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Short Article

Fusarium annulatum associated with floral malformation and causing mango branch dieback in southeastern Iran

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ABSTRACT

During a survey of mango (*Mangifera indica* L., *Anacardiaceae*) orchards in Sistan and Baluchestan Province, southeastern Iran, characteristic symptoms of branch dieback and floral malformation were observed on branches and inflorescences. A total of 14 fungal isolates, of which nine were obtained from symptomatic branches and five from malformed inflorescences, were recovered and identified as *Fusarium annulatum*, based on cultural and morphological characteristics and multilocus phylogenetic analyses of the ITS-rDNA, *tef1-α*, and *tub2* regions, using representative isolate ZD4-7. This study provides the first report of *Fusarium annulatum* associated with floral malformation and capable of causing branch dieback symptoms in pathogenicity assays in Iran and expands the current knowledge of fungal pathogens associated with mango diseases in the southeast of Iran.

KEYWORDS

Dieback, DNA barcoding, *Mangifera indica*, multilocus phylogeny, Sistan and Baluchestan.

INTRODUCTION

Mango production is constrained by a wide range of fungal and other diseases, among which diseases affecting shoots, branches, and inflorescences are of particular concern because of their direct and cumulative effects on tree health and productivity. Mango dieback is considered one of the economically important diseases of mango and is characterized by the progressive necrosis and drying of young shoots and branches, often accompanied by defoliation, vascular discoloration, gummosis, and gradual deterioration of affected branches. The disease may initially develop on individual shoots but can progressively spread to larger branches, resulting in substantial canopy loss, reduced vegetative growth, impaired fruit production, and significant yield reduction. Under favorable conditions and severe disease pressure, continued dieback may ultimately result in extensive canopy decline and, in some cases, the death of affected trees (Ploetz 2003, Khanzada et al. 2004).

In addition to shoot and branch dieback, mango floral malformation is an important disease syndrome that can severely impair flowering and fruit production. The disease is characterized by abnormal development of inflorescences, including excessive branching, shortening and thickening of the inflorescence axis, and the formation of compact clusters bearing malformed and frequently sterile flowers. These abnormalities can substantially reduce fruit set and consequently cause considerable yield losses. Mango malformation disease has been reported from major mango-producing regions in Asia, Africa, and the Americas, and has been recognized as an economically important disease of mango since its first description in India in 1891 (Marasas et al. 2006, Ansari et al. 2015).

Several *Fusarium* species have been associated with mango malformation disease. *Fusarium mangiferae* is one of the best-documented and most widely distributed causal agents, having been reported from India, Egypt, Florida (USA), Malaysia, South Africa, Oman, China, Sri Lanka, Spain, and other mango-growing regions worldwide (Britz et al. 2002, Marasas et al. 2006, Kvas et al. 2008, Zhan et al. 2012, Sinniah et al. 2013, Freeman et al. 2014). Other species associated

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with mango malformation include *F. sterilihyphosum* in Brazil and South Africa, *F. tupiense* in Brazil, Senegal, and Spain, and *F. mexicanum* and *F. pseudocircinatum* in Mexico (Marasas et al. 2006, Ansari and Tuteja 2015). Importantly, *F. proliferatum* has also been associated with malformed mango tissues. This species was reported from mango malformation in Malaysia, China, and Egypt, indicating a geographically broad association with the disease (Zheng and Ploetz 2002, Youssef et al. 2007, Mohamed Nor et al. 2013). More recently, Molina-Cárdenas et al. (2023) reported *F. proliferatum* as the causal agent of mango malformation disease in Mexico, providing further evidence for its pathogenic role in floral and vegetative malformation of mango.

However, the identity and pathogenic significance of *Fusarium* species associated with mango branch dieback and floral malformation in southeastern Iran remain poorly understood. Therefore, this study aimed to identify *Fusarium* isolates associated with these symptoms and evaluate the pathogenicity of representative isolates on mango seedlings.

MATERIALS AND METHODS

Sampling and fungal isolation

Surveys were conducted in commercial mango orchards in Sistan and Baluchestan Province, southeastern Iran. Symptomatic tissues were collected from the margins of actively progressing necrotic lesions. Tissue segments approximately 7–8 mm in length were excised and surface-disinfected in 2% sodium hypochlorite for 3 min, followed by three rinses with sterile distilled water. The disinfected pieces were then blotted dry on sterile filter paper and placed on 2% water agar (WA) in Petri dishes. Plates were incubated at 25 °C in darkness and monitored regularly for fungal growth. Emerging fungal colonies were purified using single-conidium or hyphal-tip isolation techniques. Representative pure cultures were subsequently transferred to potato dextrose agar (PDA) and maintained for morphological characterization and subsequent molecular identification and phylogenetic analyses (Pordel et al. 2024).

