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Short Article

Occurrence and pathogenicity of *Neoscytalidium dimidiatum* as the causal agent of dieback on mango in Hormozgan Province, Iran

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ABSTRACT

Mango (*Mangifera indica* L.) is one of the most valuable tropical fruit crops cultivated extensively in the southern regions of Iran, particularly in Hormozgan Province. In September and October of 2022, symptoms including leaf wilting, twig and branch dieback and necrosis were observed in several mango orchards in this region. Samples were collected from symptomatic trees, and a fungal species was isolated from the infected tissues. According to morphological features of the asexual structures, *Neoscytalidium dimidiatum* was identified. Pathogenicity was confirmed through Koch's postulates. To the best of our knowledge, this is the first report of mango dieback caused by *N. dimidiatum* in Iran.

KEYWORDS

Hormozgan, *Mangifera indica*, morphology, pathogenicity, tropical fruits.

INTRODUCTION

The mango (*Mangifera indica*) is one of the most widely cultivated and economically important tropical fruits in the world, with a history of domestication and use dating back over 4,000 years. It is grown in over 90 countries and serves as a key agricultural product that supports the economies of numerous developing nations. Its popularity is attributed not only to its appealing flavor and rich nutritional profile-particularly its high vitamin A and C content-but also to its significant role in supporting the livelihoods of millions of smallholder farmers. Global demand for mangoes, both fresh and processed, has been steadily increasing, reinforcing its status as an essential crop for export and domestic markets (Jedele et al. 2003).

In Iran, mango farming is gaining popularity, especially in the southern provinces where the climate is well-suited to its cultivation. Recent efforts have been made to enhance the quality and commercial appeal of Iranian mango varieties. Studies have indicated that the levels of heavy metals in these fruits are within safe consumption limits (Zahedi et al. 2016, Rahimzadeh and Rastegar 2017). Mango cultivation faces significant challenges due to various fungal diseases that can severely affect the health and yield of the trees. Among the most prevalent diseases are anthracnose, powdery mildew, black molds, galls, rusts, deformities, dieback, and stem-end rot, which are caused by different fungal pathogens (Ploetz and Freeman 2009). A study conducted in Azad Kashmir highlighted that dieback disease had the highest incidence rate of 33.73%, while blossom blight showed a lower incidence of 30.98% (Hussain et al. 2024). In recent years, research on fungal diseases of tropical fruit trees, including mango, has

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been conducted in Iran (Dehghani et al. 2022, 2024, Pordel et al. 2024). Based on the results of these studies, several fungal species such as *Alternaria alternata, Bartalinia pini, Beltrania rhombica, Curvularia hawaiiensis,* and *Exserohilum rostratum* have been associated with diseased plants. In addition, other fungal species such as *Alternaria destruens*, *Aspergillus niger*, *Fusarium semitectum*, *Gibberella intricans* (Karampour and Ershad 2008), *Botryodiplodia mangiferae* (Ershad 2022), *Botryosphaeria rhodina* (Mirzaee et al. 2002), *Dothiorella ladharensis* (Petrak 1956), *Glomerella cingulata* (Zebarjad and Ershad 2008), and *Oidium mangiferae* (Zakii et al. 1995) have also been reported from mango in Iran.

The fungal genus *Neoscytalidium* (*Botryosphaeriaceae*, *Botryosphaeriales*), is notable for its pathogenic effects on both plants and humans. It plays a significant role in agriculture, being linked to numerous plant diseases that impact a wide array of crop and ornamental species. Among its members, *N. dimidiatum* stands out as the most widespread and virulent species, capable of infecting over 126 plant species from 46 different families, particularly eudicot angiosperms. This pathogen has a global widespread, with high incidence in regions such as Asia and North America. Researchers in countries such as Iran and Turkey have reported severe outbreaks, underscoring their threat to crop production and plant diversity (Nourian et al. 2021, Derviş and Özer 2023). This study aimed to identify the causal agent of mango dieback in Hormozgan Province, Iran.

