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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

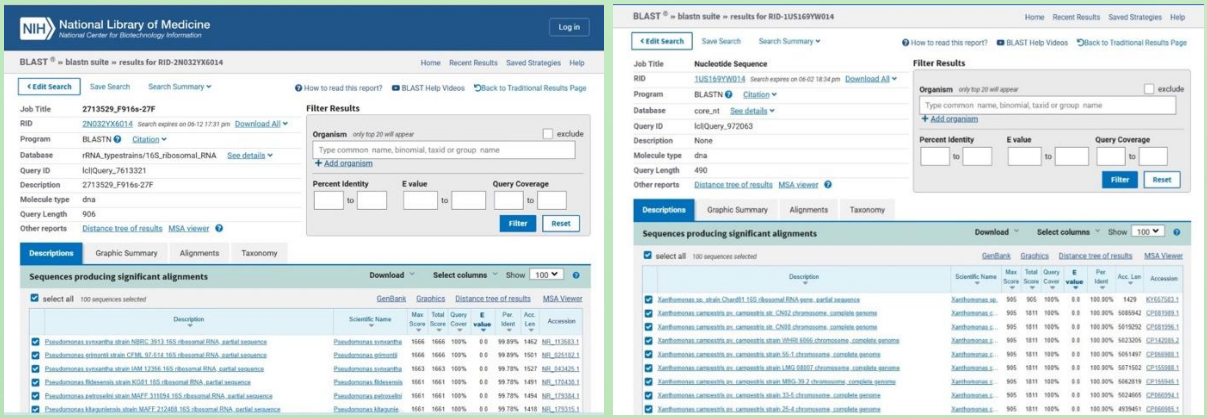
As a result of isolations made from soil and infected plant samples, yellow-pigmented, round, smooth-edged colonies developed on Nutrient Agar medium. Representative colonies are shown in Figure 1. In the hypersensitivity reaction test applied to the purified isolates, necrosis formation was observed 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.

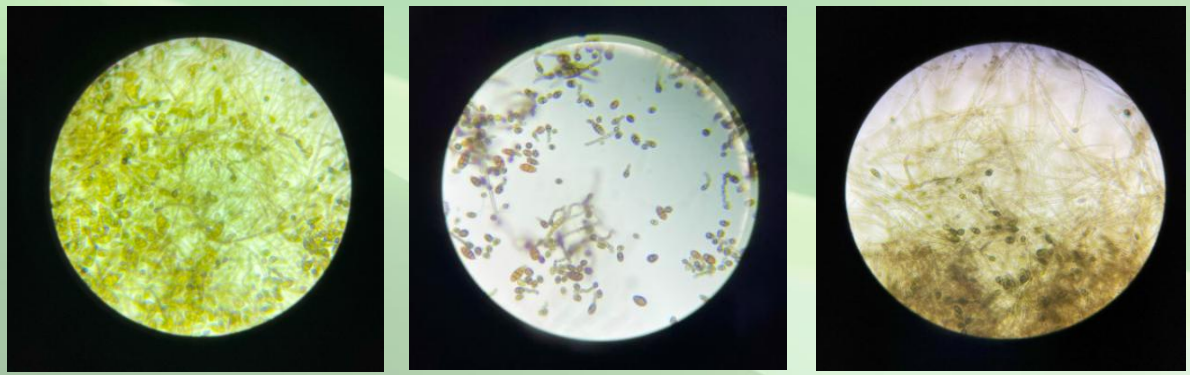


A B

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.

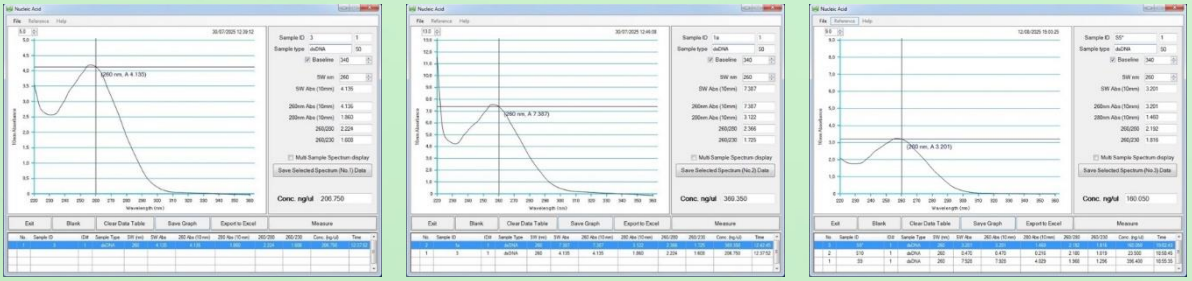


A B C

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.

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