Morphological and cultural characterization

For morphological and cultural characterization, the isolates obtained from mango trees having dieback and floral malformation were cultured on potato dextrose agar (PDA), carnation leaf agar (CLA), and synthetic low-nutrient agar (SNA) and incubated at 25 ± 2 °C. Colony characteristics, including radial growth rate, texture, margin, aerial mycelium, pigmentation, and colour of the upper and reverse surfaces, were recorded after 7 and 14 days of incubation. Colony colour was determined using the standardized colour charts of Rayner (1970).

For morphological identification, isolates were examined for the production and morphology of macroconidia, microconidia, conidiogenous cells, and chlamydospores. Particular attention was given to the shape, size, septation, curvature, and morphology of macroconidia, the shape and arrangement of microconidia, the presence and morphology of monophialides and/or polyphialides, and the occurrence and position of chlamydospores. The formation of conidia and other diagnostic structures was assessed primarily on CLA and SNA, which promote the development of characteristic sporodochia and conidiogenous structures in *Fusarium*. Measurements were obtained from 20–30 randomly selected structures for each morphological character using a compound microscope equipped with a calibrated ocular micrometer. The isolates were identified based on their cultural and morphological characteristics by comparison with descriptions provided in established taxonomic literature and identification manuals for *Fusarium* species (Ellis 1971, Booth 1971, Leslie and Summerell 2006, Crous et al. 2021, Costa et al. 2024). Fungal isolates are maintained in the fungal culture collection of the Plant Protection Research Department, Baluchestan Agricultural and Natural Resources Research and Education Center, Iran.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from a representative isolate following the protocol of Cenis (1992). The internal transcribed spacer region of the nuclear ribosomal DNA (ITS-rDNA) was amplified using the universal primers ITS1 and ITS4 (White et al. 1990). To improve species-level resolution and provide more robust phylogenetic inference, partial regions of the translation elongation factor 1-alpha (*tef1-α*) and β-tubulin (*tub2*) genes were also amplified using the primer pairs EF-1/EF-2 (O'Donnell et al. 1998) and T1/TUB4Rd (O'Donnell and Cigelnik 1997), respectively. PCR amplification of *tef1-α* and *tub2* followed the cycling conditions described by O'Donnell and Cigelnik (1997) and O'Donnell et al. (1998), as also applied in recent taxonomic studies of *Fusarium* (Costa et al. 2024). PCR products showing the expected bands were purified and commercially sequenced by Microsynth AG (Balgach, Switzerland). Raw chromatograms were assessed manually to identify low-quality bases, ambiguous positions, and potential sequencing artefacts. Forward and reverse reads were edited and assembled into consensus sequences using BioEdit v7.2 (Hall 1999). For phylogenetic reconstruction, reference sequences were selected primarily from reliably identified strains, with preference given to ex-type, ex-epitype, ex-neotype, or otherwise authenticated cultures whenever available. Sequence datasets for each locus were aligned independently, carefully inspected, and manually adjusted where necessary; the individual alignments were subsequently combined for multilocus analyses when all required loci were available for the selected taxa. Phylogenetic relationships were reconstructed under a Maximum Likelihood (ML) framework using MEGA X (Kumar et al. 2018), while Bayesian Inference (BI) was implemented in MrBayes v3.2.7 (Ronquist et al. 2012). The sequences generated in this study were deposited in the NCBI GenBank database under their respective accession numbers.

Pathogenicity tests

To verify the pathogenicity of the ZD4-7 and A6 isolates, inoculation tests were performed on healthy 12-month-old mango (*Mangifera indica* L.) seedlings following Koch's postulates (Dhingra and Sinclair 1995). Seedlings of a commonly cultivated mango cultivar were acclimatized under greenhouse conditions (28 ± 2 °C, 70–80% relative humidity, and a 15 h light/9 h dark photoperiod) for two weeks before inoculation. Five seedlings were inoculated with each isolate, with three independent experimental replicates. Mycelial plugs (8 mm in diameter) were excised from the actively growing margins of 7-day-old PDA cultures and placed onto superficial wounds (5–8 mm long) made on the apical region of young shoots after surface disinfection with 70% ethanol. The inoculation sites were sealed with Parafilm to maintain adequate moisture and facilitate fungal establishment. Control seedlings received sterile PDA plugs without fungal mycelium (Dhingra and Sinclair, 1995).

