payback periods vary. For high-value perishables (berries, seafood, cut flowers), payback is often less than 6 months. For lower-value commodities (potatoes, onions), payback can exceed 2 years, which may deter investment without subsidies [32]. The cost of nanosensors remains higher than conventional T/H sensors – approximately $5–15 per unit compared to $1–3 – but prices are falling rapidly with printed electronics.

**4. Discussion**

The evidence assembled in this review indicates that AI and IoT technologies are not speculative future solutions but operational tools that are already delivering measurable reductions in food waste across real supply chains. However, several critical barriers must be overcome to achieve widespread adoption, particularly in low- and middle-income countries where losses are highest.

**Technical barrier – model generalisation**: Most AI models are trained on specific food varieties, packaging types, and environmental conditions. An LSTM trained on cv. 'Cavendish' bananas from Ecuador may perform poorly on cv. 'Grand Naine' from the Philippines due to different respiration rates. Transfer learning and domain adaptation techniques are being developed, but they require sharing data across supply chain partners – which is often commercially sensitive. Federated learning, where models are trained locally and only parameter updates are shared, offers a promising path [36].

**Data interoperability and standards**: Currently, sensor data formats, communication protocols, and cloud platforms are highly fragmented. A farmer using a LoRaWAN network cannot easily share data with a logistics provider using a 5G-based system unless both adopt a common data schema (e.g., GS1's Electronic Product Code Information Services – EPCIS). The development of open standards for food IoT data is urgently needed, and organisations like the Open Food Chain Association (OFCA) are working in this direction [37].

**Cost and energy in low-resource settings**: In many low-income countries, the capital cost of IoT sensors (even low-cost ones) remains prohibitive for smallholder farmers. Innovative business models – such as "sensor-as-a-service" where farmers pay a small subscription fee – have shown promise in pilot projects in Kenya and Bangladesh [38]. Additionally, low-power wide-area networks (LPWAN) like LoRaWAN can operate for years on a single battery, and solar-powered gateways eliminate grid dependence.

**Integration of food safety monitoring**: A significant opportunity is to combine FLW monitoring with food safety surveillance. The same IoT platforms that track temperature and humidity can also host sensors for heavy metals, mycotoxins (aflatoxin, ochratoxin), and pesticide residues. As noted in the Iranian studies referenced earlier, contamination of medicinal plants, rice, and animal products with lead, cadmium, and other toxic elements remains a concern [12–15,17]. Alinia-Ahandani et al. (2022) systematically reviewed the safety evaluation of toxic elements in medicinal plants, emphasising that routine monitoring is insufficient in many regions [15]. If IoT-enabled nanosensors for these contaminants become affordable, they could serve a dual purpose: rejecting contaminated batches (preventing health risks) and also reducing waste by directing less-contaminated materials to appropriate uses (e.g., processing rather than raw consumption).

**Behavioural aspects – the consumer link**: Even the most sophisticated AI/IoT system cannot force a household to eat food before it spoils. However, digital nudging – using notifications, recipe suggestions, and visual feedback – has been shown to reduce household waste by 15–20% [39]. A smart fridge that tracks inventory and displays a "use by tonight" list on a smartphone app reduced self-reported waste by 28% in a six-month Swiss study [40]. Privacy concerns remain; many users are uncomfortable with cameras inside their fridge. Simpler solutions (RFID tags on grocery items paired with a door sensor) achieve similar benefits with less intrusion.

**Environmental trade-offs**: While LCA generally supports AI/IoT for FLW reduction, there are edge cases where the sensors themselves have high embodied energy (e.g., short-lived, non-recyclable sensors using rare earth metals). The electronics waste (e-waste) from discarded sensors is a growing concern. Designing sensors with biodegradable substrates (cellulose, polylactic acid) and using additive manufacturing (inkjet printing of silver nanoparticles) can reduce both cost and environmental footprint [41].

**Future directions**: We foresee five transformative developments over the next decade: (i) **ubiquitous low-cost nanosensors** – printed gas sensors below $0.10 per unit, enabling every package to have a "digital freshness indicator"; (ii) **AI-powered dynamic shelf life** – models that continuously update use-by dates based on actual

time-temperature history rather than static labels; (iii) **autonomous cold chain vehicles** – delivery drones and autonomous trucks that use real-time spoilage data to reroute to the nearest market; (iv) **blockchain integration** – immutable records of food condition for insurance claims and liability; (v) **regulatory mandates** – governments requiring IoT monitoring for high-risk perishables, similar to the EU's cold chain regulations for pharmaceuticals.

## 5. Conclusions

Food loss and waste is not an inevitable cost of doing business – it is a solvable problem. The convergence of low-cost IoT sensors, advanced machine learning, and cloud computing has created an unprecedented opportunity to monitor, predict, and mitigate waste across the entire food supply chain. This review has shown that:

- In primary production, AI-driven yield prediction and precision harvesting can reduce pre-harvest losses by 15–25%.
- In cold chain logistics, IoT-based real-time monitoring combined with LSTM spoilage models reduces waste by 20–35%, with digital twins offering additional efficiency gains.
- In retail, AI demand forecasting and dynamic pricing cut waste by 25–40%, while also improving product availability.
- Emerging nanosensor technologies (ethylene, biogenic amines, heavy metals) can be integrated with IoT platforms to provide real-time quality and safety data, addressing both waste and contamination risks – a dual benefit highlighted by recent research on toxic elements in food raw materials [12–18].

The economic and environmental returns on investment are strongly positive for high-value perishables, though lower-value commodities may require subsidy or shared infrastructure. Key barriers – cost in low-income settings, data interoperability, model generalisation, and consumer behaviour change – are being addressed through technical innovation, open standards, and behavioural science.

For the international food industry, the message is clear: AI and IoT are no longer optional enhancements but essential components of a resilient, low-waste, and safe food system. Policymakers should support the development of open data standards, invest in digital infrastructure for cold chains, and encourage the adoption of smart sensors through tax incentives or mandates for high-risk products. Researchers should focus on low-cost, biodegradable sensor platforms, transfer learning methods that work across diverse food types, and integration of food safety surveillance into waste reduction systems.

Finally, it is worth remembering that every tonne of food waste prevented avoids not only economic loss but also unnecessary GHG emissions, water consumption, and land use. In a world of finite resources, making our food supply chain intelligent is not just good business – it is a moral imperative.

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# Evaluation of the nutritional value of the cyanobacterium *Spirulina platensis* cultivated on wastewater from a recirculating aquaculture system

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## Abstract

The increasing demand for alternative sources of safe and efficient vegetable proteins and the development of sustainable solutions for the valorization of aquaculture wastewater stimulate the interest in the use of the cyanobacterium *Spirulina platensis* as a biomass with high nutritional value. The present study is based on the evaluation of the nutritional potential of *Spirulina platensis* biomass cultivated on wastewater from recirculating aquaculture systems at the Research and Development Station for Aquaculture and Aquatic Ecology Iasi of the "Alexandru Ioan Cuza" University of Iasi. The algae were cultivated on the standard nutrient medium (Zarrouk) in a proportion of 90%, supplemented with 10% wastewater from the recirculating aquaculture system. During the exponential growth phase of the cyanobacterium, the general biochemical content of the biomass and the amino acid profile were determined (using classical spectrophotometric and chromatographic methods). The results showed that the *Spirulina platensis* biomass has a high protein content, constituting 53.94%, while carbohydrates represented 13.50% and lipids 7.30%. Amino acid analysis revealed the presence of a diverse spectrum of amino acids, with high values for glutamic acid (11.59%), leucine (6.70%), arginine (5.90%), aspartic acid (5.79%) and alanine (5.51%). The biomass also contained essential amino acids such as threonine, valine, isoleucine, leucine, lysine, methionine and phenylalanine, with values that did not differ significantly from those obtained in the case of cultivation of the algae on standard nutrient medium. Cultivation of *Spirulina platensis* on aquaculture wastewater represents a promising approach for obtaining an alternative source of biomass with a significant content of biologically active substances, simultaneously contributing to the valorization of nutrients from water and the development of circular technologies in the agri-food sector. The results obtained confirm the potential of this biomass for applications in aquatic animal nutrition (wastewater generating fish), biotechnology and sustainable valorization of aquatic resources.

***Keywords:*** *Spirulina platensis, biomass, nutritional value, aquaculture wastewater*

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# Comparative Review of Conventional and Novel Extraction Methods on the Phenolic Content of Plant Extracts: Implications for Food Applications

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## Abstract

Plant-derived phenolic compounds are widely recognized for their potent antioxidant and antimicrobial properties, making them highly valuable in the food, pharmaceutical, and cosmetic industries. However, the efficiency of their extraction- and consequently their bioactivity -is critically dependent on the extraction method employed. This review provides a comparative analysis of conventional extraction techniques (maceration, Soxhlet) and novel methods, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE), with a focus on their impact on total phenolic content (TPC) and antioxidant activity. A systematic literature search was conducted using PubMed, Scopus, and Science Direct databases for articles published between 2010 and 2025. The findings indicate that novel extraction methods significantly enhance phenolic yield (typically by 30–100%) compared to conventional approaches. MAE and UAE drastically reduce extraction time (from hours to minutes) and solvent consumption while minimizing degradation of heat-sensitive compounds. SFE is the most environmentally friendly technique but requires high-capital equipment. Key parameters influencing extraction efficiency -solvent type (particularly hydroalcoholic solvents), temperature (optimum 40–60 °C), extraction time, and solvent-to-solid ratio- are discussed. Response surface methodology (RSM) is highlighted as an effective tool for simultaneous optimization of these parameters. This review concludes that the choice of extraction method directly determines the quantity and quality of phenolic extracts. For industrial food applications requiring high yield, short processing time, and scalability, MAE and UAE are superior to conventional methods. Future research should focus on developing green solvents (e.g., natural deep eutectic solvents, NADES) and integrating novel techniques to achieve efficient, economical, and environmentally sustainable extraction processes suitable for food industry adoption.

***Keywords:*** *Phenolic compounds; Microwave-assisted extraction; Ultrasound-assisted extraction; Maceration; Green solvents; Response surface methodology; Food preservation*

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# Cordyceps, Unlocking nature's benefits for health and wellness

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## Abstract

*Cordyceps* spp. are a group of entomoparasitic fungi belonging to the phylum Ascomycota. There are more than thousand species of "Cordyceps" have been identified under four genera, namely, *Cordyceps*, *Ophiocordyceps*, *Elaphocordyceps* and *Metacordyceps*. Those are describing a specific type of entomoparasitic fungi on insect orders including Lepidoptera, Hymenoptera & Hemiptera. As obligate parasites, *Cordyceps* use a mechanism that hijacks of the host nervous system. After infection, the fungi produce secondary metabolites that alter the circadian and diurnal rhythms of the host, leading it to locations with optimal environmental conditions for fungal reproduction. Over the time, the fungus has infected the body of the host and once the suitable environmental condition reached the host dies. By absorbing the nutrients of the carcass, the fungus grows continuously and produce reproductive structures. Cordyceps are usually rich form numerous secondary metabolic components including cordycepin, pentostatin, peroxides, organic acids, oosporeins and polysaccharides. Many of these components are carrying medicinal properties which is important for human health and well-being. In Traditional Chinese Medicine (TCM) a certain species of cordyceps including *Ophiocordyceps sinensis* and *Cordyceps militaris* has been used as medicines for cough, renal disfunction and failures, immunity, anti-inflammatory, antioxidant, and blood sugar regulation. Some properties of *Cordyceps* spp. have been discovered from recent researches in pharmaceuticals. Immunomodulation is one of the recently discovered property from *O. sinensis.* It can enhance the effectiveness of an anti- tuberculosis drug names PA-824. The combination has better effectiveness than the using the drug along against *Mycobacterium tuberculosis*. Apart from headed properties, many *Cordyceps* species are containing compounds which can be using in treatments of various cancer types, to maintaining the gut, liver and renal health and antioxidant compounds. Hence, further studies regarding *Cordyceps* can open a new view on the production of antibiotics and pharmaceuticals which enhance the well-being of human life.

***Keywords:*** *Antibiotics, Entomoparasites, metabolites, Fungi, Secondary*

## Introduction

*Cordyceps* are known as a genus of entomoparasitic fungi in phylum Ascomycetes which almost entirely parasitize on wide ranges of arthropods including order Coleoptera, Hemiptera, Hymenoptera, Diptera and Lepidoptera (Chen et al., 2024). It is one of the largest and most diverse genera in family Claviciputaceae which belonging to the phylum Ascomycota. Majority of these fungal species are distributed among tropical and temperature regions of the world including Europe, North America, Southeast, Eastern and Asian regions (Krishna et al., 2024). All *Cordyceps* spp. are share a common mechanism of their life cycle invading, hijacking and growing on their selected host's body tissues, in order to ensure the growth and the continuation of the generation. Vast majority of them are showing the host specificity, while several species has the ability of growing on considerably broader range of host species. Fewer cordyceps species are selecting a narrow range of fungal species (Hart's truffels) as their hosts. During the parasitic process, *Cordyceps* are maintaining a complex mechanism to facilitate its attachment to the host's body and avoiding the destruction from it's immune system. Through this entire procedure, *Cordyceps* are producing several unique secondary metabolic components which is important in the several stages of its life cycle (Chen et al., 2024; Chou et al., 2024). As secondary components, *Cordyceps* spp. are secreting nucleotides, polysaccharides, steroids, terpenes, proteins derivatives, sugar alcohols, organic acids, and fatty acid derivatives (Chen et al., 2024). Naturally, most of these complex organic metabolic components are responsible with the physiological and structural functions, nutrient requirements, and active metabolic pathways ( Chan et al., 2015). Existence of these metabolic components ensure the parasitic life cycle of the fungus while existing as a rich source of organic components, which widely using for studies to discover new drugs with immunomodulatory, antitumor, and hypoglycemic functions (Xiao and Zhong, 2007).

## Traditional Chinese Medicine (TCM)

Traditional Chinese medicine or TCM is known as a systematic healthcare system which developed from clinical experiences (Matos et al., 2021; Wu et al., 2025). Even though the origin of TCM remains unclear, most of the archeological structures which belonging to the TCM dating back to several millennia ( Matos et al., 2021). In the core modalities of TCM, one important part is using the herbal medicines to treat diseases. As the herbal medicines in TCM, plant parts, animal parts, mineral remedies and fungal structures has been used through several thousand years of history. Within the fungal ingredients which commonly using for the TCM, *DongChongXiaCao*

(Winter worm-summer grass) is referring for the famous "Caterpillar fungi" or *Ophiocordyceps sinensis*. It is a rare species of cordyceps family which surviving in high altitudes (3,000-4,000 meters) such as Qinghai and Tibet plateau (Chellapandi and Saranya, 2024). With the gradual growth, the fungi parasitize the larvae of ghost moth species which belonging to the order *Lepidoptera*, under the genus *Thitarodes* (Formerly classified under genus *Hepialus*). After infecting the fungal parasite, the larva is turned into a sclerotium which covered by the intact exoskeleton of the insect to withstand the winter, which is regarded as "winter worm". In the late spring or early of the summer of the next year, a clavate stroma of the fungus grows from the sclerotium and emerged from the ground appearing as a herb, which is regarded as "summer grass" (Wang and Yao , 2011). It was one of the high-demanding medicinal ingredients in TCM, which has been used for treating lung diseases, boost immunity, enhancing vitality and strengthen the immune system (Paterson, 2008; Chellapandi and Saranya, 2024). Instead of *O. sinensis*, *Cordyceps militaris*, *Cordyceps purinosa* and *Cordyceps ophiglossoides* has been used in various aspects in TCM such as a tonic, herbal drink, reduce inflammations and a medicine for blood sugar management (Paterson, 2008).