MATERIALS AND METHODS

Sample collection, isolation, and identification of the pathogens

Sampling was carried out in mango orchards in Minab, Hormozgan Province, southern Iran, during September and October 2022. Mango trees showing typical symptoms of dieback, including wilting, drying of twigs and branches, and necrotic lesions, were chosen for sample collection. Affected twigs and branches were cut and placed in polyethylene bags. Each sample was properly labeled with details such as tree number, orchard location, collection date, and observed symptoms. The samples were surface disinfected by immersing them sequentially in 70% ethanol for 30 seconds, 1% sodium hypochlorite (NaOCl) solution for 2 minutes, and rinsed three times with sterile distilled water. The samples were blotted on sterile filter paper within a laminar flow cabinet. Small pieces (approximately 5 × 5 mm) taken from the margins of necrotic areas were aseptically placed onto potato dextrose agar (PDA) plates containing streptomycin (100 mg/L) to suppress bacterial growth. These plates were incubated in the dark at 25 ± 1°C for 3 to 5 days. Emerging fungal colonies were purified using the single-spore method on fresh PDA plates. The pure cultures were deposited in the Herbarium of the Mycology Laboratory at the University of Jiroft, Kerman (UJFCC).

For morphological identification, the fungal isolates were examined based on colony traits and microscopic characteristics. Colony color, texture, and diameter were recorded after 7 days of incubation on PDA at 25 ± 1°C. Microscopic observations were conducted on slide cultures using a CH2 microscope (Olympus, Japan) equipped with a Microbin 5 camera (Germany). Special focus was given to arthrospores, assessing their shape, dimensions, septation, and pigmentation. Additionally, pycnidia and pycnidiospores were studied to aid species-level identification. To induce pycnidia formation, agar plugs from actively growing cultures were transferred onto water agar (WA) plates supplemented with sterilized pine needles. These plates were incubated at 25 ± 1°C under a 12-hour light/dark cycle for 2 to 3 weeks. The developed pycnidia and released pycnidiospores were then examined and documented microscopically (Rayner 1970, Crous et al. 2006).

Pathogenicity test

One-year-old mango seedlings were selected for the pathogenicity test. The seedlings were grown individually in pots under controlled conditions. For inoculation, a spore suspension was prepared at a concentration of 1×10^6 spores/mL, and 10 µL of this suspension was used per seedling. Inoculation was performed by first creating a small wound at the apical shoot region, into which the spore suspension was injected. The inoculation site was then sealed with parafilm to prevent contamination and desiccation. Control plants were treated with sterile distilled water following the same procedure. After inoculation, the seedlings were maintained at 27°C. To maintain initial humidity, each pot was covered with plastic wrap for 24 hours post-inoculation. After removing the plastic covers, the relative humidity was maintained at approximately 70%. Disease symptoms were monitored and recorded following the fulfillment of Koch's postulates (Sharma et al. 2024).

RESULTS AND DISCUSSION

Disease symptoms and morphology of the pathogen

Branch cankers linked to dieback were commonly observed across mango orchards in Minab, Hormozgan Province. These cankers primarily affected mature trees over 10 years old and were often associated with physical injuries, such as wind-induced fractures. The cankers extended longitudinally along the branches, leading to bark peeling, tissue desiccation, and eventual dieback. In advanced stages, spore masses became visible beneath the bark (Fig. 1). In this study, a total of eight samples were obtained from different locations in Minab, Hormozgan Province. Morphological characterization of the isolates confirmed their identity as *Neoscytalidium dimidiatum*. A brief description and illustration of the species are provided below.

***Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, Stud. Mycol. 55: 244 (2006)**

On PDA, the colony surface initially appeared white to hyaline, gradually turned olive green to gray, and

became dark gray to black after 7 days; the reverse side of the colony also showed a dark gray to black coloration. The colony exhibited rapid growth, fully covering the surface of a 6 cm Petri dish after 72 hours at 25 ± 1°C. The colonies produced mycelium that fragmented into cylindrical to round arthroconidia with 0 to 2-septate. Arthrospores were observed as cylindrical to round in shape, occurring either singly or in arthric chains, and ranged in size from 3–10(–12) × (1.5–)2–6 µm, with coloration varying from hyaline to brown (Fig 2). Pycnidiospores were hyaline, ellipsoidal to ovoid or fusiform, and 4–5 × 11–12(–3) µm.

Specimen examined: Iran, Hormozgan Province, Minab, *Mangifera indica*, 14 Sept. 2022, A.R. Amirmijani, (UJFCC1202).