RESULTS

Morphological and molecular identification

***Fusarium annulatum* Bugnic., Rev. Gén. Bot. 59: 17 (1952)**

The colony surface on PDA was cottony, whereas the reverse was generally pale to colourless, occasionally becoming yellowish-cream toward the centre while retaining a white margin (Fig. 1A). Sporodochia were produced on CLA media and appeared as discrete, pale orange to orange structures, often associated with the formation of macroconidia (Fig. 1B). Macroconidia were slender, falcate to sickle-shaped, hyaline, 17–53 × 2–4 µm, and typically 3–5-septate, with a tapering to slightly curved apical cell and a poorly developed to foot-shaped basal cell (Fig. 1C–D). Conidiophores proliferate sympodially and produce both mono- and polyphialides. Microconidia with truncate bases grouped in moderately long chains and false heads, hyaline, aseptate, and predominantly clavate, oval to slender, 3.5–11.5 × 1–2 µm (Fig. 1E). Chlamydospores were absent.

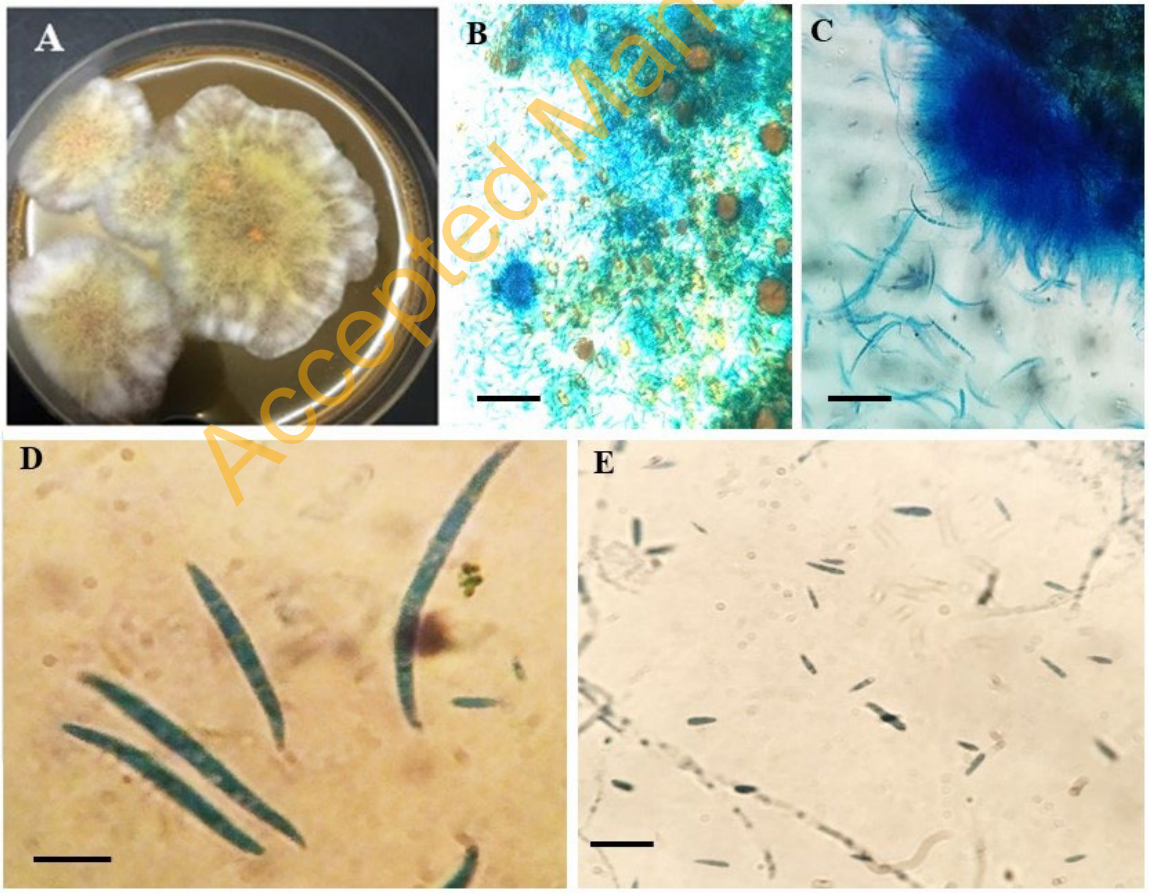

Fig. 1. Morphological characteristics of *Fusarium annulatum*. (A) colony on PDA, (B) sporodochium, (C, D) macroconidia, (E) microconidia. Scale bars: (B–E) 10 µm.