**Modern research outcomes- Immunomodulatory properties of *Cordyceps* spp.**

Immune system is one of the most complex systems in a human body which has complicated defense mechanisms against the infective agents. Immunomodulation is a process of modifying immune responses in a positive or negative administration of a drug or certain components (Saroj et al., 2012). In recent years, researches regarding the immunostimulatory and immunomodulatory properties of cordyceps species as well as the secondary metabolite extracts has been significantly increased. From recent researches, mycelial extractions and bioactive compounds of various cordyceps species have been studied with the production of various cytokinin production such as interleukin (IL)-1β, IL-2, IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor (TNF)-α and phagocytosis stimulation of immune cells of the immune system (DAS et al., 2020). Immunomodulation is a broader process rather than immunostimulation, it alters the immune system, whether by turning it up or turning it down. Many cordyceps species extractions including *O. sinensis* has the immunomodulation properties too. The extractions of *O. sinensis* has the potential of splenocyte stimulation and enhanced Th1-type cytokine expression from the splenocytes. Importantly, the same treatment significantly enhanced the natural killer cell activity of the splenocytes and enhance the effectiveness of an anti- tuberculosis drug names PA-824 (Jang et al., 2015). Another widely using cordyceps species for pharmaceutical industry is *Cordyceps militaris*. Mycelial extractions of *C. militaris* has the ability of activation of macrophages and enhancement of phagocytosis. As the bioactive components which responsible for immunomodulation were polysaccharides in *C. militaris*, including a mixture of arabinose, galactose, glucose, mannose, and Xylose (Liu et al., 2024).

**Antitumor properties**

The amount of people who are suffering from cancers and tumors in the world will continue to grow steadily, according to the latest report about the trend of global cancer released by the World Health Organization (WHO). According to the official reports of WHO, nearly 10 million deaths has been observed from various types of cancers and each year, nearly 400,000 children has been develop various types of cancers (WHO, 2026). Developing drugs against cancers and tumors are incredibly challenging because cancer cells are containing mutants, vary from the structure and the function even inside the same tumor and sharing many similar functions with normal cells in the body. When developing anti cancerous treatments, various *Cordyceps* spp. have been provided promising results. Especially, the component which known as cordycepin has discovered as a successful anti -tumor agent, specially against several cancer types (Jin et al., 2018). Cordycepin has the ability of induce cancer cell apoptosis in caspase-dependent pathways, a primary mechanism of apoptosis, which governing by a family of protease enzymes named caspases. Apoptosis of Human liver cancer (HepG2) cells were induced by the activation of caspase, interaction between Fas and FADD, and modulation of the protein levels of Bid and tBid (Shao et al., 2016). Cordycepin also decreased human bladder carcinoma cells (T24 cells) survival. That was regulating by the activation of A3 adenosine receptor of the cells and the subsequent inactivation of Akt pathways, leading to the increases in cleaved Caspase-3 and apoptosis (Cao et al., 2017). Instead of cordycepin, polysaccharides, Beauvericin, Ergosterol and cordycemaides are also responsible for the activation of tumor suppression pathways. ( Xiao and Zhong , 2007).

**Health benefits and human well being**

Rather than using for pharmaceutical extractions and production of new medicines, *Cordyceps* are much popular among people as a nutrient rich source from minerals, proteins, amino acid derivatives and phenolic components (Liu et al., 2019; Ashraf et al., 2020). Abundant number of bioactive components and secondary metabolites are present in *Cordyceps* such as proteins derivatives, carbohydrates such as arabinose, fatty acids, volatile compounds, carotenoids, phenolic compounds, flavonoids, minerals (Fe, Ca, Mg, Ni, Sr, Na, Ti, Pi, Se, Mn, Zn, Al, Si, K, Cr, Ga, V and Zr) and vitamins (B1, B2, B12, E and K) (Hur, 2008; Elkhateeb et al., 2019; Ashraf et al., 2020). Because of that, *Cordyceps* spp. are now using as a dietary supplement as well as a herbal medicine by various states including United States and Eastern Asian countries such as China (Das et al., 2010).

**Cultivation of *Cordyceps***

In natural environments, *Cordyceps* spp. are growing on specific host species. But, when comes to mass production, cereal-based substrates such as rice, wheat, oats, and barley, which provide essential nutrients for mycelial colonization and metabolite biosynthesis for cordyceps cultvation. Supplementation with suitable nitrogen sources and trace elements further enhances growth, fruiting body formation, and cordycepin accumulation (Sharma *et al.*, 2024; Shrikhandia *et al.*, 2026). In contrast, submerged liquid fermentation is primarily used for rapid mycelial biomass production under controlled environmental conditions, including optimized pH, temperature, aeration, agitation, and nutrient availability. It offers advantages such as shorter cultivation periods, reduced contamination risks, easier process control, and scalability for industrial production, although fruiting body development is generally absent under submerged conditions (Paterson, 2008; Shrikhandia *et al.*, 2026). The selection of cultivation substrates, carbon and nitrogen sources, light exposure, temperature, and oxygen availability significantly influences fungal growth and the synthesis of secondary metabolites. Recent advances have focused on optimizing cultivation media using agro-industrial residues, mixed grain substrates, and insect-derived materials to improve productivity while reducing production costs. Furthermore, the integration of bioprocess engineering, controlled fermentation systems, and strain improvement has enhanced biomass yield and metabolite production, supporting the commercial development of *Cordyceps*-derived nutraceuticals and pharmaceuticals. Nevertheless, the establishment of standardized cultivation protocols and efficient large-scale production systems remains a major research priority to ensure consistent product quality and maximize the accumulation of valuable bioactive compounds (Sharma *et al.*, 2024; Shrikhandia *et al.*, 2026).

**Conclusion**

*Cordyceps* is a group of entomoparasitic fungal species which belonging in to the phylum Ascomycota. As an obligate parasite, *Cordyceps* are using various types of metabolic compounds for ensure their life cycle. They are usually rich form numerous secondary metabolic components including cordycepin, Pentostatin, peroxides, organic acids, polysaccharides, volatile components, and mineral nutrients. In Traditional Chinese Medicine (TCM) a certain species of cordyceps and their extractions including *O. sinensis*, *C. militars* and *C. purinosa* has been used as medicines for cough, renal disfunction and failures, immunity, anti-inflammatory, antioxidant, and blood sugar regulation. Some properties of *Cordyceps* spp. have been discovered from recent researches in pharmaceuticals. Immunomodulatory is one of the recently discovered property from *O. sinensis.* It can enhance the effectiveness of an anti -tuberculosis drug names PA-824. The combination has better effectiveness than the using the drug along against *Mycobacterium tuberculosis*. Apart from that, many *Cordyceps* spp. are containing compounds which can be used in treatments of various cancers including several types of hepatic cancers. Because of being a high profitable industry, selected *Cordyceps* spp. are now cultivated in media, including cereal based and liquid fermentation methods. Further researches and studies regarding the bioactive compounds, metabolite extraction methods and cultivation optimization techniques can enhance the *Cordyceps* biotechnological applications.

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# Effects of Egg Weight Classes on Egg Characteristics, Chick Quality and Hatchery Performance in Different Layer Lines

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## Abstract

This study investigated the effects of different layer lines and egg weight classes on egg characteristics, chick quality traits, and hatchery performance. Four layer lines, namely BAR-1, RIR-1, BLUE, and MAROON, were evaluated at the Ankara Poultry Research Institute. A total of 960 eggs, including 240 eggs from each line, were used and classified into light, medium, and heavy weight classes according to the mean egg weight of each line. Egg weight, egg weight loss, width, length, shape index, day-18 egg weight, chick weight, chick length, chick quality, hatching time, fertility, hatch of fertile eggs, and hatchability were determined. Data were analyzed using SPSS version 26, and $P<0.05$ was considered statistically significant. Genotype significantly affected almost all of the investigated traits ($P<0.05$). The RIR-1 line had the highest initial egg weight (61.38 g), chick weight (44.02 g), chick length (17.28 cm), and chick quality score (97.54), whereas BAR-1 showed the highest fertility (97.99%), hatch of fertile eggs (92.19%), and hatchability (90.36%). In RIR-1, these values were 78.45%, 81.66%, and 64.34%, respectively. Increasing egg weight significantly increased chick weight and chick length; however, hatchability was lower in heavy eggs (75.36%) than in light eggs (86.25%). A strong positive correlation was found between egg weight and chick weight ($r=0.804$; $P<0.01$). Overall, the findings indicate that both layer genotype and egg weight play important roles in chick development and hatchery performance. Therefore, genotype-specific hatchery performance, together with an appropriate egg weight range, should be considered when selecting hatching eggs.

***Keywords:*** *Layer line, egg weight, hatchability, chick quality, fertility*

## Farklı Yumurtacı Hatlarda Yumurta Ağırlık Sınıflarının Yumurta Özellikleri, Civciv Kalitesi ve Kuluçka Sonuçları Üzerine Etkileri

## Özet

Bu çalışmada, farklı yumurtacı hatlar ile yumurta ağırlık sınıflarının yumurta özellikleri, civciv kalite özellikleri ve kuluçka sonuçları üzerine etkileri araştırılmıştır. Çalışmada Ankara Tavukçuluk Araştırma Enstitüsünde yetiştirilen BAR-1, RIR-1, BLUE ve MAROON olmak üzere dört yumurtacı hat kullanılmıştır. Her hattan 240 olmak üzere toplam 960 yumurta değerlendirilmiş ve yumurtalar, her hattın ortalama yumurta ağırlığı dikkate alınarak hafif, orta ve ağır olmak üzere üç ağırlık sınıfına ayrılmıştır. Yumurta ağırlığı, yumurta ağırlık kaybı, genişlik, uzunluk, şekil indeksi, 18. gün yumurta ağırlığı, civciv ağırlığı, civciv uzunluğu, civciv kalitesi, çıkım zamanı, fertilite, fertil yumurtalardan çıkış gücü ve kuluçka randımanı belirlenmiştir. Veriler SPSS 26 paket programı kullanılarak analiz edilmiş ve $P<0,05$ istatistiksel önem düzeyi olarak kabul edilmiştir. Genotip, incelenen özelliklerin tamamına yakınında önemli farklılıklara neden olmuştur ($P<0,05$). RIR-1 hattı en yüksek başlangıç yumurta ağırlığı (61,38 g), civciv ağırlığı (44,02 g), civciv uzunluğu (17,28 cm) ve civciv kalite skoruna (97,54) sahip olurken, BAR-1 hattı en yüksek fertilite (%97,99), fertil yumurtalardan çıkış gücü (%92,19) ve kuluçka randımanını (%90,36) göstermiştir. RIR-1 hattında ise bu değerler sırasıyla %78,45, %81,66 ve %64,34 olarak belirlenmiştir. Yumurta ağırlık sınıfının artması civciv ağırlığı ve uzunluğunu önemli düzeyde artırmış; ancak kuluçka randımanı ağır yumurtalarda (%75,36), hafif yumurtalara (%86,25) göre daha düşük bulunmuştur. Yumurta ağırlığı ile civciv ağırlığı arasında güçlü ve pozitif bir ilişki belirlenmiştir ($r=0,804$; $P<0,01$). Sonuç olarak, yumurtacı hat ve yumurta ağırlığının hem civciv gelişimi hem de kuluçka performansı üzerinde önemli rol oynadığı ve damızlık yumurtaların seçiminde yalnızca yumurta ağırlığının değil, genotipe özgü kuluçka performansının da dikkate alınması gerektiği sonucuna varılmıştır.

***Anahtar Kelimeler:*** *Yumurtacı hat, yumurta ağırlığı, kuluçka randımanı, civciv kalitesi, fertilite*

## Giriş

Kuluçkalık yumurtanın özellikleri, elde edilen civcivlerin kalite ve gelişim performansı ile kuluçka sonuçlarının önemli belirleyicilerinden biridir. Özellikle yumurta ağırlığı; civciv ağırlığı, yumurta ağırlık kaybı, embriyonik gelişim ve çıkım sonuçları ile yakından ilişkilidir. Bununla birlikte, optimum yumurta ağırlığı tüm genotipler için aynı olmayabilir.

Yumurta ağırlığının artması genellikle daha yüksek civciv ağırlığı ve daha uzun civciv elde edilmesini sağlarken, çok ağır veya uygun olmayan ağırlıktaki yumurtalarda kuluçka randımanının olumsuz etkilenebileceği bildirilmektedir. Bunun yanında genetik yapı; yumurta büyüklüğü, şekli, kabuk özellikleri, embriyonik gelişim ve civciv kalitesi gibi birçok özelliği etkileyebilmektedir.

Yumurtacı hatlarda genetik farklılıkların kuluçka özellikleriyle birlikte değerlendirilmesi, damızlık yumurta seçiminde daha doğru kriterlerin belirlenmesine katkı sağlayabilir. Bu çalışmada, dört farklı yumurtacı hattın ve üç farklı yumurta ağırlık sınıfının yumurta özellikleri, civciv kalite özellikleri ve kuluçka sonuçları üzerine etkilerinin belirlenmesi amaçlanmıştır.

## Materyal ve Yöntem

### *Hayvan materyali*

Araştırmada Ankara Tavukçuluk Araştırma Enstitüsünde yetiştirilen dört farklı yumurtacı hat kullanılmıştır. Bunlar iki kahverengi yumurtacı hat olan BAR-1 ve RIR-1 ile iki beyaz yumurtacı hat olan BLUE ve MAROON'dur.

Her genotipten 240 adet olmak üzere toplam 960 kuluçkalık yumurta araştırmaya dahil edilmiştir.

### *Yumurta ağırlık sınıfları*

Yumurtalar, her genotipin kendi ortalama yumurta ağırlığı dikkate alınarak üç sınıfa ayrılmıştır:

- **Hafif:** 54,75 g ortalama
- **Orta:** 58,77 g ortalama
- **Ağır:** 63,09 g ortalama

Genotip × ağırlık sınıfı düzeyindeki değerler değerlendirilirken Tablo 2'de verilen veriler esas alınmıştır. Buna göre RIR-1 hattında hafif, orta ve ağır yumurtaların ortalama ağırlıkları sırasıyla 56,85, 61,53 ve 65,78 g olarak belirlenmiştir.

### *İncelenen özellikler*

Yumurtalarda başlangıç ağırlığı, 18. gün ağırlığı, ağırlık kaybı, yumurta genişliği, yumurta uzunluğu ve şekil indeksi belirlenmiştir.

Çıkıştan sonra civcivlerin bireysel olarak canlı ağırlıkları ve uzunlukları ölçülmüş; civciv kalitesi değerlendirilmiştir. Ayrıca çıkım zamanı belirlenmiştir.

Kuluçka performansı kapsamında fertilite, fertil yumurtalardan çıkış gücü, kuluçka randımanı ile erken, orta ve geç dönem embriyonik ölümler değerlendirilmiştir.

### *İstatistiksel analiz*

Elde edilen veriler Microsoft Excel programında düzenlenmiş ve SPSS 26 paket programında analiz edilmiştir. Genotip, yumurta ağırlık sınıfı ve genotip × yumurta ağırlık sınıfı etkileşiminin etkileri değerlendirilmiştir. Ortalamalar arasındaki farklılıkların belirlenmesinde uygun çoklu karşılaştırma testi kullanılmış ve $P<0,05$ değeri istatistiksel önem sınırı olarak kabul edilmiştir.

## Bulgular ve Tartışma

### Yumurta özellikleri

Genotip, başlangıç yumurta ağırlığı, 18. gün yumurta ağırlığı, yumurta genişliği, yumurta uzunluğu, şekil indeksi ve ağırlık kaybı bakımından önemli farklılıklara neden olmuştur ($P<0,001$).

RIR-1 hattı 61,38 g ile en yüksek başlangıç yumurta ağırlığına sahip olurken, BLUE 60,65 g ile ikinci sırada yer almıştır. BAR-1 ve MAROON hatlarında başlangıç yumurta ağırlıkları sırasıyla 56,60 ve 56,84 g olarak belirlenmiştir.

