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Occurrence of Fusarium sp. and Lasiodiplodia sp. Causing Root Rot in Grapevine (Vitis vinifera L.) in Sri Lanka

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Abstract

A decline of various grapevines (*Vitis vinifera* L.) associated with root rot symptoms was observed during a field survey conducted from January to March 2024 in vineyards in the Jaffna district, Northern Province, Sri Lanka. Despite the significance of identifying the causative agents for effective disease management, no prior reports exist on the pathogens responsible for grapevine root rot in Sri Lanka. In this study, based on morphological characterization, we identified *Fusarium* sp. and *Lasiodiplodia* sp. as potential causal agents of grapevine root rot. Koch's postulates were confirmed by the successful re-isolation of both pathogens from healthy plants inoculated with the conidial suspensions under controlled conditions. To the best of our knowledge, this is the first report of *Fusarium* sp. and *Lasiodiplodia* sp. causing grapevine root rot in Sri Lanka. This study highlights the need for species-level identification using molecular data and lays the foundation for future research aimed at developing sustainable disease management strategies.

Key Words: *Fungal diseases, Pathogenicity, Sordariomycetes, Botryosphaeriaceae*

Introduction

Grapevine (*Vitis vinifera* L.) is an economically significant crop worldwide, cultivated for fresh fruit, wine production, and other value-added products (Perestrelo et al., 2014; Parihar & Sharma, 2021). Global grape production spans about 7.9 million hectares, with 51.42% of the total coming from China (12.85%), Italy (11.5%), the USA (9.24%), Spain (9.07%), and France (8.69%) (Kulwijila et al., 2018). In Sri Lanka, grapes are primarily grown for fresh consumption on about 100 hectares (Champa, 2015), mainly in the Dry Zone areas of the Northern and Eastern regions (Champa, 2015; Ramanathan & Sivapalan, 1988). During a survey of the vineyards in the Jaffna district, Northern Province, Sri Lanka, a widespread vine declining was noted with root rot symptoms. Root rot is primarily caused by soil-borne pathogens and is particularly challenging to manage because initial symptoms develop below ground, becoming visible only after significant damage has occurred, which compromises yield and jeopardizes plant survival (Bodah, 2017; Williamson-Benavides & Dhingra, 2021).

Among the root rot causing pathogens, *Fusarium* spp. are common (Alves et al., 2023; Bodah, 2017; Williamson-Benavides & Dhingra, 2021), and *Lasiodiplodia* spp. are also frequently associated with root rot in various crops (El-Ganainy et al., 2022; Gnanesh et al., 2022; Latha et al., 2009; Xie et al., 2014). The genus *Fusarium* belongs to the phylum Ascomycota, class Sordariomycetes, order hypocreales, and family Nectriaceae (Crous et al., 2021). *Fusarium* spp. are characterized by aerial phialides or sporodochial conidiophores and conidiogenous cells; aerial microconidia produced on long, narrow phialides; mesoconidia; thick-walled septated macroconidia with blunt, rounded apical cells and inconspicuous foot-shaped basal cells; and chlamydospores (Leslie & Summerell, 2006; Crous et al., 2021). The genus *Lasiodiplodia* belongs to the phylum Ascomycota, class Dothideomycetes, order Botryosphaeriales, and family Botryosphaeriaceae (Phillips et al., 2013). *Lasiodiplodia* spp. are characterized by subglobose or oval, smooth, thick-walled conidia that are initially hyaline, and become dark brown and striated upon maturity (Phillips et al., 2013). *Lasiodiplodia* spp. are distinguished from related genera by the presence of pycnidial paraphyses and longitudinal striations on mature conidia (Abdollahzadeh et al., 2010).

Fusarium spp. and *Lasiodiplodia* spp. have been reported to cause grapevine root rot disease in various parts of the world, including China (Li et al., 2023; Zhang et al., 2023), Egypt (El-Ganainy et al., 2022; El-Mohamedy et al., 2010; Hemida et al., 2017, 2024), Iraq (Abdullah et al., 2015), South Africa (Marais, 1979), and Turkey (Akgül et al., 2024). Despite their global significance, there has been no documented evidence of these pathogens affecting grapevines in Sri Lanka. Studying new occurrences of fungal pathogens in various hosts offers valuable insights into host-pathogen interactions and helps expand knowledge of their geographical distribution. This study presents the first report of *Fusarium* sp. and *Lasiodiplodia* sp. causing root rot in grapevines in Sri Lanka. The findings contribute to the existing knowledge on grapevine root rot and highlight the need for integrated disease management strategies to ensure sustainable grape production in the region.