Note: The morphological characteristics of the fungal isolate obtained from mango dieback matched the description of *Neoscytalidium dimidiatum* (Crous et al. 2006, Nouri et al. 2018). As noted by Huang et al. (2016), *Hendersonula toruloidea* was originally described by Nattrass in 1933. Later on, *Scytalidium hyalinum* was identified as a species with comparable pathogenic traits. In a taxonomic reassessment, Sutton and Dyko (1989) reclassified *H. toruloidea* under the genus *Nattrassia*, naming it *Nattrassia mangiferae* as the type species and recognizing *S. dimidiatum* as its synanamorph. Subsequently, Crous et al. (2006), highlight the polyphyletic nature of *Scytalidium*, established the genus *Neoscytalidium* and reassigned the species as *N. dimidiatum*. Later, Phillips et al. (2013) considered *N. dimidiatum* and *N. hyalinum* to be conspecific, and due to nomenclatural priority, *N. dimidiatum* remained the valid name.

Pathogenicity test

Characteristic disease symptoms were observed on one-year-old mango seedlings within 18 days of inoculation with *N. dimidiatum*. Initial symptoms, including wilting, appeared around seven days post-inoculation, followed by progressive tissue necrosis and eventual dieback, leading to the complete death of the seedlings (Fig. 3). The fungus was re-isolated from the necrotic tissues, and the recovered isolates matched the original isolate used for inoculation, confirming its pathogenicity. No symptoms developed in the control seedlings, and no fungal isolates were re-isolated from them. Although *N. dimidiatum* has previously been reported as the causal agent of dieback and canker in mango trees (Ray et al. 2010, Youssef et al. 2023, Armand et al. 2025), there have been no prior reports of this fungus causing dieback in mango in Iran. To the best of our knowledge, this is the first report of mango dieback caused by *N. dimidiatum* in Iran. A review of previous studies indicates that several members of the family *Botryosphaeriaceae* have been isolated and reported from various parts of mango trees in both Iran

Fig. 1. Symptoms of mango dieback observed in the field. Wilting and necrosis in the leaves and branches (left). Typical longitudinal canker and dieback symptoms associated with *Neoscytalidium dimidiatum* (right).