Yumurta ağırlık sınıfının artmasıyla başlangıç yumurta ağırlığı, 18. gün yumurta ağırlığı, yumurta genişliği ve yumurta uzunluğu doğrusal olarak artmıştır. Hafif, orta ve ağır yumurtalarda başlangıç ağırlıkları sırasıyla 54,75, 58,77 ve 63,09 g olmuştur ($P<0,001$).

Buna karşılık yumurta ağırlık sınıfının şekil indeksi ve ağırlık kaybı üzerindeki etkisi önemli bulunmamıştır. Genotip × ağırlık sınıfı etkileşimi başlangıç yumurta ağırlığı, 18. gün ağırlığı, yumurta uzunluğu ve ağırlık kaybı bakımından önemli bulunmuştur ($P<0,001$).

Tablo 1. Genotip ve yumurta ağırlık sınıfına göre yumurta özellikleri

| *Grup* | *Başlangıç ağırlığı (g)* | *18. gün ağırlığı (g)* | *Genişlik (mm)* | *Uzunluk (mm)* | *Şekil indeksi (%)* | *Ağırlık kaybı (%)* |
|---|---|---|---|---|---|---|
| BAR-1 | 56,60$^{d}$ | 50,72$^{d}$ | 41,95$^{d}$ | 56,47$^{b}$ | 74,34$^{c}$ | 10,35$^{c}$ |
| BLUE | 60,65$^{b}$ | 53,39$^{b}$ | 43,01$^{b}$ | 57,12$^{a}$ | 75,33$^{ab}$ | 12,07$^{a}$ |
| MAROON | 56,84$^{c}$ | 50,80$^{c}$ | 42,22$^{c}$ | 56,23$^{b}$ | 75,15$^{b}$ | 10,89$^{b}$ |
| RIR-1 | 61,38$^{a}$ | 54,16$^{a}$ | 43,31$^{a}$ | 57,17$^{a}$ | 75,82$^{a}$ | 11,73$^{a}$ |
| Hafif | 54,75$^{c}$ | 48,62$^{c}$ | 41,61$^{c}$ | 55,63$^{c}$ | 74,85 | 11,39 |
| Orta | 58,77$^{b}$ | 52,24$^{b}$ | 42,61$^{b}$ | 56,65$^{b}$ | 75,27 | 11,04 |
| Ağır | 63,09$^{a}$ | 55,93$^{a}$ | 43,64$^{a}$ | 57,96$^{a}$ | 75,36 | 11,35 |

*Not: Aynı sütunda farklı üst simgelerle gösterilen ortalamalar arasındaki farklılıklar önemlidir ($P<0,05$).*

***Civciv özellikleri***

Genotip, civciv uzunluğu, civciv ağırlığı, civciv verimi, civciv kalitesi ve çıkım zamanı bakımından önemli farklılıklara neden olmuştur (P<0,001).

RIR-1 hattından elde edilen civcivler 44,02 g ile en yüksek canlı ağırlığa ve 17,28 cm ile en yüksek uzunluğa sahip olmuştur. Aynı hat 97,54 ile en yüksek civciv kalite skorunu göstermiştir.

Buna karşılık BLUE hattında civciv kalite skoru 94,04 ile en düşük düzeyde bulunmuştur. BAR-1 civcivlerinde çıkım zamanı 481,43 saat ile en kısa, BLUE hattında ise 491,59 saat ile en uzun olarak belirlenmiştir.

Yumurta ağırlığının artması civciv ağırlığı ve civciv uzunluğunu önemli ölçüde artırmıştır. Hafif, orta ve ağır yumurtalardan elde edilen civcivlerin ortalama ağırlıkları sırasıyla 38,65, 41,61 ve 44,05 g; civciv uzunlukları ise 16,88, 17,05 ve 17,25 cm olmuştur.

Genotip × yumurta ağırlık sınıfı etkileşimi civciv ağırlığı bakımından önemli bulunmuştur (P=0,004).

Tablo 2. Genotip ve yumurta ağırlık sınıfına göre civciv özellikleri

| *Grup* | *Civciv uzunluğu (cm)* | *Civciv ağırlığı (g)* | *Civciv verimi (%)* | *Civciv kalitesi* | *Çıkım zamanı (saat)* |
|---|---|---|---|---|---|
| BAR-1 | 16,83$^{c}$ | 40,65$^{bc}$ | 71,85$^{a}$ | 96,02$^{b}$ | 481,43$^{d}$ |
| BLUE | 17,11$^{b}$ | 41,14$^{b}$ | 67,79$^{c}$ | 94,04$^{c}$ | 491,59$^{a}$ |
| MAROON | 17,02$^{b}$ | 39,94$^{c}$ | 70,09$^{b}$ | 95,54$^{b}$ | 488,53$^{b}$ |
| RIR-1 | 17,28$^{a}$ | 44,02$^{a}$ | 71,87$^{a}$ | 97,54$^{a}$ | 485,23$^{c}$ |
| Hafif | 16,88$^{c}$ | 38,65$^{c}$ | 70,44$^{ab}$ | 96,18 | 486,41$^{ab}$ |
| Orta | 17,05$^{b}$ | 41,61$^{b}$ | 70,81$^{a}$ | 95,92 | 485,52$^{b}$ |
| Ağır | 17,25$^{a}$ | 44,05$^{a}$ | 69,96$^{b}$ | 95,26 | 488,15$^{a}$ |

***Kuluçka sonuçları***

Kuluçka sonuçları genotipler arasında önemli farklılıklar göstermiştir. BAR-1 hattında fertilite %97,99, fertil yumurtalardan çıkış gücü %92,19 ve kuluçka randımanı %90,36 ile en yüksek değerlerde bulunmuştur.

BLUE hattında fertilite %94,62, çıkış gücü %89,48 ve kuluçka randımanı %84,50 olarak belirlenmiştir. MAROON hattında bu değerler sırasıyla %96,12, %85,01 ve %81,79 olmuştur.

RIR-1 hattı ise %78,45 fertilite, %81,66 çıkış gücü ve %64,34 kuluçka randımanı ile tüm genotipler içerisinde en düşük kuluçka performansını göstermiştir.

Genotipin fertilite, fertil yumurtalardan çıkış gücü ve kuluçka randımanı üzerindeki etkisi sırasıyla P=0,001, P=0,009 ve P=0,001 düzeyinde önemli bulunmuştur.

Yumurta ağırlık sınıfı da fertilite ve kuluçka randımanını önemli düzeyde etkilemiştir. Hafif yumurtalarda fertilite %95,98 ve kuluçka randımanı %86,25 iken, ağır yumurtalarda bu değerler sırasıyla %87,88 ve %75,36 olarak belirlenmiştir. Bu sonuç, yumurta ağırlığının artmasının civciv gelişimini desteklemesine karşın kuluçka randımanı bakımından her zaman avantaj sağlamadığını göstermektedir.

Tablo 3. Genotip ve yumurta ağırlık sınıfına göre kuluçka sonuçları

| *Grup* | *Fertilite (%)* | *Fertil yumurtalardan çıkış (%)* | *Kuluçka randımanı (%)* | *Erken ölüm (%)* | *Orta dönem ölüm (%)* | *Geç ölüm (%)* |
|---|---|---|---|---|---|---|
| BAR-1 | 97,99$^{a}$ | 92,19$^{a}$ | 90,36$^{a}$ | 2,32$^{b}$ | 1,09 | 4,40 |
| BLUE | 94,62$^{a}$ | 89,48$^{ab}$ | 84,50$^{ab}$ | 3,15$^{b}$ | 0,00 | 7,37 |
| MAROON | 96,12$^{a}$ | 85,01$^{bc}$ | 81,79$^{b}$ | 6,68$^{ab}$ | 1,27 | 7,04 |
| RIR-1 | 78,45$^{b}$ | 81,66$^{c}$ | 64,34$^{c}$ | 9,61$^{a}$ | 1,66 | 7,07 |
| Hafif | 95,98$^{a}$ | 85,66 | 86,25$^{a}$ | 5,29 | 0,46 | 4,17 |
| Orta | 91,53$^{ab}$ | 86,24 | 79,13$^{b}$ | 4,55 | 1,66 | 7,55 |
| Ağır | 87,88$^{c}$ | 85,36 | 75,36$^{b}$ | 6,48 | 0,89 | 7,70 |

***Özellikler arasındaki ilişkiler***

Korelasyon analizinde başlangıç yumurta ağırlığı ile yumurta genişliği (r=0,831), yumurta uzunluğu (r=0,674), 18. gün yumurta ağırlığı (r=0,936) ve civciv ağırlığı (r=0,804) arasında güçlü ve pozitif ilişkiler belirlenmiştir (P<0,01).

Yumurta ağırlığı ile civciv verimi arasında negatif bir ilişki bulunmuştur (r=-0,102; P<0,05). Ayrıca yumurta ağırlık kaybı ile civciv ağırlığı (r=-0,229) ve civciv verimi (r=-0,536) arasında negatif ilişkiler belirlenmiştir (P<0,01).

Civciv ağırlığı ile civciv verimi arasında pozitif ilişki (r=0,508; P<0,01) bulunurken, civciv verimi ile çıkım zamanı arasında negatif ilişki (r=-0,434; P<0,01) belirlenmiştir. Civciv kalitesi ile çıkım zamanı arasındaki ilişki de negatif bulunmuştur (r=-0,193; P<0,01).

Bu sonuçlar, yumurta ağırlığının özellikle civciv ağırlığının önemli bir belirleyicisi olduğunu, ancak yüksek yumurta ağırlığının kuluçka başarısı açısından tek başına olumlu bir kriter olarak değerlendirilemeyeceğini göstermektedir.

**Genel Değerlendirme**

Çalışmada genotipler arasında belirgin farklılıklar ortaya çıkmıştır. RIR-1 hattı daha ağır yumurtalar üretmiş ve bu yumurtalardan daha ağır, daha uzun ve daha yüksek kalite skoruna sahip civcivler elde edilmiştir. Bununla birlikte, aynı hat fertilite ve kuluçka randımanı bakımından en düşük performansı göstermiştir.

BAR-1 hattı ise daha düşük yumurta ağırlığına rağmen en yüksek fertilite ve kuluçka randımanını sağlamıştır. Bu durum, yüksek yumurta ağırlığının kuluçka performansı için tek başına yeterli bir seçim kriteri olmadığını göstermektedir.

Yumurta ağırlık sınıfları değerlendirildiğinde, ağır yumurtalardan daha ağır ve uzun civcivler elde edilmiştir. Ancak ağır yumurtalarda kuluçka randımanının hafif yumurtalara göre daha düşük olması, damızlık yumurta seçiminde optimum ağırlık aralığının belirlenmesinin önemini ortaya koymaktadır.

**Sonuç ve Öneriler**

Bu çalışmada, yumurtacı hat ve yumurta ağırlığının yumurta özellikleri, civciv gelişimi ve kuluçka performansı üzerinde önemli etkilerinin bulunduğu belirlenmiştir.

RIR-1 hattı civciv ağırlığı, civciv uzunluğu ve civciv kalitesi bakımından öne çıkarken, BAR-1 hattı fertilite ve kuluçka randımanı bakımından daha başarılı olmuştur. Yumurta ağırlığının artması civciv ağırlığı ve uzunluğunu artırmış, ancak kuluçka randımanında azalmaya yol açmıştır.

Bu nedenle kuluçkalık yumurta seçiminde yalnızca yüksek yumurta ağırlığı hedeflenmemeli; **genotip, yumurta ağırlığı, fertilite ve kuluçka randımanı birlikte değerlendirilmelidir**. Özellikle farklı yumurtacı hatlar için optimum kuluçkalık yumurta ağırlığının ayrı ayrı belirlenmesi, hem kuluçka verimliliğinin hem de kaliteli civciv üretiminin artırılmasına katkı sağlayabilir.

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# The Structure of Poultry Production in Benin

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## Abstract

Poultry production is an important livestock activity in Benin in terms of animal protein supply, diversification of rural household incomes, and food security. The poultry sector in the country has a mixed structure in which low-input traditional production systems coexist with more intensive and commercially oriented production systems. This study aimed to evaluate the current structure of poultry production in Benin in terms of poultry population, production systems, indigenous genetic resources, farm size, feeding, animal health, biosecurity, and socioeconomic characteristics. The study was conducted as a review based on national and international scientific publications, official statistics, and institutional reports. A literature search was performed using Web of Science, Scopus, ScienceDirect, and Google Scholar focusing on studies related to poultry production in Benin, indigenous chickens, commercial broiler and layer production systems, biosecurity, and poultry diseases. Available evidence indicates that poultry production in Benin is largely based on traditional systems involving indigenous chickens. The chicken population was reported to reach approximately 19.049 million birds in 2024. Traditional systems are particularly important for rural households because of their low investment and input requirements; however, they are constrained by low productivity, inadequate feeding, disease risks, and insufficient biosecurity practices. In contrast, modern commercial poultry production has been developing, particularly in the egg sector, and offers greater production potential, although high feed costs, capital requirements, and disease risks remain important constraints. Indigenous chicken ecotypes in Benin represent valuable genetic resources because of their adaptation to local environmental conditions and resilience under low-input production systems. In conclusion, sustainable development of poultry production in Benin requires the conservation and improvement of indigenous genetic resources, strengthening of producer organizations, efficient utilization of local feed resources, wider implementation of vaccination and biosecurity measures, and development of commercial poultry production infrastructure.

***Keywords:*** *Benin, Poultry production, Indigenous chicken, Commercial poultry farming, Poultry production systems*

# Benin'de Tavukçuluğun Yapısı

## Özet

Tavukçuluk, Benin'de hayvansal protein üretimi, kırsal hane halklarının gelir kaynaklarının çeşitlendirilmesi ve gıda güvenliğinin sağlanması açısından önemli bir hayvansal üretim faaliyetidir. Ülkede tavukçuluk sektörü, düşük girdili geleneksel üretim sistemleri ile daha yoğun ve ticari nitelikteki modern üretim sistemlerinin bir arada bulunduğu karma bir yapı göstermektedir. Bu çalışmada, Benin'de tavukçuluğun mevcut yapısının; kanatlı varlığı, üretim sistemleri, yerli genetik kaynaklar, işletme büyüklükleri, besleme, hayvan sağlığı, biyogüvenlik ve sosyoekonomik özellikler açısından değerlendirilmesi amaçlanmıştır. Çalışma, ulusal ve uluslararası bilimsel yayınlar, resmi istatistikler ve kurumsal raporlar kullanılarak gerçekleştirilen bir derleme niteliğindedir. Literatür taraması Web of Science, Scopus, ScienceDirect, ve Google Scholar veri tabanlarında gerçekleştirilmiş ve Benin'de tavukçuluk, yerli tavuk, ticari etlik ve yumurtacı üretim sistemleri, biyogüvenlik ve kanatlı hastalıklarıyla ilgili çalışmalar değerlendirilmiştir. Mevcut veriler, Benin'deki tavukçuluğun büyük ölçüde yerli tavukların yetiştirildiği geleneksel üretim sistemlerine dayandığını göstermektedir. 2024 yılında tavuk varlığının yaklaşık 19,049 milyon başa ulaştığı bildirilmiştir. Geleneksel sistemler düşük yatırım ve girdi gereksinimleri nedeniyle özellikle kırsal haneler açısından önemli olmakla birlikte düşük verim, yetersiz besleme, hastalıklar ve biyogüvenlik eksiklikleri ile sınırlanmaktadır. Buna karşılık modern ticari tavukçuluk, özellikle yumurta üretiminde gelişmekte olup daha yüksek üretim kapasitesine sahip olmakla birlikte yem maliyetleri, sermaye gereksinimi ve hastalık riskleri gibi sorunlarla karşı karşıyadır. Benin'de bulunan yerli tavuk ekotipleri, yerel çevre koşullarına uyumları ve düşük girdili üretim sistemlerindeki dayanıklılıkları nedeniyle önemli genetik kaynaklar olarak değerlendirilmektedir. Sonuç olarak, Benin tavukçuluğunun sürdürülebilir gelişimi için yerli genetik kaynakların korunması ve ıslahı, üretici örgütlenmesinin güçlendirilmesi, yem kaynaklarının etkin kullanılması, aşılama ve biyogüvenlik uygulamalarının yaygınlaştırılması ve ticari üretim altyapısının geliştirilmesi gerekmektedir.