Material and Methods

Sample collection and isolation of pathogens

Samples were collected from the grapes fields at Jaffna district from January to March 2024. Infected roots were collected from the grape vines showing disease symptoms. The root samples were thoroughly washed with tap water to remove adhered soil and surface dirt. Then the diseased root tissues were cut into small sections and surface sterilized using 1% sodium hypochlorite solution for 2 minutes. After washing three times in sterile distilled water, the sections were dried using sterile blotting papers. The sections were then placed on potato dextrose agar (PDA) medium and incubated at 28 ℃. After 2-3 days, mycelium tips of resulting fungi were transferred into fresh PDA in order to obtain pure cultures.

Identification of pathogens

Fusarium sp. and *Lasiodiplodia* sp. isolates were identified based on the morphology of fungal colonies and conidia described by Crous et al. (2021) and Phillips et al. (2013), respectively. Morphological characters were observed and documented using a compound microscope (Olympus BH-2, Japan), equipped with a Canon EOS 600D (Japan) digital camera. Measurements were made using Image Focus Plus Version 2.0, and the photographs were processed with Adobe Photoshop Version 22.0.

Pathogenicity test

The isolated *Fusarium* sp. and *Lasiodiplodia* sp. were tested for pathogenicity following Koch's postulates. Healthy grapevines were transplanted in sterilized pots filled with sterilized soil and maintained in a greenhouse at the University of Jaffna. Two methods, such as wounded and unwounded were followed to assess the infection ability and the virulence of the isolates on grapevine roots. In wounded method, soil at the crown area was removed carefully, cleared the prominent roots and a small wound was created by using a sterile needle. Then, a 5 ml conidial suspension was applied into the root zone of each wounded and unwounded plants. Control tests were done by applying 5 ml of sterilized distilled water. The inoculated plants were observed daily for disease symptom development. After appearing the symptoms, the pathogen was re-isolated and confirmed by morphological characterization.

Results and Discussion

Identification of pathogens

Two pathogenic fungi were isolated from diseased grapevine root tissues and identified as *Fusarium* sp. and *Lasiodiplodia* sp. based on their morphological characteristics. *Fusarium* sp. colonies on PDA were moderately dense with whitish mycelium and a yellowish reverse, reaching 90 mm in diameter after 3 days at 25°C. The macroconidia were slightly curved to straight, elongated, tapering at both ends with 2-4 septa, measuring 22.7-39.10 × 4.93-5.9 μm. The microconidia were oval to fusiform, hyaline, smooth, and either aseptate or septate, measuring 10.9-15.4 × 3.13-4.19 μm (Figure 1).

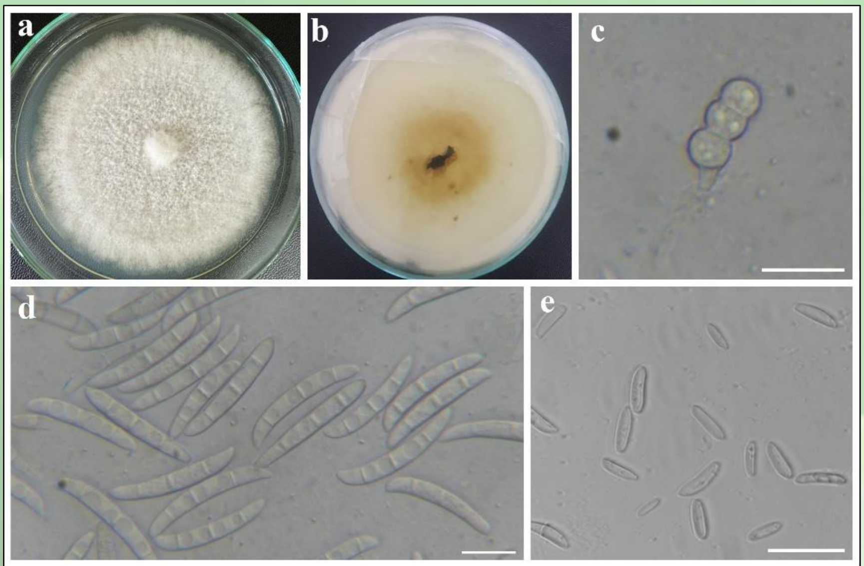

Figure 1: Morphological characters of *Fusarium* sp. (a, b) Colony morphology on potato dextrose agar (PDA) (c) chlamydospore (d) macroconidia (e) microconidia. Scale bars: 10 μm.

Lasiodiplodia sp. colonies were initially whitish, later becoming dark olivaecious with a yellowish reverse, and reached 90 mm in diameter after 3 days at 25°C. The conidia were initially hyaline, becoming dark brown with one septum and longitudinal striations upon maturity, measuring 14.94-19.36 × 10.21-12.53 µm (Figure 2).