All isolates showed similar cultural and morphological characteristics consistent with *F. annulatum*. The ZD4-7 isolate was selected for multilocus sequence analyses. The combined ITS, *tef1-α*, and *tub2* dataset provided sufficient phylogenetic resolution to distinguish the isolate obtained in this study from closely related taxa with high statistical

support. The *Fusarium* isolate obtained in the present study clustered with the corresponding type strains with 99% bootstrap support (Fig. 2). The sequences of the ITS, *tef1-α*, and *tub2* gene regions were deposited in GenBank and assigned the following accession numbers: ITS (QB013052), *tef1-α* (QB017908), and *tub2* (QB017909).

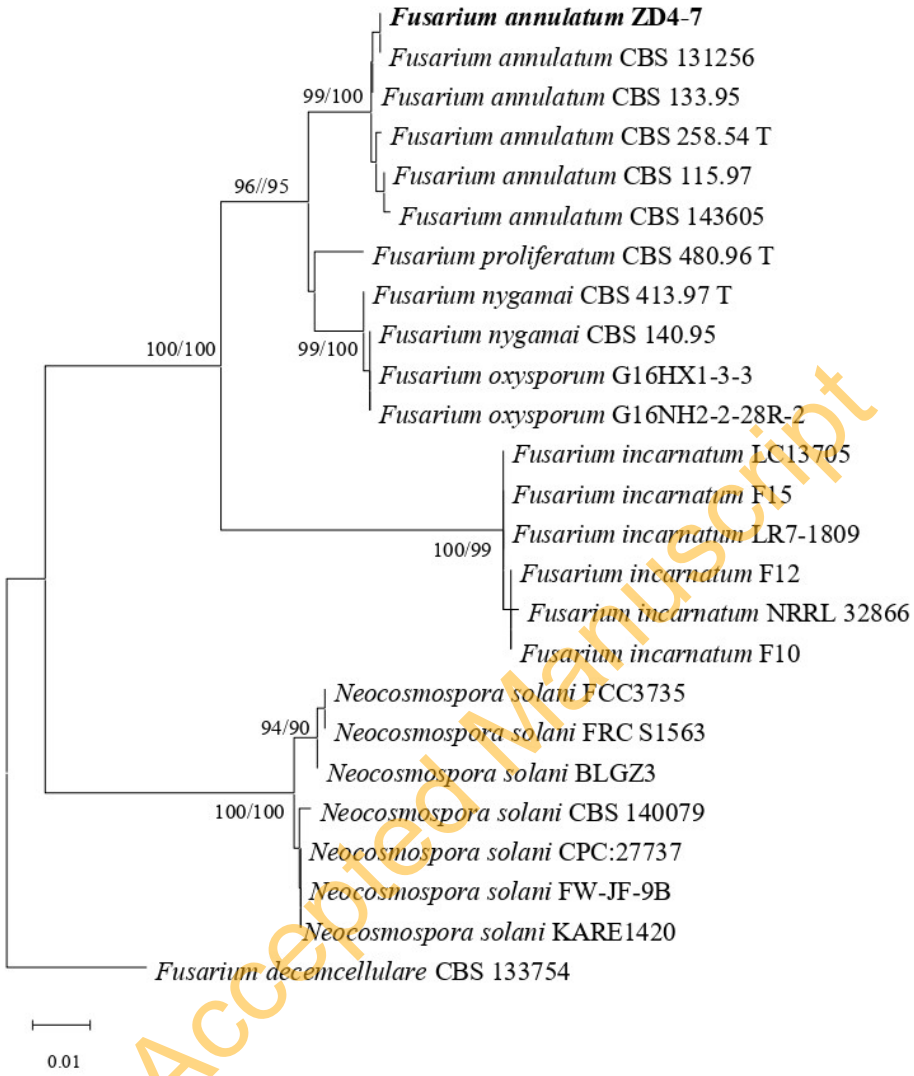

Fig. 2. Phylogenetic tree inferred from the concatenated ITS/*tef1-α*/*tub2* sequence dataset using the Maximum Likelihood (ML) method, showing the phylogenetic relationships among *Fusarium* species. Bootstrap support values and Bayesian Posterior Probability (ML/BI) are indicated at the nodes. Isolates obtained in the present study are shown in bold, whereas ex-type strains are indicated by their corresponding collection numbers. *Albonectria rigidiuscula* CBS 133754 was used as the outgroup.