***Anahtar Kelimeler:*** *Benin, tavukçuluk, Yerli tavuk, Ticari tavukçuluk, Kanatlı üretim sistemleri*

## Giriş

Tavukçuluk, dünya genelinde kısa üretim süresi, yüksek biyolojik etkinliği, nispeten düşük başlangıç sermayesi gerektirmesi ve et ile yumurta gibi yüksek biyolojik değere sahip ürünler sağlaması nedeniyle hayvansal üretimin önemli dallarından biridir (Hoffmann, 2005). Özellikle gelişmekte olan ülkelerde tavuk yetiştiriciliği yalnızca hayvansal protein üretimi açısından değil, küçük ölçekli üreticilerin gelir elde etmesi, kırsal istihdamın desteklenmesi ve hane halklarının ekonomik dayanıklılığının artırılması bakımından da önem taşımaktadır (FAO, 2020).

Benin'de de tavukçuluk, hayvansal üretim sistemlerinin önemli bir bölümünü oluşturmaktadır. Ülkede tavuklar, özellikle kırsal alanlarda küçük aile sürüleri şeklinde yetiştirilmekte ve çoğunlukla yerli genetik kaynaklardan yararlanılmaktadır (Rodríguez vd., 2011). Geleneksel tavukçuluk, düşük girdi ve düşük çıktı karakteri taşıyan bir üretim sistemi olmakla birlikte kırsal hanelerin geçim kaynakları içerisinde önemli bir yere sahiptir. Benin'de yapılan bir çalışmada, yerli tavuk yetiştiriciliğinde en yaygın üretim sisteminin serbest dolaşımlı ekstansif sistem olduğu ve işletmelerin yaklaşık %70'inin bu sistemi kullandığı bildirilmiştir (Camus vd., 2020; Dessie vd., 2012; Rodríguez vd., 2011).

Benin'de tavukçuluğun diğer önemli özelliği, geleneksel üretim sistemlerinin yanında ticari ve daha yoğun üretim yapan işletmelerin de bulunmasıdır. Bu iki üretim sistemi; kullanılan genetik materyal, yemleme, barındırma, veteriner hizmetleri, biyogüvenlik, sürü büyüklüğü ve üretim amacı bakımından önemli farklılıklar göstermektedir (FAO, 2015). Yerli tavukların ekonomik ve biyolojik önemi yalnızca mevcut üretim performanslarıyla sınırlı değildir. Bu hayvanlar, yerel çevre koşullarına adaptasyon, düşük kaliteli yemlerden yararlanabilme ve çeşitli çevresel streslere dayanıklılık gibi özellikleri nedeniyle gelecekteki ıslah programları için önemli genetik kaynaklar olarak değerlendirilmektedir (Dessie vd., 2012). Benin'de yerli tavukların korunmasının, bu genetik çeşitliliğin gelecekte kullanılabilmesi açısından önemli olduğu bildirilmektedir (Rodríguez vd., 2011).

Bu çalışmanın amacı, Benin'de tavukçuluğun mevcut yapısını genel bir çerçevede değerlendirmek; geleneksel ve modern üretim sistemlerini, yerli tavuk genetik kaynaklarını, işletme özelliklerini, üretim sorunlarını, hayvan sağlığı ve biyogüvenlik uygulamalarını ve sektörün geliştirilme olanaklarını ortaya koymaktır.

## Materyal ve Yöntem

Bu çalışma, Benin'de tavukçuluğun mevcut yapısını, üretim sistemlerini, kanatlı genetik kaynaklarını, işletme özelliklerini, üretim performansını ve sektörün karşı karşıya olduğu temel sorunları ortaya koymak amacıyla gerçekleştirilen bir derleme çalışmasıdır. Çalışmada deneysel bir uygulama yapılmamış; ulusal ve uluslararası bilimsel yayınlar, resmi istatistikler ve konuya ilişkin kurumsal raporlardan yararlanılmıştır.

### *Çalışma alanı*

Benin, Batı Afrika'da Gine Körfezi boyunca y alan ve yaklaşık 114.763 km² yüzölçümüne sahip bir ülkedir. Ülkenin 2025 yılına ait tahminler yaklaşık 14,8 milyon kişidir. iyi huylu; Batıda Togo, Kuzeyde Burkina Faso ve Nijer, doğuda ise Nijerya ile sınır komşusudur. Ülkenin güney sınırı Atlas Okyanusu'nun Gine Körfezi'ne uzanan kıyı kesimi boyunca uzanmaktadır. Porto-Novo ülkesinin başkenti iken, Cotonou geniş ekonomik ve ticari merkez konumundadır. Bu durumda doğru çözümü seçebileceksiniz ancak daha sonra da kullanabileceksiniz. Ancak ne olacağını bilmek istiyorsanız bunu başarabilirsiniz. Benin, Sudan ve Sudan-Sahel bölgesindeki ülkelerin çoğunda ağırlıklı olarak tarım kullanılıyor. Son olarak ekonomiler farklı şekillerde kullanılıyor ama sanat aynı değil ama aynı zamanda sanat sanatının güzel bir örneği. katkı bulunmaktadır. Ancak, yağ pompasını yerinden kaldırmak için birkaç dakika ayırmak gerekebilir.

### *Literatür Taraması*

Literatür taraması Web of Science, Scopus, ScienceDirect ve Google Scholar veri tabanlarında gerçekleştirilmiştir. Taramalarda "Benin poultry", "poultry production in Benin", "indigenous chicken Benin", "commercial poultry Benin", "broiler production Benin", "layer production Benin", "poultry farming systems Benin", "chicken genetic resources Benin", "poultry disease Benin" ve "poultry biosecurity Benin" anahtar kelimeleri tek başına veya çeşitli kombinasyonlarda kullanılmıştır. Literatür seçiminde özellikle Benin'de gerçekleştirilen saha araştırmaları, üretim sistemlerini inceleyen çalışmalar, yerli tavukların genetik ve fenotipik özelliklerini değerlendiren araştırmalar ve hayvan sağlığı ile biyogüvenliğe ilişkin çalışmalar dikkate alınmıştır.

### *Veri Kaynakları*

Benin'in kanatlı varlığı ve üretim yapısına ilişkin verilerin değerlendirilmesinde Benin'in resmi tarım ve hayvancılık istatistiklerinden yararlanılmıştır. Bunun yanında Food and Agriculture Organization (FAO), FAOSTAT, World Organisation for Animal Health (WOAH) ve ilgili uluslararası kuruluşların veri ve raporları değerlendirilmiştir. Bilimsel yayınlardan elde edilen bilgiler; üretim sistemi, sürü büyüklüğü, genetik kaynaklar, yumurta ve et üretimi, büyüme ve üreme performansı, hastalıklar, biyogüvenlik uygulamaları ve üreticilerin karşılaştıkları sorunlar açısından sınıflandırılmıştır.




Resim 1. Benin'in coğrafi haritası
Kaynak: UN Geospatial

***Verilerin Değerlendirilmesi***

Elde edilen bilgiler öncelikle geleneksel ve modern tavukçuluk sistemleri olmak üzere iki ana üretim grubu altında değerlendirilmiştir. Geleneksel üretim sistemi; düşük girdili, küçük ölçekli, aile emeğine dayalı ve çoğunlukla serbest dolaşımlı yetiştiricilik özellikleri bakımından incelenmiştir. Modern üretim sistemi ise ticari etlik ve yumurtacı işletmeler, yoğun yetiştirme, ticari yem kullanımı, aşılama, veteriner hizmetleri ve biyogüvenlik uygulamaları açısından değerlendirilmiştir.

Yerli tavuklara ilişkin çalışmalar; ekotip, üretim amacı, büyüme, yumurta verimi, yumurta özellikleri, fertilite, kuluçka randımanı ve çevresel adaptasyon özellikleri bakımından karşılaştırılmıştır. Sektörün temel sorunları; üretim, ekonomik yapı, yem kaynakları, hayvan sağlığı, biyogüvenlik, pazarlama ve üretici örgütlenmesi başlıkları altında değerlendirilmiştir. Farklı araştırmalarda kullanılan örneklem büyüklüğü, üretim koşulları ve araştırma yöntemlerindeki farklılıklar nedeniyle çalışma kapsamında meta-analiz gerçekleştirilmemiş; bulgular niteliksel ve karşılaştırmalı derleme yaklaşımı ile değerlendirilmiştir.

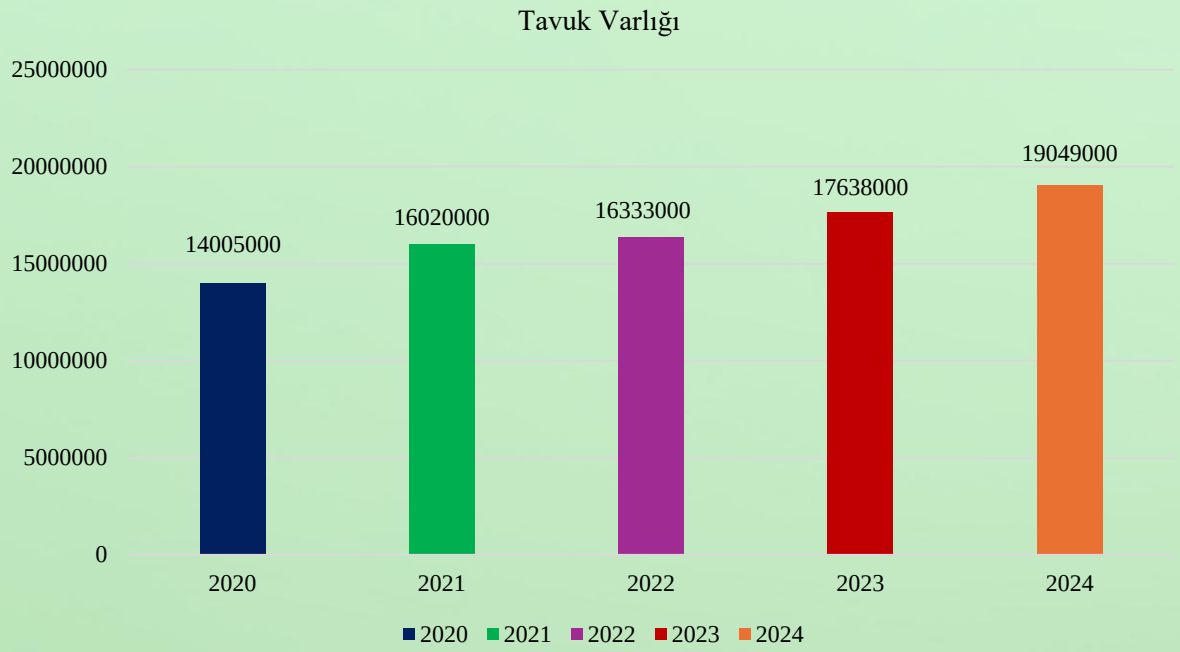
***Çalışmanın Kapsamı***

Çalışma, Benin Cumhuriyeti sınırları içerisindeki tavukçuluk faaliyetleri ile sınırlandırılmıştır. Değerlendirmede özellikle yerli tavuk yetiştiriciliği ile ticari etlik ve yumurtacı tavuk üretimine odaklanılmıştır. Bununla birlikte tavukçuluk sektörünü doğrudan etkileyen yem, hayvan sağlığı, biyogüvenlik, genetik kaynaklar, pazarlama ve sosyoekonomik faktörler de kapsam içerisinde değerlendirilmiştir.

**Benin'de Tavukçuluğun Genel Yapısı**

Benin'de tavukçuluk, geleneksel ve modern üretim sistemlerinin birlikte bulunduğu heterojen bir yapı göstermektedir. Sektörün niceliksel olarak büyük bölümünü küçük ölçekli geleneksel üretim oluşturmaktadır. FAOSTAT 2024 yılı verilerine göre Benin'de tavuk varlığı yaklaşık 19,049 milyon başa ulaşmıştır. Bu değer, yerli tavukların ülkenin kanatlı sektöründeki ağırlığını açık biçimde göstermektedir. Ayrıca yerli tavuk sayısının bir önceki yıla göre yaklaşık %7,4 arttığı bildirilmiştir.

Bu yapı, tavukçuluğun Benin'de yalnızca ticari işletmeler üzerinden değerlendirilmemesi gerektiğini göstermektedir. Küçük aile işletmeleri, kırsal geçim ekonomisi ve yerli genetik kaynaklar sektörün temel unsurlarıdır. Bununla birlikte kentleşme, nüfus artışı ve hayvansal protein talebindeki yükseliş, ticari tavukçuluğun gelişmesini teşvik etmektedir. Böylece ülkede geleneksel ve modern üretim sistemleri aynı üretim zinciri içerisinde birlikte varlığını sürdürmektedir.




Şekil 1: Benin'de Yıllara Göre Tavuk Varlığı (2019-2024)
Kaynak : FAOSTAT, 2026

**Geleneksel Tavukçuluk Sistemi**

Benin tavukçuluğunun temelini geleneksel veya serbest dolaşımlı üretim sistemi oluşturmaktadır (Faustin vd., 2010). Bu sistemde tavuklar çoğunlukla küçük aile sürülerinde yetiştirilmekte ve işletme yönetimi profesyonel ticari üretimden ziyade hane halkının ihtiyaçlarına göre şekillenmektedir (Houngbo vd., 2026). Benin'de 269 üreticiyle gerçekleştirilen bir araştırmada üç temel üretim sistemi belirlenmiş; en yaygın sistemin serbest dolaşımlı ekstansif sistem (%70) olduğu bildirilmiştir. Bunun yanında arka bahçe ekstansif ve yarı entansif sistemlerin de kullanıldığı belirlenmiştir (Camus vd., 2020).

Geleneksel sistemlerde yemleme çoğunlukla doğal kaynaklardan sağlanan yem materyallerine, evsel artıkların kullanımına ve sınırlı miktarda ek yeme dayanmaktadır. Bu nedenle üretim maliyetleri düşük olmakla birlikte hayvanların genetik üretim potansiyelinin tam olarak kullanılabilmesi çoğu zaman mümkün olmamaktadır (Houngbo vd., 2026; Rodríguez vd., 2011). Bu sistemin en önemli avantajlarından biri, yerli tavukların çevresel koşullara yüksek düzeyde uyum göstermesidir. Yerli tavuklar düşük girdili koşullarda yaşayabilmekte, doğal yem kaynaklarından yararlanabilmekte ve farklı çevresel streslere karşı dayanıklılık gösterebilmektedir (Dessie vd., 2012; Faustin vd., 2010; Houngbo vd., 2026; Rodríguez vd., 2011).