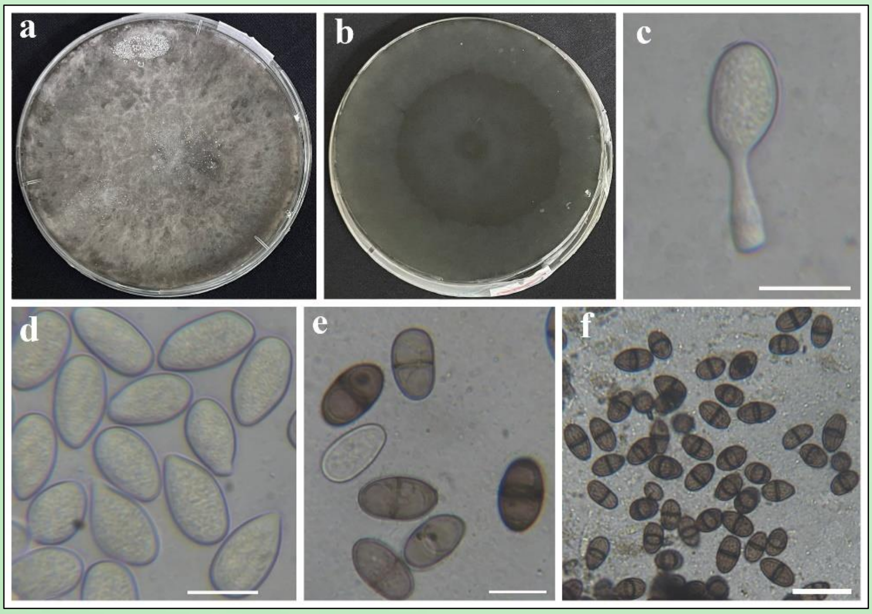

Figure 2: Morphological characters of *Lasiodiplodia* sp. (a, b) Colony morphology on potato dextrose agar (PDA) (c) a conidium developing on a conidiogenous cell (d) hyaline, immature conidia (e, f) mature conidia in two different magnifications to show the longitudinal striations. Scale bars: 10 µm (c-e); 20 µm (f).

This is the first report of *Fusarium* sp. and *Lasiodiplodia* sp. as root rot pathogens of grapevines in Sri Lanka. However, in other regions, various *Fusarium* species have been documented to cause grapevine root rot, including *F. oxysporum* (Highet & Nair 2008; Ziedan 2003; Ziedan et al., 2020), *F. solani* (Hemida et al., 2024; Ziedan 2003; Ziedan et al., 2020), *F. avenaceum* (Ziedan et al., 2020), *F. commune* (Zhang et al., 2023), *F. brachygibbosum*, *F. chlamydosporum*, and *F. ipomoeae* (Hemida et al., 2024). Similarly, though less frequent than *Fusarium* spp., *Lasiodiplodia* spp., such as *L. theobromae* (Hemida et al., 2024; Ziedan et al., 2020) and *L. exigua* (Hemida et al., 2024), have also been reported to cause grapevine root rot. Beyond root rot, *Fusarium* spp. are implicated in other grapevine diseases like vine decline (Bustamante et al., 2024; Reveglia et al., 2018), vascular wilt (Ziedan et al., 2011), basal rot, and wood cankers (Akgül et al., 2024). Likewise, *Lasiodiplodia* spp. can cause vine decline (Aroca et al., 2008) and dieback (Correia et al., 2016; Rangel Montoya et al., 2021) in addition to root rot.

Pathogenicity test

Grapevine plants inoculated with spore suspensions of *Fusarium* sp. and *Lasiodiplodia* sp. developed root rot symptoms approximately after 3-4 weeks and 2 weeks, respectively. No symptoms were observed in control plants. *Fusarium* sp. primarily caused leaf necrosis along the length of the shoot, while *Lasiodiplodia* sp. led to chlorosis of the bottom-to-top leaves in grapevine plants, eventually causing wilting and plant death (Figure 3).

Figure 3: Disease symptoms of grapevine root rot on the shoot system, under soil inoculated with *Fusarium* sp. (c) and *Lasiodiplodia* sp. (b), compared to the control (a).

Both pathogens caused rotting of the secondary and feeder roots, with brown discoloration observed and also negatively impacted overall root system growth (Figure 4). These symptoms align with previous studies (Hemida et al., 2024; Zhang et al., 2023).

Figure 4: Disease symptoms of grapevine root rot on the root system, under soil inoculated with *Fusarium* sp. (c) and *Lasiodiplodia* sp. (b), compared to the control (a).

Therefore, it is evident that these two pathogens are among the causative agents of grapevine root rot, a serious threat to grapevine cultivation in Sri Lanka. This finding highlights the need for species-level identification using molecular data and provides a foundation for future studies aimed at managing this disease. Given the significant threat posed to grapevine cultivation, implementing integrated disease management strategies, including biological control measures, will be vital to safeguarding vineyards in the region. This study contributes to the growing knowledge base and calls for continued research to mitigate the impact of grapevine root rot in Sri Lanka.

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