Pathogenicity test

Pathogenicity tests demonstrated that the representative isolates ZD4-7 and A6 were able to induce disease symptoms on inoculated mango seedlings, whereas no disease symptoms were observed in the non-inoculated control plants. The first symptoms appeared approximately twenty-one days after inoculation and initially consisted of necrosis around the inoculation sites. Subsequently, the lesions expanded and developed into bark discoloration and dieback, resulting in wilting. Fungal re-isolation from symptomatic tissues resulted in the recovery of fungi morphologically consistent with the respective inoculated isolates. The recovered isolates showed cultural and morphological characteristics comparable to the original ZD4-7 and A6 cultures, and their identity was further confirmed by molecular sequencing. In contrast, neither the inoculated fungi nor corresponding disease symptoms were detected in the control seedlings. These results fulfilled the pathogenicity requirement and confirmed the pathogenic potential of ZD4-7 and A6 on mango seedlings (Fig. 3).

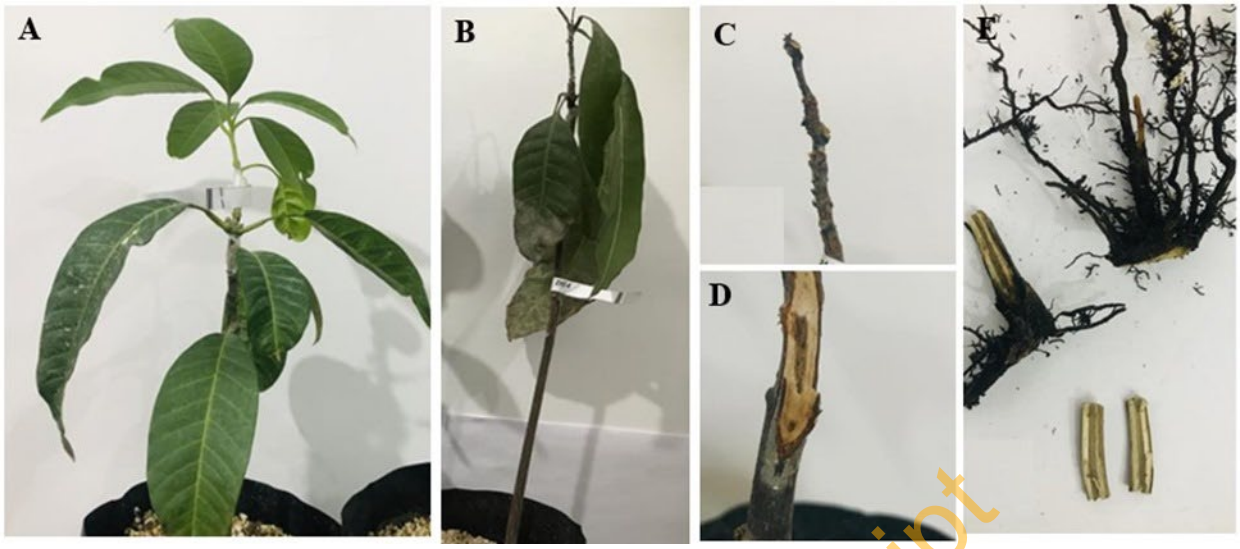

Fig. 3. Pathogenicity test of *Fusarium* isolates on mango seedlings. (A) Control, (B) leaf discoloration and defoliation at the shoot tips, (C) dieback of the apical bud, (D, E) vascular discoloration.

DISCUSSION

The present study provides evidence that *Fusarium annulatum* is associated with mango branch dieback and malformed inflorescences in Iran. The fungus was consistently recovered from symptomatic dieback tissues and from malformed inflorescences, and its identity was confirmed using morphological and molecular characteristics. The recovery of *F. annulatum* from both vegetative and reproductive tissues suggests that this species may be associated with more than one disease syndrome in mango, particularly branch dieback and floral malformation.