**Benin'de Yarı Yoğun ve Yoğun Kanatlı Hayvan Yetiştiriciliği Sistemleri**

Benin'de yarı yoğun ve yoğun kanatlı hayvan yetiştiriciliği sistemleri hâlen sınırlı düzeyde gelişmiş olup, ağırlıklı olarak ülkenin güneyindeki kentsel ve kentsel çevre bölgelerde yoğunlaşmaktadır (Batonon-Alavo vd., 2015; Onibon & Sodégla, 2005). Yarı yoğun sistem, çoğunlukla "yarı modern" olarak tanımlanmakta ve geleneksel yetiştiricilik ile ticari modern yetiştiricilik arasında bir geçiş modeli oluşturmaktadır. Bu sistem; barınma koşullarının iyileştirilmesi, ek yemleme uygulamalarının kullanılması, veterinerlik hizmetlerinden daha düzenli yararlanılması ve bazı durumlarda yerel ırkların ithal genotip veya hatlarla melezlenmesi ile karakterize edilmektedir (Kulla vd., 2021). Ayrıca, geleneksel ekstansif kanatlı yetiştiriciliğine kıyasla sermaye yatırımlarının ve satın alınan girdilerin kullanımında belirgin bir artış söz konusudur. Geleneksel sistem ise ağırlıklı olarak öz tüketim amacıyla yürütülen ekstansif yetiştiricilik biçimidir (Kulla vd., 2021; Onibon & Sodégla, 2005). Bununla birlikte, yarı modern sistem üretici sayısı bakımından oldukça sınırlıdır. Kulla vd. (2021), 2015 yılında Benin'de yalnızca yaklaşık 650 modern üretici bulunduğunu, buna karşılık 500.000'den fazla kırsal hanenin geleneksel

"poulet bicyclette" olarak adlandırılan yerel tavuk yetiştiriciliğiyle uğraştığını bildirmiştir. Yarı modern işletmeler, geleneksel işletmelere kıyasla genellikle daha büyük sürülere sahip olmakta ve ulusal düzeyde gerçekleştirilen bir çalışmada işletme başına ortalama yaklaşık 500 kanatlı hayvan bulunduğu bildirilmektedir; ancak bu işletmeler yatırım düzeyi ve teknik kapasite açısından hâlen orta düzeyde kalmaktadır (Kulla vd., 2021). Buna karşılık, yoğun veya modern yetiştiricilik sistemi, özellikle etlik piliç ve yumurta üretiminde, büyük ölçüde geliştirilmiş veya ithal genetik hatların kullanımına dayanmaktadır (Batonon-Alavo vd., 2015; Kulla vd., 2021). Bu sistemde ticari yemlerin, uzmanlaşmış kümeslerin ve sağlık koruma uygulamalarının kullanımı daha yaygındır. Ticari bir üretim yapısına sahip olmasına rağmen, bu segment Benin'de hâlen nispeten sınırlı bir gelişme düzeyindedir (Kinkpe vd., 2026; Kulla vd., 2021). Mevcut tahminlere göre modern üretim, toplam kanatlı üretiminin yaklaşık %8'ini, yarı modern sistem ise %2'sinden daha azını oluştururken, geleneksel sistem sektörde açık ara baskınlığını sürdürmektedir (Kulla et al., 2021; Kinkpe et al., 2026).

**Yerli Tavuk Genetik Kaynakları**

Benin, farklı çevresel ve sosyoekonomik koşullara adapte olmuş çeşitli yerli tavuk popülasyonlarına sahiptir. Yerli tavukların genetik çeşitliliği, ülkenin önemli hayvansal genetik kaynaklarından biri olarak değerlendirilmektedir (Camus vd., 2020; Houngbo vd., 2026; Rodríguez vd., 2011). Yerli tavukların önemli özelliklerinden biri, düşük girdili üretim sistemlerine uyum sağlamalarıdır. Bu nedenle yalnızca mevcut üretim kapasitesi açısından değil, gelecekte değişen iklim ve üretim koşullarına uyum sağlayabilecek genetik özellikler bakımından da önemlidirler (Dessie vd., 2012; Faustin vd., 2010).

Benin'de yapılan son araştırmalardan birinde Koklo yaya, Holli ve Fulani ekotiplerinin üreme performansları karşılaştırılmıştır. Aynı yoğun yetiştirme koşullarında gerçekleştirilen çalışmada Fulani ekotipinin daha ağır yumurtalara ve daha yüksek kuluçka randımanına sahip olduğu, Koklo yaya ekotipinin ise daha yüksek yumurtlama oranı gösterdiği belirlenmiştir. Fertilite oranları ekotipler arasında yaklaşık %74 düzeyinde bulunmuştur (Houngbo vd., 2026).

Bu sonuçlar, yerli tavuk ekotiplerinin aynı üretim koşullarında dahi farklı avantajlara sahip olabileceğini göstermektedir. Dolayısıyla tek bir genotipi bütün üretim amaçları için kullanmak yerine, belirli üretim hedeflerine uygun ekotiplerin seçilmesi daha etkili bir ıslah yaklaşımı olabilir.



Resim 2. Koklo Yaya tavuğu



Resim 3. Holli tavuğu



Resim 4. Fulani ekotiplerinin

**Modern Ticari Tavukçuluk**

Benin'de modern ticari tavukçuluk, özellikle yumurta ve et üretimine yönelik işletmelerden oluşmaktadır. Geleneksel üretimden farklı olarak bu işletmelerde ticari genetik materyal, dengeli yem, kontrollü barınak, aşılama, veteriner hizmetleri ve biyogüvenlik uygulamalarına daha fazla önem verilmektedir. Modern üretim sistemleri daha yüksek üretim kapasitesine sahip olmakla birlikte üretim maliyetleri geleneksel sistemlere göre daha yüksektir. Özellikle yem maliyetleri, civciv ve damızlık materyali, ilaç ve veteriner hizmetleri ile enerji giderleri ticari işletmelerin ekonomik sürdürülebilirliğini etkileyen önemli unsurlardır. Etlik üretimde hızlı canlı ağırlık artışı ve kısa üretim süresi temel avantajlar iken, yumurtacı işletmelerde düzenli yumurta üretimi ve sürü devamlılığı temel ekonomik göstergeler arasında yer almaktadır (Camus vd., 2020; Dessie vd., 2012; Houngbo vd., 2026).

**İşletme Büyüklüğü ve Üretici Profili**

Benin'de tavuk işletmeleri sürü büyüklüğü, üretim amacı ve profesyonellik düzeyi bakımından önemli farklılıklar göstermektedir(Batonon-Alavo vd., 2015; Onidje vd., 2024). Kuzey Benin'deki kentsel ve kentsel çevre bölgelerde yapılan araştırmalar, küçük aile işletmelerinden daha büyük ve ticari nitelikteki işletmelere kadar farklı üretici gruplarının bulunduğunu ortaya koymuştur (Onidje vd., 2024). Geleneksel işletmelerde sürü büyüklüğünün düşük olması, üretimin temel olarak aile ihtiyaçları ve küçük ölçekli satışlar için yapılmasıyla ilişkilidir (Houndjo vd., 2018; Onidje vd., 2024). Buna karşılık ticari işletmelerde üretim daha çok pazar talebine yönelik olarak planlanmaktadır. Bu farklılıklar, Benin'de tavukçuluk politikalarının bütün işletmeleri tek bir üretim modeli içerisinde değerlendirmemesi gerektiğini göstermektedir (Kulla vd., 2021; Onidje vd., 2024).

**Tavukçuluğun Sosyoekonomik Önemi**

Tavukçuluk, Benin'de özellikle kırsal haneler için önemli bir gelir ve geçim kaynağıdır. Küçük sürüler, gerektiğinde satılarak nakde dönüştürülebilen bir ekonomik varlık işlevi görmektedir (Houndjo vd., 2018; Houngbo vd., 2026).

Yerli tavukların ekonomik değerinin yalnızca et ve yumurta üretiminden oluşmadığı; kültürel değer, hane güvenliği ve ekonomik tasarruf fonksiyonu da taşıdığı bildirilmektedir. Benin'deki hane halkları üzerinde gerçekleştirilen araştırmalar, yerli tavukların çeşitli ekonomik ve kültürel özelliklerinin üretici tercihlerini etkilediğini göstermiştir. Ayrıca yerli tavuk etinin belirli tüketici gruplarında daha yüksek fiyatlarla satılabilmesi, yerli genetik kaynakların korunmasına yönelik ekonomik bir teşvik oluşturmaktadır (Rodríguez vd., 2011). Bir modelleme çalışmasında, yerli tavukların korunmasında tüketicilerin yerli tavuk etine yönelik özel pazar talebinin önemli olduğu gösterilmiştir (Faustin vd., 2010).

**Yemleme ve Besleme**

Benin'de tavukçuluk üretiminin önemli sınırlılıklarından biri beslemedir. Geleneksel sistemlerde hayvanların yem gereksinimleri çoğunlukla serbest dolaşım sırasında elde edilen doğal yem kaynakları ile karşılanmakta olup, bu rejimin özellikle protein bakımından yetersiz kaldığı bildirilmiştir (Edenakpo vd., 2020). Bu uygulama üretim maliyetlerini azaltmakla birlikte özellikle büyüme döneminde enerji, protein, mineral ve vitamin gereksinimlerinin tam olarak karşılanamamasına neden olabilmektedir. Sürü büyüklüğü arttıkça doğal yem kaynaklarının yetersiz kalması da önemli bir sorun hâline gelmektedir. Benin'de yerli ve egzotik tavuk üretiminin ekonomik sonuçlarını inceleyen modelleme çalışmasında, sürü büyüklüğü arttıkça yem kaynaklarının önemli bir sınırlayıcı faktör hâline geldiği bildirilmiştir (Rodríguez vd., 2011). Ticari işletmelerde ise dengeli yem kullanımı daha yaygın olmakla birlikte yem maliyetleri üretim ekonomisinin en önemli unsurlarından biridir. Ayrıca Benin'deki yumurta tavukçuluğu değer zinciri üzerine yapılan bir çalışma, hammadde fiyatlarındaki istikrarsızlık ve yerel yem hammaddelerinin sınırlı bulunabilirliğinin sektörün kârlılığını zayıflattığını göstermiştir (Batonon-Alavo vd., 2015). Bu nedenle yerel yem hammaddelerinin değerlendirilmesi ve bölgesel koşullara uygun yem formülasyonlarının geliştirilmesi önem taşımaktadır.

**Hayvan Sağlığı ve Hastalıklar**

Hastalıklar, Benin'de hem geleneksel hem de ticari tavukçuluğun sürdürülebilirliğini etkileyen temel sorunlardan biridir (Camus vd., 2020; Kulla vd., 2021). Özellikle Newcastle hastalığı (ND) ve avian influenza (AI), küçük ölçekli kanatlı işletmeleri açısından önemli riskler oluşturmaktadır (Faustin vd., 2010). Kuzey Benin'de 118 küçük üretici üzerinde gerçekleştirilen 2024 tarihli bir çalışmada, üretim sistemleri, biyogüvenlik uygulamaları ve bu hastalıkların görülme durumu arasındaki ilişkiler incelenmiştir. Çalışma, kanatlı yetiştiriciliğinin bölgede geçim ve gıda güvenliği açısından önemli olduğunu, ancak Newcastle hastalığı ve kuş

gribi gibi hastalıkların önemli tehdit oluşturduğunu göstermiştir (Onidje vd., 2024). Geleneksel işletmelerde veteriner hizmetlerine erişimin sınırlı olması, aşılamanın düzenli yapılamaması ve farklı sürüler arasındaki hayvan hareketlerinin kontrol edilememesi hastalıkların yayılma riskini artırmaktadır. Benin'de gerçekleştirilen araştırmalarda hastalık ve mortalite, üreticilerin karşılaştığı başlıca sorunlar arasında gösterilmiştir (Rodríguez vd., 2011).

**Biyogüvenlik**

Biyogüvenlik, özellikle yoğun üretim sistemlerinin sürdürülebilirliği açısından temel bir yönetim aracıdır. Bununla birlikte küçük ölçekli geleneksel işletmelerde biyogüvenlik uygulamalarının sınırlı olması önemli bir risk oluşturmaktadır (FAO, 2015). Başlıca biyogüvenlik eksiklikleri arasında yeni hayvanların karantinadan sürüye katılması ve ölü hayvanların uygun olmayan şekilde uzaklaştırılması (Allanonto vd., 2023), farklı sürüler arasında hayvan hareketleri, yabani hayvanlarla temas, günlük kontrol eksikliği ve yem ve su döngüsü kontaminasyonu (Onidje vd., 2024). Bu nedenle biyogüvenlik uygulamalarının yalnızca büyük ticari işletmelerde değil, küçük aile işletmelerinde de yaygınlaştırılması gerekmektedir.

**Üretimin Önündeki Temel Sorunlar**

Benin'de tavukçuluğun karşı karşıya olduğu sorunlar üretim sistemine göre farklılık göstermektedir.

- Geleneksel işletmeler açısından başlıca sorunlar:
- Hastalıklar ve mortalite,
- Yetersiz esleme,
- Düşük üretim performansı,
- Biyogüvenlik eksiklikleri,
- Yetersiz barınak koşulları,
- Hırsızlık
- Yırtıcı hayvanlar,
- Krediye erişim güçlüğü,
- Teknik bilgi eksikliği ve
- Sınırlı yatırım olanaklarıdır.

269 üreticiyle yapılan araştırmada hırsızlık, banka kredisine erişim, eğitim eksikliği, sınırlı yatırım ve hastalıkların yerli tavuk üretiminin başlıca sorunları olduğu bildirilmiştir. Ticari işletmelerde ise yem maliyetleri, kaliteli civciv ve damızlık materyaline erişim, sermaye gereksinimi, hastalık riski ve pazar koşulları daha belirgin sorunlardır (Camus vd., 2020).

**Benin Tavukçuluğunun Geliştirilme Olanakları**

Benin tavukçuluğunun sürdürülebilir biçimde geliştirilmesi için geleneksel ve modern üretim sistemlerinin birbirinin alternatifi olarak değil, birbirini tamamlayan üretim modelleri olarak ele alınması gerekmektedir.

İlk olarak yerli genetik kaynakların korunması ve karakterizasyonu önem taşımaktadır. Koklo yaya, Holli ve Fulani gibi yerli ekotiplerin farklı üretim özellikleri göstermesi, ıslah programlarında genotip × üretim amacı ilişkisinin dikkate alınması gerektiğini göstermektedir.

İkinci olarak, üretici örgütlenmesinin güçlendirilmesi gerekmektedir. Kooperatifler aracılığıyla yem, aşı, veteriner hizmetleri ve genetik materyalin daha uygun koşullarda temin edilmesi küçük üreticilerin rekabet gücünü artırabilir.

Üçüncü olarak, aşılama ve biyogüvenlik programlarının yaygınlaştırılması gerekmektedir. Özellikle Newcastle hastalığına karşı düzenli aşılama programları ve üretici eğitimleri hastalık kaynaklı ekonomik kayıpların azaltılmasına katkı sağlayabilir.

Dördüncü olarak, yerel yem kaynaklarının daha etkin kullanılması ve düşük maliyetli dengeli rasyonların geliştirilmesi gerekmektedir.

Son olarak, modern tavukçuluğun gelişimi yalnızca işletme sayısının artırılması şeklinde değerlendirilmemelidir. Kuluçkahaneler, damızlık işletmeleri, yem üretimi, veteriner hizmetleri, kesimhaneler, soğuk zincir ve pazarlama kanallarını kapsayan bütünleşik bir tavukçuluk değer zincirinin geliştirilmesi gerekmektedir.

**Sonuç**

Benin'de tavukçuluk, geleneksel ve modern üretim sistemlerinin bir arada bulunduğu çok katmanlı bir yapıya sahiptir. Sektörün temelini, özellikle kırsal hanelerde yetiştirilen yerli tavuklar oluşturmaktadır. 2024 yılında tavuk

varlığının yaklaşık 19,049 milyon başa ulaşması, bu üretim sisteminin ülkedeki önemini açık biçimde göstermektedir. Geleneksel tavukçuluk düşük sermaye ve girdi gereksinimi nedeniyle kırsal haneler açısından önemli avantajlara sahip olmakla birlikte, düşük verim, hastalıklar, yetersiz besleme ve biyogüvenlik eksiklikleri nedeniyle önemli sınırlamalar taşımaktadır. Modern ticari tavukçuluk ise daha yüksek üretim potansiyeli sunmakta, ancak yem maliyetleri, sermaye ihtiyacı ve hastalık riskleri nedeniyle daha yüksek işletme riski taşımaktadır. Benin'in yerli tavuk genetik kaynakları, düşük girdili üretim koşullarına uyumları nedeniyle önemli bir genetik rezerv oluşturmaktadır. Güncel araştırmalar, farklı yerli ekotiplerin yumurta verimi, yumurta özellikleri, kuluçka performansı ve civciv kalitesi bakımından farklı avantajlara sahip olduğunu göstermektedir.