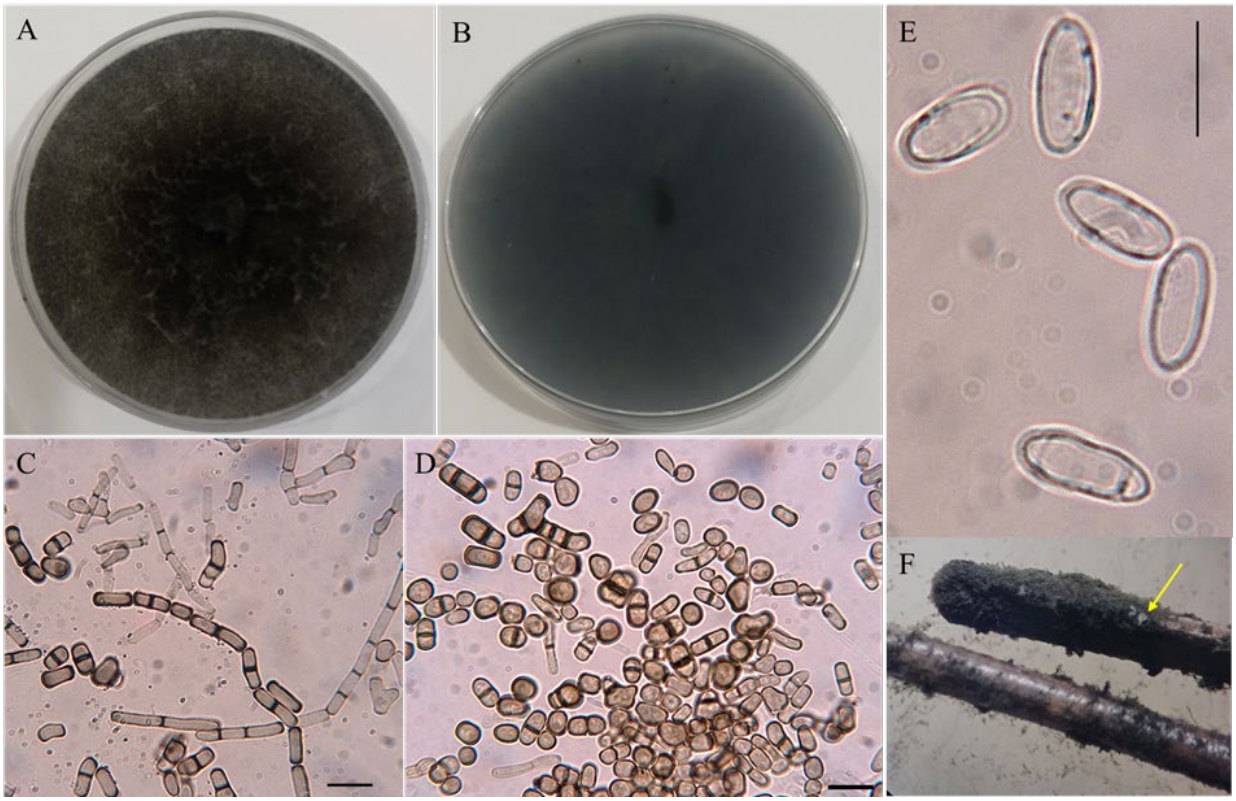

Fig. 2. Morphological characteristics of *Neoscytalidium dimidiatum*. (A, B) Surface and reverse of colonies on potato dextrose agar, after 7 days at 25 ± 1°C., (C, D) Hypha and arthrospores, (E) Pycnidiospores, (F) Conidial mass exuding from pycnidia. Scale bars = 10 µm.

Fig. 3. Pathogenicity of *Neoscytalidium dimidiatum* on one-year-old mango seedlings after 18 days inoculation.

and Australia, including branches and trunks (Abdollahzadeh et al. 2010, Sakalidis et al. 2011), as well as from fruits, buds, and seeds of mango trees in Iran (Raeisi and Mohammadi 2025), and from walnut trees (Antony et al. 2023). Considering the climatic conditions of Hormozgan Province—such as high temperature and humidity—and the diverse means by which these pathogens can spread (e.g., through seeds, vegetative propagules, soil, and wind), along with their ability to persist in contaminated plant debris in the soil, the potential for disease outbreaks across various hosts and locations is significant. These factors must be carefully considered in the development of effective disease management strategies.

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AUTHOR CONTRIBUTION

AA, AP: Identification of the producer strain, review and editing; **KS, NS:** Isolation of the strains, writing - original draft preparation.

DATA AVAILABILITY

Requests for data and materials should be addressed to AR Amirmijani**.**

DECLARATION

The authors declare that there is no conflict of interest.

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The authors declare that no financial support was received during this research.

ETHICS APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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شیوع و بیماریزایی *Neoscytalidium dimidiatum* به عنوان عامل سرخشکیدگی انبه در استان هرمزگان، ایران

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چکیده

انبه (*Mangifera indica* L.) یکی از ارزشمندترین میوه‌های گرمسیری است که به طور گسترده در مناطق جنوبی ایران، به ویژه در استان هرمزگان کشت می‌شود. در ماههای شهریور و مهر ۱۴۰۱، علائم سرخشکیدگی - شامل پژمردگی برگ، خشک شدن و نکروز شاخه - در چندین باغ انبه در این منطقه مشاهده شد. نمونه‌ها از درختان دارای علائم جمع‌آوری شدند و یک گونه قارچی به طور مداوم از بافت‌های آلوده جدا شد. بررسی ریخت‌شناسی پرگنه‌های جدایه‌های به دست آمده، همراه با بررسی میکروسکوپی ریسه‌ها و آرتروسپورها، منجر به شناسایی قارچ *Neoscytalidium dimidiatum* شد. بیماری‌زایی جدایه به دست آمده از طریق اصول کخ تأیید شد. بر اساس اطلاعات موجود، این اولین گزارش از سرخشکیدگی انبه ناشی از *N. dimidiatum* در ایران است.

کلمات کلیدی: هرمزگان، ، ریخت‌شناسی، بیماری‌زایی، میوه‌های استوایی، *Mangifera indica*.

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