Mango floral malformation is a complex and economically important disease syndrome characterized by abnormal development of vegetative and reproductive shoots. Several *Fusarium* species have previously been reported in association with mango malformation in different mango-growing regions (Ploetz 1994, Britz et al. 2002, Haggag and Hassan 2011). In particular, *F. subglutinans* and related *Fusarium* species have been repeatedly associated with malformed mango inflorescences, supporting the importance of the *Fusarium* genus in this disease complex (Ploetz 1994, Britz et al. 2002). The detection of *F. annulatum* in malformed mango inflorescences in the present study expands the range of *Fusarium* species found in association with mango floral malformation and provides evidence of its occurrence in malformed inflorescences in Iran. However, because pathogenicity tests were not conducted on mango inflorescences, the role of *F. annulatum* in the development of floral malformation remains to be determined. Importantly, the pathogenicity tests demonstrated that representative *F. annulatum* isolates, including ZD4-7 and A6, induced dieback symptoms on mango seedlings, whereas the non-inoculated control plants remained asymptomatic. The development of symptoms following inoculation, together with the successful re-isolation of the fungus from symptomatic tissues, supports the pathogenic role of *F. annulatum* in mango branch dieback.

The occurrence of *F. annulatum* in association with both branch dieback and malformed inflorescences may have important implications for mango production. Branch dieback can directly affect tree health and productivity, while floral malformation interferes with normal inflorescence development and may consequently reduce fruit production. Previous studies have emphasized the importance of Fusarium species in mango malformation and their potential involvement in the abnormal development of reproductive tissues (Ploetz 1994, Haggag et al. 2011). The presence of *F. annulatum* in these two types of symptomatic tissues in the present study warrants further investigation to determine its epidemiological role and whether it contributes directly to the development and progression of floral malformation under field conditions.

To our knowledge, this study represents the first report of *F. annulatum* associated with malformed inflorescences and causing mango branch dieback in Iran. The combination of isolation from naturally symptomatic tissues, molecular identification, and pathogenicity testing provides strong evidence for the involvement of *F. annulatum* in mango disease in Iran.

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AUTHOR CONTRIBUTION

Farzaneh Damani Zamani: Investigation, Methodology, Data curation, Writing – original draft. **Mojtaba Keykhasaber:** Project administration, Supervision, Methodology, Investigation, Validation, Writing – review and editing**. Mahdi Pirnia:** Investigation, Data curation, Formal analysis, Writing – review and editing**. Shir Ahmad Sarani:** Investigation, advisor, Writing – review and editing. **Adel Pordel:** Project administration, Conceptualization, Methodology, Validation, Writing – review and editing.

DATA AVAILABILITY

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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DECLARATION

The authors declare no competing interests.

ETHICAL APPROVAL

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

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مقاله کوتاه

گونه قارچی *Fusarium annulatum* جدا شده از گل آذین‌های بدشکل و عامل بیماری خشکیدگی سرشاخه درختان انبه در جنوب شرق ایران

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چکیده

در طی بررسی باغ‌های انبه (*Mangifera indica* L., *Anacardiaceae*) در استان سیستان و بلوچستان، جنوب‌شرق ایران، علائم خشکیدگی سرشاخه و بدشکلی گل‌آذین مشاهده شد. در مجموع، ۱۴ جدایه قارچی شامل ۹ جدایه از سرشاخه‌های دارای علائم و ۵ جدایه از گل‌آذین‌های بدشکل جداسازی شد. تمامی جدایه‌ها بر اساس ویژگی‌های ریخت‌شناختی و تجزیه‌های فیلوژنتیکی چندژنی نواحی ITS-rDNA، *tef1-α* و *tub2* با استفاده از جدایه نماینده ZD4-7، به‌عنوان *Fusarium annulatum* شناسایی شدند. نتایج آزمون‌های بیماری‌زایی نشان داد که این گونه قادر به ایجاد علائم خشکیدگی در شاخه‌های انبه است. با این حال، آزمون بیماری‌زایی روی گل‌آذین‌های انبه در مطالعه حاضر انجام نشد. این مطالعه نخستین گزارش از حضور *F. annulatum* همراه با گل‌آذین‌های بدشکل انبه و توانایی آن در ایجاد علائم خشکیدگی سرشاخه در آزمون‌های بیماری‌زایی در ایران می‌باشد که به گسترش دانش موجود درباره قارچ‌های مرتبط با بیماری‌های انبه در ایران کمک می‌کند.

واژگان کلیدی: بارکدگذاری DNA، خشکیدگی سرشاخه، سیستان و بلوچستان، فیلوژنی چندژنی، *Mangifera indica*

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