Sonuç olarak Benin tavukçuluğunun gelecekteki gelişimi; yerli genetik kaynakların korunması ve ıslahı, küçük üreticilerin örgütlenmesi, yem kaynaklarının etkin kullanılması, aşılama ve biyogüvenliğin geliştirilmesi, veteriner hizmetlerine erişimin artırılması ve modern üretim değer zincirinin güçlendirilmesine bağlıdır. Yerli genetik kaynakların korunması ile verim artırmaya yönelik ıslah çalışmalarının birlikte yürütülmesi, Benin'in hem gıda güvenliğine hem de kırsal kalkınmasına önemli katkılar sağlayabilir.

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# olorimeter and total volatile nitrogen base assessments of *Kebabs* produced from meat of broiler fed cloves and garlic as additives in diet

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## Abstract

The poultry industry has recently geared towards the incorporation of natural additives into diets in order to improve the quality of meat products. One-day-old 308 Ross broiler chicks (n= 200; r = 5, 10 birds/r) were randomly allotted to four diets: T1 (Basal Diet (BD), T2 (BD + 0.25 % garlic), T3 (BD + 0.15 % cloves) and T4 (BD + 0.25 % garlic+ 0.15 % cloves (GC). On 42 day, 3birds/replicate were randomly selected, euthanized, defeathered and meat excised from primal cuts and ground. Minced meat (each treatment) was used to produced chicken kebabs. Colorometric assessment (L* a* b*) and Total Volatile Basic Nitrogen (TVBN (mg/100g)) on days 0 and 7 were assessed following standard procedure. No significant differences (P>0.05) in colour parameters, L*(1.13-1.85), a* (1.04-1.32) and b* (1.50-1.71). On day 7, TVBN (7.48) in kebabs from cloves fed broiler was lower (P>0.05) than 7.94 (garlic), 7.65 (GC) and 8.15 (BD). Garlic and cloves inclusion in broiler diet improved the quality of the meat which is reflected in the shelf stability of chicken kebabs.

***Keywords:*** *Antioxidants, natural additives, kebabs, protein oxidation, shelf stability*

## Introduction

Poultry meat particularly broiler meat is highly perishable due to its high moisture and nutrient contents, which provide favorable conditions of biochemical deterioration and microbial proliferation during processing and storage (Katiyo et al., 2020). This is usually addressed by incorporating additives in thediet the aim of enhancing the overall production efficiency as well as producing safe and quality meat and meat products (Al-Baadani et al., 2023). However, the recent campaign and consumer interest in natural products has made producers to shift their interest towards the use of natural plant-based additives in broiler diets as alternatives to synthetic additives. Garlic (*Allium sativum*) and cloves (*Syzygium aromaticum*) contains some bioactive compounds particularly allicin and eugenol respectively which are known for their antimicrobial and antioxidant potentials (Tareq et al., 2018). Although, there has been scientific reports on the use of garlic and cloves as additives in broiler production, however, there is limited information on the quality attributes of ready- to- meat products produced from meat of broiler chickens fed these additives. Therefore, this study evaluated the colourimetric characteristics and Total Volatile Basic Nitrogen (TVBN) qualities of chicken kebabs produced from meat of broiler chickens fed garlic and cloves either singly or in combination in their diets.

## Materials and Methods

*Test ingredients*

Garlic and cloves were procured from a market within the University of Ibadan environs and processed into powder using standard procedures and kept in air tight container until further use.

*Experimental Birds and Management*

One-day-old 308 Ross broiler chicks procured from a reputable hatchery in Ibadan were randomly allotted (n=200, r- 5, 10 birds/r) to four diets: T1= Basal diet (BD) only; T2= BD + 0.25 % Garlic; T3= BD + 0.15 % cloves, T4= BD+0.25 % Garlic + 0.15 % Cloves. The chicks were reared on a deep litter at the Poultry Unit, Teaching and Research Farm, University of Ibadan, fed *ad libitum* with water and feed for a period of six weeks. All vaccination schedules and routine management were carried out effectively.

*Production of chicken kebabs*

On day 42, three birds from each replicate were randomly selected euthanized, defeathered and meat was excised carefully from all primal cuts and ground. Minced meat was then mixed with other *kebabs* ingredients and then make into kebabs following Ekhlas et al. (2023) procedures.

*Data collected*

*Colorimeter assessment of kebabs*

Colorimeter assessment of chicken kebab was carried out in accordance to Sedaroglu et al. (2018) procedures using a colorimeter Model LS 173

*Total Volatile Basic Nitrogen (TVBN)*

Protein degradation in kebabs during storage was assessed using the titrimetric procedure (Bekhit et al., 2021) and expressed as TVBN/mg/100g.

## Results

*Colorimeter assessment of kebabs*

The results showed that there were no significant differences ($p > 0.05$) in all the colour parameters (Table 1) measured. The values were 1.13-1.85 (L* Lightness), 1.04-1.29 (a* redness) and 1.50-1.71 (b* yellowness).

*Total Volatile Basic Nitrogen in kebabs during storage*

On the first day of storage (zero), the TVBN (5.21 mg/100g) in kebabs produced from meat of broilers fed cloves and those fed combined additives (5.19 mg/100g) were significantly lower ($P<0.05$) while on the 7th day of storage, kebabs produced from meat of broiler chickens fed only cloves exhibited significantly ($P<0.05$) the lowest (7.48 mg/100g) TVBN value.

**Table 1:** Colourimetric assessment of chicken *kebabs* produced from meat of broiler supplemented with garlic, cloves and their combination in the diet

| *Parameters* | *Control* | *Garlic* | *Cloves* | *GC* | *SEM* | *P-Value* |
|---|---|---|---|---|---|---|
| L | 1.45 | 1.85 | 1.13 | 1.58 | 0.38 | 0.62 |
| a* | 1.29 | 1.04 | 1.08 | 1.32 | 0.13 | 0.38 |
| b* | 1.71 | 1.50 | 1.51 | 1.68 | 0.21 | 0.83 |

Means with same superscripts on the same row are not significantly different ($p > 0.05$)
L* = lightness; a* = redness; b* = yellowness; GC = garlic and cloves combination

**Table 2:** Interaction effect of dietary inclusion of garlic and cloves in broiler diets and storage duration on Total Volatile Basic Nitrogen of chicken *kebabs*

| *Storage Day* | *Treatment* | *TVBN (mg/100g)* | *P-value* |
|---|---|---|---|
| 7 | Control | $8.15^{a}$ | <.0001 |
| 7 | Garlic | $7.94^{b}$ | <.0001 |
| 7 | GC | $7.65^{c}$ | <.0001 |
| 7 | Cloves | $7.48^{d}$ | <.0001 |
| 0 | Control | $7.03^{e}$ | <.0001 |
| 0 | Garlic | $6.83^{f}$ | <.0001 |
| 0 | Cloves | $5.21^{g}$ | <.0001 |
| 0 | GC | $5.19^{g}$ | <.0001 |

$^{a,b,c,d}$ Means with different superscripts on the same column are significantly different ($p < 0.05$)
GC: garlic and cloves combination

## Discussion

Colour is a critical physical property shaping consumer acceptance and directly impact their purchasing decisions (Kyakma et al., 2023). The insignificant in the colour parameters of al the *kebabs* implied that the inclusion of garlic and cloves either singly or in combination broiler diet will not impart any undesirable colour on the raw meat that will subsequently affect the appearance of the chicken *kebabs* produced from it.

The concentration of volatile basic nitrogenous compounds serves as an important indicator of protein breakdown and microbial spoilage in meat products (Zhang et al., 2022). This implied that addition of cloves and garlic to the broiler diet probably enhanced the muscle tissue by slowing down the rate of protein degradation which translates into lower formation of volatile amines as reflected in the kebabs produced from such meat. These lower TVB-N values suggests better freshness and stability of the product which could be attributed to reduced microbial activity which is a major factor in protein degradation (Bekhit et al., 2021) in these *kebabs*. This may be linked to the antimicrobial potentials of cloves and garlic (Batiha et al., 2020; El Faqer et al., 2022) which inhibit microbial growth thus reduces the microbial activities in the kebabs thereby preventing protein degradation and subsequently inhibiting the formation of volatile nitrogenous bases (Ahmed et al., 2022).

## Conclusion

Garlic and cloves either singly or in combination in broiler diets enhanced the quality of the raw meat which is further reflected in the improved shelf stability of kebabs produced from these meats. This highlights the efficacy of natural feed additives as effective strategy in reducing dependence on synthetic additives thus prioritizing food safety and improving meat products quality.

## Declarations

### *Ethical Approval Certificate*

The experimental procedures of this study were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC) approved on 31/07/2025 with number UI-ACUREC/090-0525/29.

### *Author contribution statement*

Olayemi Awodoyin: Project administration, supervision, conceptualization, investigation methodology, writing the final draft, reviewing and editing.

Blessing Adekunle: Data collection, formal analysis, and writing the original draft.

Peter Ojolo: Data collection and formal analysis.

### *Conflict of Interest*

The authors declare no conflict of interest in the study.

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# Foods Produced Using Nanotechnology Methods and Their Halal Evaluation

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## Abstract

In the global food industry, the demand for halal-certified products is rapidly increasing, driven by religious sensitivities as well as the search for healthy and ethical food. In Islamic law, food consumption must not only avoid prohibitions (haram) but also meet the highest standards of safety, quality, and hygiene according to the "Halalan-Tayyiban" (halal and pure) principle. In this context, nanotechnology offers revolutionary advantages in enhancing the nutritional value of foods, nano-encapsulation of bioactive components, and active/smart packaging systems. However, the potential cytotoxicity risks of nanomaterials and the origin of stabilizers used in production (such as pork-derived proteins) create serious ethical dilemmas and health concerns regarding the "tayyib" (pure/pure) element in halal assessment. Furthermore, sensor-based technologies and molecular detection methods play a critical role in the post-production inspection of food, food supplements, and packaging produced using nanotechnology methods. Olfactometric devices such as "e-nose" (electronic nose) can rapidly distinguish the characteristic volatile organic compounds of haram ingredients like lard. Furthermore, gold nanoparticle-based biosensors and nano-bioprops can detect even fragmented DNA sequences in processed foods with high sensitivity. While alternative sources are gaining importance in determining the halal status of animal-derived intermediates such as gelatin, advanced techniques such as nano UPLC-MS/MS can detect "marker peptides," enabling 100% accuracy in distinguishing between pork and beef. In conclusion, nanotechnology enhances efficiency in halal food production, while tools such as e-noses and nanobiosensors ensure the prevention of food fraud and monitoring compliance with religious requirements. This study aims to evaluate the halal suitability of raw materials used in the production of food, food supplements, and packaging materials, as well as those produced using nanotechnological methods, and to assess molecular detection methods.

***Keywords:*** *Nanotechnology, Halal Food, Halalan-Tayyiban, Gelatin, Biosensors*

## Nanoteknolojik Yöntemlerle Üretilen Gıdalar ve Helal Uygunluk Açısından Değerlendirilmesi

## Özet

Küresel gıda endüstrisinde helal sertifikalı ürünlere olan talep, dini hassasiyetlerin yanı sıra sağlıklı ve etik gıda arayışıyla hızla artmaktadır. İslam Hukuku'nda gıda tüketimi, sadece yasaklardan (haram) kaçınmayı değil, aynı zamanda "Halalan-Tayyiban" (helal ve temiz) prensibi gereği en yüksek güvenlik, kalite ve hijyen standartlarını da karşılamalıdır. Bu bağlamda nanoteknoloji, gıdaların besin değerini artırma, biyoaktif bileşenlerin nano-enkapsülasyonu ve aktif/akıllı paketleme sistemleri ile devrim niteliğinde avantajlar sunmaktadır. Ancak nanomalzemelerin potansiyel sitotoksisite riskleri ve üretimde kullanılan stabilizatörlerin (domuz kaynaklı proteinler gibi) menşei, helallik değerlendirmesinde "tayyib" unsuru açısından ciddi etik ikilemler ve sağlık endişeleri yaratmaktadır. Ayrıca nanoteknolojik yöntemlerle üretilen gıda, gıda takviyeleri ve ambalajların üretim sonrası denetiminde ise sensörlü teknolojiler ve moleküler tespit yöntemleri kritik rol oynamaktadır. "E-nose" (elektronik burun) gibi olfaktometrik cihazlar, domuz yağı gibi haram bileşenlerin karakteristik uçucu organik bileşenlerini hızla ayırt edebilmektedir. Ayrıca, altın nanopartikül tabanlı biyosensörler ve nano-biyoproplar, işlenmiş gıdalarda parçalanmış DNA dizilerini bile yüksek hassasiyetle saptayabilmektedir. Jelatin gibi hayvansal kaynaklı ara bileşenlerin helalliğinin saptanmasında, alternatif kaynaklar önem kazanırken; nano UPLC-MS/MS gibi ileri tekniklerle "marker peptid" tespiti yapılarak domuz ve sığır ayrımı %100 doğrulukla gerçekleştirilebilmektedir. Sonuç olarak nanoteknoloji, helal gıda üretiminde verimliliği artırırken; e-nose ve nanobiyosensörler gibi araçlar, gıda sahteciliğinin önlenmesinde ve dini gerekliliklerin denetiminde bir güvence sağlamaktadır. Bu çalışmada, nanoteknolojik yöntemlerle üretilen gıdalar, gıda takviyeleri, ambalaj maddeleri üretiminde kullanılan hammaddelerin helal açıdan uygunluğu ve moleküler tespit yöntemlerinin değerlendirilmesi amaçlanmıştır.

***Anahtar Kelimeler:*** *Nanoteknoloji, Helal Gıda, Helal ve Temiz, Jelatin, Biyosensörler*

## Giriş

Küresel gıda pazarında helal gıda tüketimi, sadece Müslüman nüfus için değil, aynı zamanda güvenli ve kaliteli gıda arayan geniş kitleler için de kritik bir öneme sahiptir. . İslam dinine göre, Kuran-ı Kerim'de geçen Arapça "helal" kelimesi İngilizceye "izin verilebilir" olarak çevrilebilmektedir (Mortas ve ark 2022;Usman ve ark 2024). İslam hukukunda gıda tüketimi "Halalan-Tayyiban" (helal ve temiz/iyi) prensibi üzerine kuruludur; bu da ürünün hem dini kurallara uygun olmasını hem de sağlık, hijyen ve etik standartları en üst düzeyde karşılamasını gerektirir (Bujang ve ark 2020; Hanapi ve Khairuldin, 2017). Bakara, Maide, En'am ve Nahl surelerinde yer alan domuz, kan, Allah'tan başkası adına kesilen, boğulan ve yırtıcı hayvanlar tarafından parçalanan hayvanlardan elde edilen ürünlerin tüketilmesi haram kılınmakta, bireylerin beden ve ruh sağlığının korunması amaçlanmaktadır (Karaman, 2012; Özgen ve Hazarhun 2019). Bu kapsamda gıda katkı maddelerinin de helal olabilmesi için uygun olmayan hammaddelerden elde edilmemesi ve sağlığa zararlı olmaması gerekmektedir Gıda işlemede nanoteknoloji gıda kalitesini (lezzet, renk ve doku) artırmayı, katkı maddelerinin ve besin maddelerinin kontrollü salınımını sağlamak için nanoemülsiyonlar ve nanoenkapsülasyon kullanarak gıda stabilitesini iyileştirmeyi, daha verimli gıda işleme için enzim immobilizasyonu, antimikrobiyal amaçlı aktif ve akıllı ambalajlar oluşturmayı amaçlamaktadır (Momin ve Joshi 2015; Patel ve ark 2021) Nanoteknolojinin kullanımı büyük avantajlar sunsa da, bu teknolojilerin helal sertifikasyon süreçlerindeki yeri ve potansiyel riskleri yeni tartışmaları beraberinde getirmektedir (Aghwan ve ark 2024; Basir ve ark. 2020). Bu çalışmada, nanoteknolojinin helal gıda üretimindeki rolü, kromatografik ve moleküler tespit yöntemleri ve özellikle jelatin gibi kritik bileşenlerin analizindeki teknolojik ilerlemeler incelenmektedir.

## İnançlar Ekseninde Gıdaların Sınıflandırılması

Gıdaların sınıflandırılması, dini açıdan manevi bir temizlik aracı kabul edilmektedir. İslam dininde domuz, alkol ve usulüne uygun kesilmemiş hayvanlar "haram" kabul edilirken; Yahudilik'te (Kosher) domuz yasağının yanı sıra et ve sütün ayrılması gibi ek kurallar mevcuttur **(Azmi ve ark 2024)**. Hinduizm'de sığır eti yasağı, Budizm'de ise genel bir vejetaryen beslenme eğilimi hakim durumdadır **(Uddin ve ark 2021)**. Bu dini çeşitlilik, gıdaların üretim süreçlerinde menşei tayininin teknolojik olarak yapılmasını zorunlu kılmaktadır.

## Nanoteknolojik Yöntemlerle Üretilen Gıda ve Ambalajlarda Kullanılan Hammadde ve Yardımcı Maddeler

Hayvansal bağ dokudan elde edilen jelatin, kaynağı en çok tartışılan ara bileşendir. Balık ve sığır kemikleri şu anda helal jelatinin başlıca kaynağı olarak yaygın olarak kullanılmaktadır. Helal alternatif olarak deve derisinden veya balık derisinden jelatin üretimi üzerine çalışmalar bulunmaktadır (Ahmed ve ark 2020; Syariena ve Cheman, 2009). Karmaşık gıda matrislerinde domuz ve sığır jelatinini ayırt etmek için nanoUPLC-MS/MS gibi tekniklerle "marker peptid" tespiti yapılmakta ve bu yöntemler %100'e yakın doğruluk sağlamaktadır (Rohman ve ark 2025; Yilmaz ve ark, 2013).

Gıda ambalajı için uygun helal biyomalzemeler arasında nişastadan elde edilen biyoplastikler, selüloz bazlı malzemeler, karides ve yengeç kabuklarından elde edilen kitosan ve bambu lifleri yer almaktadır. Bu malzemeler, biyolojik olarak parçalanabilir ve geri dönüştürülebilir oldukları için geleneksel gıda ambalajlarına göre çevre dostu, sürdürülebilir ve helal alternatifler sunmaktadır. (Mohd Hatta ve ark 2023)

## Gıda Üretiminde Nanoteknolojinin Avantaj ve Dezavantajları

Nanoenkapsülasyon yöntemiyle vitamin ve minerallerin etkinliğinin korunması, nanoemülsiyonlar ile sindirilebilirliğin artırılması ve akıllı paketleme ile gıda tazeliğinin izlenmesi (Hammed, 2018; Khoshdouni Farahani, 2021) önemli avantajlar sağlarken, nanopartiküllerin insan hücreleri üzerindeki potansiyel toksisite etkileri ve üretimde kullanılan yardımcı maddelerin (örneğin domuz kaynaklı stabilizatörler) helal durumunun belirsizliği en büyük dezavantajlara neden olmaktadır (Aghwan ve ark 2024).

Silika dioksit ($SiO_2$) (E551), magnezyum oksit (MgO) (E530) ve titanyum dioksit ($TiO_2$) (E171) gibi yaygın olarak kullanılan inorganik oksit kimyasalları (geleneksel veya nano parçacık boyutunda), birçok ülkede topaklanmayı önleyici madde, gıda aroması taşıyıcısı ve gıda renklendirici katkı maddesi olarak yönetmeliklerle izin verilmektedir. Bununla birlikte, $TiO_2$ nanopartiküllerinin kullanımının, hayvanlarda akciğer iltihabına, fibrozise, epitel hiperplazisine ve tümör oluşumuna neden olduğunu ve yeterince yüksek dozda veya önemli miktarda $TiO_2$ nanopartikülüne maruz kalma süresi nedeniyle akciğer yüküne yol açtığını bildirilmiştir (Bujang ve ark 2020)

Athinarayanan ve ark. (2014) tarafından yapılan çalışmada gıdalarda topaklanmayı önleyici madde olarak kullanılan silikaya maruz kalmanın insan akciğer hücrelerinde sitotoksik olduğu tesit edilmiştir. Mahler ve ark. (2012), toksik olmayan polistiren nanopartiküllere akut maruz kalmanın demir taşınmasını bozduğunu ve kronik maruz kalmanın bağırsak villuslarında (kaust) yeniden yapılanmaya neden olduğunu gözlemlemiştir.

**Helal Gıda Tespitinde Kullanılan Yöntemler**

Gıdalardaki helal olmayan bileşenlerin tespit edilmesinde geleneksel yöntemler düşük maliyetleri nedeniyle uzun süredir kullanılmaktadır. Bununla birlikte numune hazırlama süreçlerinin uzun sürmesi gibi kısıtlamalar, biyoteknolojik gelişmeler ile hızlı ve etkili yeni teknikler geliştirilmesine yol açmıştır (Zia ve ark. 2020). Kromatografik, spektroskopik ve immünolojik testler gibi gelişmiş tekniklerin yanı sıra, biyosensörler olarak bilinen DNA tabanlı yöntemler, Fourier dönüşümlü kızılötesi spektroskopisi (FTIR), Polimeraz zincir reaksiyonu (PCR), gaz kromatografisi, yüksek performanslı sıvı kromatorafisi, diferansiyel tarama kalorimetrisi (DSC) , elektronik burun (E-nose), biyosensörler kullanılmaktadır (Kua ve ark 2022; Tao vd. 2022; Wu vd. 2022)

***Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR)***

Fourier dönüşümlü kızılötesi spektroskopisi (FTIR), katı, sıvı veya gaz halindeki maddelerin absorpsiyon, emisyon ve fotokondüktivitesinin kızılötesi spektrumu ile helal gıda kontaminasyonunu tespit etmek için kullanılan modern bir yöntemdir (Candoğan ve ark. 2021). Numune hazırlama aşamalarının daha az olması, yüksek hassasiyeti, çözücülerin sınırlı kullanımı ile bir parmak izi tekniği olarak kullanılmaktadır (Domingos ve ark. 2012; Usman ve ark. 2023). FT-IR spektrumları ile yapılan çalışmalarda, domuz jelatin tipinin sığır jelatin tipine kıyasla daha keskin piklere sahip olduğu tespit edilmiştir (Farahani, 2021)

***Polimeraz Zincir Reaksiyonu (PCR)***

Polimeraz zincir reaksiyonu (PCR), tek bir veya istenen sayıda DNA dizisini birden fazla sıra üzerinden çoğaltarak binlerce ve milyonlarca kopya üreten, hızlı sonuçlar veren ve hassas moleküler bir işlemdir (Erwanto ve ark., 2018; Zhu ve ark, 2020; Alkay 2022). Kang ve ark. (2018), domuz jelatinin tespiti üzerine yaptıkları çalışmada sonuçların güvenilir bir tanımlama sağladığı, Sultana ve ark., (2020) tarafından yapılan çalışmada sığır, domuz ve balık jelatinlerine, domuz türü jelatin karıştırıldığında saptama sınırının, 0.005 ng/µL olduğu belirtilmiştir.

***Enstrümental Yöntemler***

Gaz kromatografisi-kütle spektrometrisi (GC-MS), helal olmayan bileşenlerin varlığını tespit etmek için gıdalarda bulunan yağ asidi kompozisyonun tespiti amacıyla kullanılmaktadır. Yağ asitlerinden türetilen yağ asidi metil esterlerinin tutunma süresini ve pik alanları değerlendirilerek tağşiş tespit edebilmektedir (Uddin ve ark, 2021). Yapılan bir çalışmada domuz yağı ve sığır yağındaki yağ asidi içeriği GC-MS kullanarak tespit edilmiştir. Sığır yağındaki doymuş yağ asitlerinin tekli doymamış yağ asitlerine oranının (SFA: MUFA) (3.6) domuz yağına göre çok daha yüksek olduğu, domuz ve yaban domuzu köftelerinde bu oran sırasıyla 1.06 ve 1.2 olarak kaydedilmiştir. Bu, SFA ve MUFA oranının gıdadaki et türünü belirlemek için kullanılabileceğini göstermiştir (Ahda ve ark. 2021; Kua ve ark 2022).

Yüksek Performanslı Sıvı Kromatografisi (HPLC) , termal olarak kararsız, aşırı polar ve yüksek moleküler kütleye sahip gıda sistemlerindeki bileşenlerin kaynağını tespit etmek için de uygulanabilmektedir. Bu yöntem birçok avantajı sebebiyle helal doğrulama için gıdalarda yasaklanmış kontaminasyonu veya helal olmayan katkı maddelerini tespit etmek için büyük ilgi görmektedir (Verma ve Pant, 2021; Usman ve ark 2023). Yılmaz ve ark. (2025) tarafından yapılan çalışmada LC-MS/MS-TQ ile jelatin tür tayinine yönelik bir metot geliştirilerek, validasyon çalışmalarında tespit limiti (LOD) %0.012 olarak saptanmıştır. Yöntemin doğruluk, hassasiyet ve güvenirliğinin %100 olduğu belirlenmiştir. Bu metot ile, yurtiçi menşeili 8 adet ve yurtdışı menşeili 17 adet gıda takviyesinde jelatin tür tayini yapılmıştır. Türkiye menşeili ürünlerin etiketlerinde sığır jelatini olduğu ifade edilmesine rağmen bir üründe domuz jelatini tespit edilirken, bir tanesinde ise jelatinin türü tespit edilememiştir. Yurtdışı menşeili 8 adet gıda takviyesinde etiketlerinde belirtilmemesine rağmen domuz jelatini bulunduğu tespit edilmiştir.

Diferansiyel tarama kalorimetrisi (DSC), ısı değişimlerini tespit ederek fiziksel veya kimyasal özelliklerdeki değişiklikleri izleyen, İşleme ve depolama sırasında proteinlerin, karbonhidratların, yağların, alkolün, su içeriğinin ve gıda ambalajlarının fiziksel davranışını analiz etmek için kullanılan termo-analitik bir tekniktir. (Karim ve Muhamad, 2018). Bir numunenin ve referans malzemenin aynı oranda ısıtıldığında sıcaklıklarını artırmak için meydana gelen ısı değişim miktarı arasındaki karşılaştırmaya dayanmaktadır (Nur Azira ve Amin, 2016) Gerçek ve hileli palmiye yağının DSC soğutma profilleri incelendiğinde -26°C ve 34.5°C'de iki küçük tepe noktası ortaya çıkmıştır. 34.5°C'de, kakao yağındaki domuz yağı konsantrasyonu %3'ten %30'a çıktıkça, DSC analizinin ısıtma termogramında küçük bir tepe noktası komşu büyük tepe noktasıyla örtüştüğü, soğutma termogramının, küçük bir tepe noktasının -36°C'den - 41.5°C'ye kaydığı tespit edilmiştir (Azir ve ark. 2017; Kua ve ark 2022).

Gıdalarda domuz yağı gibi bileşenlerin tespiti için "Elektronik Burun" (E-nose) sistemleri geliştirilmiştir. Arduino tabanlı ve MQ gaz sensörleri içeren bu cihazlar, domuz yağının karakteristik kokusunu (uçucu bileşenler) PCA analiziyle saptayabilmektedir (Khadafi ve Putra, 2020). Altın nanopartiküller (AuNPs) kullanılarak geliştirilen optik sensörler, domuz DNA'sı varlığında renk değiştirerek hızlı ve yerinde analiz imkanı sunmaktadır (Abd Hamid ve ark. 2014; Farahani ve Farahani, 2021).

## Sonuç

Nanoteknoloji, helal gıda üretiminde verimliliği ve kaliteyi artıran bir güç olsa da, "Helal ve Temiz" prensibi gereği insan sağlığına etkileri ve etik boyutu titizlikle denetlenmelidir. E-nose gibi cihazlar ve nanoteknoloji tabanlı biyosensörler, geleneksel laboratuvar yöntemlerine göre daha hızlı, ucuz ve güvenilir çözümler sunmaktadır. Gelecekte, helal sertifikasyon süreçlerinin bu dijital ve nanoteknolojik araçlarla entegre edilmesi, küresel helal gıda güvenliğini sarsılmaz bir zemine oturtacağı düşünülmektedir.

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# Anthocyanins as a Natural Feed Additive for Methane Mitigation in Ruminant Nutrition

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## Abstract

Ruminant animal enteric methane emmissions contribute significantly to the accumulation of greenhouse gas and also represent a great loss of dietary energy. Natural feed additives that can reduce methane emissions without any negative effect on animal performance have been the focus of recent researches to enhance the sustainability of livestock production. Anthocyanins are naturally occuring flavonoid pigments richly available in purple corn, grape pomace, purple rice, black cane and other plants. They have antioxidant, antimicrobial, and rumen-modulating characteristics making them a potential option for enteric methane emissions mitigation. Anthocyanins have been shown to boost fermentation efficiency, decrease methanogenic archaea, interfere with microbial ecology of the rumen, and reroute hydrogen use toward propionate synthesis. This review examine the function of anthocyanins as a natural methane mitigation agents in ruminant nutrition. Their mode of action, efffects on rumen microbiota and fermentation characteristics, effects on animal performance, and potential contributions to sustainable ruminant animal production are all given a careful emphasis. Limitations and future research directions are also addressed.

***Keywords:*** *Methane mitigation, anthocyanins, rumen fermentation, ruminants, methane emissions*

## Introduction

The ruminant industry contributes significantly to global food security by transforming fibrous feed supplies unfit for human consumption in to quality meat and milk. However, ruminant production systems are the main generators of anthropogenic greenhouse gas emissions, especially methane which has a global warming potential roughly 28 times larger than carbon dioxide over a 100-year period (Legg, 2021). According to Moss et al. (2000), enteric methane produced during fermentation represents an energy loss of 2-12% of gross dietary energy intake and accounts for 30-40% of global agricultural methane emissions. Methane is produced in the rumen naturally from microbial fermentation. Rumen bacteria ferment carbohydrates during feed digestion to produce hydrogen ($H_2$), carbon dioxide ($CO_2$), and volatile fatty acids (VFAs). By using carbon dioxide and hydrogen to create methane, methanogenic archaea keep the partial pressure of hydrogen in the rumen low (Moss et al., 2000; Dijkstra et al,. 2005). This process lowers feed efficiency and significantly contributes to environmental contamination, yet being necessary for ruminal homeostasis. Methane emissions from livestock production have drawn a lot of attention because they generate approximately 14.5% of global anthropogenic greenhouse gas emissions, with enteric fermentation accounting for the highest share (Gerber et al., 2013). In addition to its negative effects on the environment, methane production is a major inefficiency in ruminant production systems since the energy wasted as a methane cannot be used for growth, lactation, or reproduction (Johnson & Johnson, 1995). As a result, all efforts towards lowering enteric methane emissions are now essential to sustainable agricultural and climate-smart ruminant production. Several methods of methane emission prevention have been evaluated which includes; nitrate feeding, ionophores, dietary lipid supplementation, probiotics, essential oils, and plant secondary metabolites (Beauchemin et al., 2008; Gerber et al., 2013; Vasta et al., 2019). Phytochemicals have drawn more attention among these strategies because they are naturally occuring substances that can both enhance animal performance and lessen environmental effects (Patra & Saxena, 2011). Anthocyanins are among the water-soluble flavonoid pigments, these pigments are responsible for the red, blue, and purple coloration in many vegetables, forage plants, fruits, and cereals (He & Giusti, 2010). Purple corn, purple rice, black cane, purple sweet potatoes, grape pomace and purple neem leaves are common feed sources that are rich in anthocyanins (Hosoda et al., 2009; Taethaisong et al., 2022). Anthocyanins have a strong antioxidant, antibacterial, anti-inflammatory and immunomodulatory functions in addition to their function as natural pigments (Han et al., 2006; Aboonabi & Singh, 2015). Recent findings showed that anthocynin rich feed have the ability to enhance rumen fermentation, modulate rumen microbiota, improve antioxidant status, enhance nutrient digestibility, and boost animal productivity (Tian et al., 2019; Tian et al., 2021; Suong et al., 2022). A study using a purple corn anthocyanin supplementation in goats reveal an altered ruminal fermentation functions and overall rumen microbial composition (Tian et al., 2021). Similar study by Taethaisong et al. (2022) reported an improvements in rumen fermentation efficiency, growth performance, and plasma antioxidant activity in goats supplemented with anthocyanin-rich purple neem foliage. In addition, polyphenol-rich feed resources containing anthocyanins, such as grape pomace, have been shown to reduce methane emissions in dairy cattle (Moate et al., 2014). Despite growing interest in anthocyanin-rich feed resources, most available studies have focused on their

antioxidant properties, animal health benefits, and product quality improvements (Tian & Lu, 2022). Comparatively fewer studies have comprehensively examined their role as methane mitigation agents and the specific mechanisms through which anthocyanins influence methanogenesis. Furthermore, available findings remain fragmented across different animal species, feed resources, and production systems. Therefore, there remains a need to synthesize current knowledge regarding the potential of anthocyanins as natural methane mitigation agents in ruminant nutrition. This paper reviews the sources, biological properties, and mechanisms through which anthocyanins influence rumen fermentation and methane production. Particular attention is given to their effects on rumen microbial ecology, hydrogen metabolism, animal performance, and environmental sustainability.

**Anthocyanins: Sources and Biological Characteristics**

Anthocyanins belong to the flavonoid family and are glycosylated derivatives of anthocyanidins. More than 600 anthocyanin compounds have been identified, with cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin being the most common forms found in nature (He & Giusti, 2010). Anthocyanins are abundant in numerous feed resources used in ruminant production, including: Purple corn (Zea mays L.), Purple corn Stover silage, Purple rice silage, Black cane silage, Purple neem foliage, Grape seed and grape pomace, Berry by-products, Purple sweet potato. Hosoda et al. (2009) reported that anthocyanin-rich corn silage retained considerable anthocyanin stability during ensiling, suggesting its suitability as a practical feed resource. Likewise, Suong et al. (2022) demonstrated that anthocyanin-rich black cane silage can be successfully incorporated into goat diets while improving rumen fermentation and animal performance. Anthocyanins possess strong free radical scavenging capacity and are recognized as potent natural antioxidants (Brewer, 2011). According to Tian and Lu (2022), anthocyanins can enhance antioxidant defenses, improve immune responses, and reduce oxidative stress in ruminants. These biological properties may indirectly contribute to improved rumen function and methane mitigation.

**Anthocyanin-Rich Feed Resources**

**Purple Corn:** Purple corn is one of the most extensively studied anthocyanin-rich feed resources. Tian et al. (2019) reported that feeding anthocyanin-rich purple corn silage improved nutrient utilization, rumen fermentation, plasma antioxidant capacity, and mammary gland gene expression in dairy goats. Furthermore, Tian et al. (2019) observed improvements in productivity and blood superoxide dismutase concentrations in lactating cows receiving purple corn silage. Purple corn anthocyanins have also been shown to improve ruminal fermentation and modify rumen microbial populations in goats (Tian et al., 2021). **Black Cane Silage**: Black cane silage contains high concentrations of anthocyanins and has received increasing attention as a functional feed resource. Suong et al. (2022) demonstrated improvements in growth performance, blood biochemical indices, rumen bacterial communities, and carcass characteristics in goats fed anthocyanin-rich black cane silage. **Purple Neem Foliage:** Taethaisong et al. (2022) reported that consumption of purple neem foliage rich in anthocyanins improved rumen fermentation, growth performance, and plasma antioxidant activity in goats.

**Anthocyanins and Polyphenols in Ruminant Nutrition**

Anthocyanins belong to the broader group of plant polyphenols, which have long been recognized for their biological activities in animal systems (Acosta-Estrada et al., 2014). Polyphenols can modulate rumen fermentation through direct interactions with microbial populations and indirect effects on nutrient metabolism (Vasta et al., 2019). Recent evidence suggests that anthocyanins may exert stronger antioxidant activities than vitamins C and E because of their unique molecular structure and high free radical scavenging capacity (Han et al., 2006). According to Brewer (2011), anthocyanins are among the most potent naturally occurring antioxidants identified in plant materials. In ruminants, anthocyanins have been associated with improved antioxidant status, reduced inflammatory responses, enhanced immune function, and improved production performance (Tian & Lu, 2022). These effects are particularly important during periods of metabolic stress such as early lactation, heat stress, and disease challenge (Xiao et al., 2021).

**Methane Production in the Rumen**

Methane production is a normal component of ruminal fermentation and is primarily carried out by methanogenic archaea. These microorganisms utilize hydrogen generated during carbohydrate fermentation to reduce carbon dioxide and produce methane (Moss et al., 2000). The primary methanogenesis reaction can be summarized as:

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

Methane production represents a loss of dietary energy and decreases feed conversion efficiency (Dijkstra et al., 2005). Factors influencing methane emissions include diet composition, rumen pH, forage-to-concentrate ratio, feed digestibility, microbial populations, and animal species (Moss et al., 2000; Vasta et al., 2019). Consequently, nutritional interventions capable of reducing hydrogen availability or suppressing methanogenic populations have become important research priorities.

**Mechanisms through Which Anthocyanins Mitigate Methane Emissions**

**1. Modification of Rumen Fermentation Patterns**: Anthocyanins influence rumen fermentation by altering microbial activity and fermentation end-products. Tian et al. (2021) observed significant changes in ruminal fermentation characteristics following supplementation with purple corn anthocyanins. According to Tian and Lu (2022), anthocyanins may promote propionate production while reducing acetate-to-propionate ratios. Because propionate formation consumes metabolic hydrogen, this pathway competes directly with methanogenesis. Increased propionate production therefore decreases hydrogen availability for methane-producing archaea, ultimately reducing methane emissions.

**2. Suppression of Methanogenic Archaea:** Plant polyphenols are known to possess antimicrobial activity against selected rumen microorganisms (Vasta et al., 2019). Anthocyanins appear capable of inhibiting methanogenic populations through disruption of microbial cell membranes and interference with enzymatic activity. Tian and Lu (2022) proposed that anthocyanins may decrease populations of methanogens while simultaneously promoting beneficial fermentative bacteria. Such microbial shifts reduce methane production without negatively affecting rumen function.

**3. Alteration of Rumen Microbial Ecology:** Recent advances in sequencing technologies have revealed significant effects of anthocyanins on rumen microbial communities. Tian et al. (2021) demonstrated that purple corn anthocyanin supplementation altered rumen microbiota composition and improved fermentation efficiency in goats. Similarly, Suong et al. (2022) reported changes in rumen bacterial communities in goats receiving anthocyanin-rich black cane silage. Vasta et al. (2019) emphasized that plant polyphenols can modify microbial populations responsible for fiber degradation, biohydrogenation, and methane production, thereby influencing overall fermentation efficiency.

**4. Improvement of Antioxidant Status and Rumen Health:** Anthocyanins possess strong antioxidant properties due to their ability to scavenge reactive oxygen species and activate antioxidant defense pathways (Han et al., 2006; Tian & Lu, 2022). Tian and Lu (2022) noted that anthocyanins activate Nrf2 signaling pathways and increase the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Improved antioxidant status may enhance microbial stability and rumen function, indirectly contributing to lower methane production.

**5. Biohydrogenation process modulation:** Apart from influencing methane production directly, anthocyanins may alter ruminal biohydrogenation processes. Vasta et al. (2019) reported that plant polyphenols can affect microbial species responsible for fatty acid biohydrogenation, leading to changes in ruminal lipid metabolism. Alterations in biohydrogenation pathways may influence hydrogen utilization patterns within the rumen. Because methanogenesis competes for available hydrogen, changes in hydrogen partitioning may contribute to reductions in methane production (Vasta et al., 2019).

**6. Influence on Fiber Degradation:** Anthocyanins may also affect fiber digestion through interactions with cellulolytic microorganisms. Similar to other polyphenols, anthocyanins can bind to plant cell walls and microbial enzymes, altering degradation kinetics (Acosta-Estrada et al., 2014). Moderate reductions in fiber degradation may decrease hydrogen production and methane formation without negatively affecting overall animal performance. However, excessive inclusion rates may impair digestibility, emphasizing the need for optimal supplementation levels (Patra & Saxena, 2011).

**Anthocyanins and Oxidative Stress Reduction**

Oxidative stress is a major challenge in modern dairy production systems, particularly during transition periods and heat stress conditions (Sordillo & Aitken, 2009). Excessive production of reactive oxygen species (ROS) can impair immune function, reduce feed intake, and compromise productivity (Miller et al., 1993). Anthocyanins are capable of activating nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of cellular antioxidant defense systems (Aboonabi & Singh, 2015). Activation of Nrf2 stimulates the expression of antioxidant enzymes including superoxide dismutase, glutathione peroxidase, catalase, and NAD(P)H quinone oxidoreductase (Tian & Lu, 2022). Simultaneously, anthocyanins inhibit activation of the nuclear factor-kappa B (NF-κB) signaling pathway, thereby reducing inflammatory responses and oxidative damage (Xiao, 2004; Kuntz et al., 2015). Such effects may contribute indirectly to improved rumen health and fermentation efficiency.

**Heat Stress, Methane Emissions and Anthocyanins**

Heat stress represents one of the most important environmental challenges affecting dairy cattle worldwide (Das et al., 2016). Elevated temperatures reduce feed intake, alter rumen fermentation patterns, impair immune responses, and decrease milk production. Anthocyanins may help alleviate heat stress through their antioxidant and anti-inflammatory properties. Tian et al. (2019) demonstrated that improved oxidative status during the postpartum period enhanced productivity and metabolic health in dairy cows. Because oxidative stress and methane production are both linked to rumen function, anthocyanin supplementation may offer dual benefits by reducing oxidative damage and improving fermentation efficiency.

Table 1. Effects of Anthocyanins in Ruminant Nutrition

| Area of Effect | Observed Effects of Anthocyanins | Mechanism of Action | Animal Species/Studies | References |
|---|---|---|---|---|
| Antioxidant Status | Increased T-AOC, SOD, GSH-Px; reduced MDA | Scavenging free radicals; enhancing antioxidant enzymes | Dairy cows, sheep, goats | Tian et al. (2019) |
| Immune Function | Improved immune response; reduced TNF-α and IL-6 | Modulation of NF-κB signaling and cytokine production | Dairy cows and lambs | Tsuda (2012); |
| Rumen Fermentation | Enhanced VFA production, especially propionate | Selective modulation of rumen microbial populations | Sheep and cattle | Makmur et al. (2022) |
| Methane Emission | Reduction in enteric methane production | Inhibition of methanogens and shift toward propionate | In vitro and in vivo ruminant studies | Patra et al. (2017); Makmur et al. (2022) |
| Nutrient Digestibility | Improved DM, OM and CP digestibility | Improved microbial efficiency and reduced oxidative stress | Sheep and dairy cattle | Tian et al. (2021) |
| Feed Intake and Palatability | Neutral to slightly positive effects on intake | Improved feed quality | Sheep and dairy cows | Tian et al. (2021) |
| Growth Performance | Increased ADG and feed efficiency | Better nutrient utilization and health status | Lambs and growing cattle | Tian et al. (2019) |
| Heat Stress Mitigation | Reduced oxidative stress during heat stress | Antioxidant and anti-inflammatory effects | Dairy cows and goats | Tian et al. (2021) |
| Gut Health and Microbiome | Improved beneficial microbial populations | Antimicrobial effects supporting beneficial microbes | Sheep and dairy cows | Makmur et al. (2022) |
| Blood Metabolites | Improved lipid profile and glucose regulation | Regulation of lipid metabolism and antioxidant pathways | Sheep, goats, dairy cows | Tsuda (2012); Tian et al. (2019) |

**Environmental Benefits**

Anthocyanins contribute to environmentally sustainable ruminant production systems primarily through their potential to reduce enteric methane emissions, a greenhouse gas with approximately 28 times higher global warming potential than $CO_2$ over a 100-year horizon (Legg, 2021). By modifying rumen fermentation pathways and decreasing methanogenesis, anthocyanin supplementation helps lower the overall carbon footprint of livestock production systems (Vasta et al., 2019). In addition to direct methane mitigation, anthocyanin-rich feed resources enhance the utilization of agricultural by-products such as grape pomace, black cane, and berry residues, thereby promoting circular bioeconomy approaches in livestock feeding systems (Tayengwa & Mapiye, 2018). Furthermore, since enteric methane production represents a loss of 2–12% of gross dietary energy (Moss et al., 2000), reducing methane emissions also improves feed energy efficiency, indirectly enhancing productivity and reducing environmental pressure per unit of animal product.

**Challenges and Limitations**

Despite their promising potential, several limitations hinder the widespread application of anthocyanins in ruminant nutrition. One of the major constraints is the variability in anthocyanin composition and concentration, which depends on plant species, environmental conditions, maturity stage, and post-harvest processing methods (Tian & Lu, 2022). This variability makes standardization of dietary supplementation difficult. Another significant limitation is the low bioavailability of anthocyanins, as these compounds are often extensively metabolized or degraded in the digestive tract before reaching systemic circulation or exerting maximal ruminal effects (Fang, 2014). Additionally, inconsistencies in experimental outcomes have been reported across studies due to differences in animal species, diet composition, dosage levels, and anthocyanin sources. These inconsistencies make it challenging to develop universally applicable feeding recommendations. Finally, most existing studies are short-term and conducted under controlled experimental conditions, limiting the understanding of long-term productivity, adaptation, and sustainability outcomes under field conditions.

## Research Gaps and Future Perspectives

Future research on anthocyanins in ruminant nutrition should focus on optimizing their application under practical feeding systems. Determining optimal dietary inclusion levels for different animal species and production stages remains a key priority. Advanced metagenomic and metabolomic approaches should be used to better characterize shifts in rumen microbial populations and functional pathways associated with anthocyanin supplementation (Vasta et al., 2019). Long-term in vivo studies under commercial farming conditions are also required to evaluate sustained effects on productivity, methane emissions, and animal health. Furthermore, exploring synergistic interactions between anthocyanins and other plant secondary metabolites such as tannins, essential oils, and saponins may provide enhanced methane mitigation strategies. From a sustainability perspective, life cycle assessment (LCA) approaches should be integrated into future studies to quantify the full environmental benefits of anthocyanin-based feeding strategies. In addition, improving extraction, preservation, and feed formulation technologies will be essential for increasing the stability and cost-effectiveness of anthocyanin-rich feed additives.

## Conclusion

Anthocyanins represent a promising class of natural plant secondary metabolites with significant potential to mitigate enteric methane emissions in ruminant livestock systems. Through modulation of rumen microbial populations, enhancement of propionate synthesis, suppression of methanogenic archaea, and improvement of fermentation efficiency, anthocyanins contribute to both reduced greenhouse gas emissions and improved nutrient utilization. Their incorporation into ruminant diets through anthocyanin-rich feed resources such as purple corn, grape pomace, and black cane offers a sustainable strategy for improving livestock productivity while reducing environmental impact. However, challenges such as variability in compound composition, limited bioavailability, and insufficient long-term field validation must be addressed before large-scale implementation. Overall, anthocyanins hold strong promise as part of integrated climate-smart livestock production strategies aimed at improving efficiency and reducing the environmental footprint of ruminant agriculture.

***Author Contribution Statement***

Abdulhamid Muhammad GARBA: Data collection, and writing the original draft
Sema YAMAN FIRINCIOĞLU: review and editing

***Conflict of Interest***

The authors declare no conflict of interest.

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