Identification of *Canariomyces notabilis* associated with *Ficus carica* branch bark discoloration

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Abstract

This study, reports the first isolation and characterization of *Canariomyces notabilis* from Iran, an emerging pathogenic fungus associated with bark discoloration of branches in fig trees (*Ficus carica*). Morphological, cultural, and molecular analyses were employed to identify isolates obtained from symptomatic bark. Phylogenetic analysis of *tub2* gene sequences using Bayesian inference and maximum likelihood methods robustly placed the isolates within the *C. notabilis* clade, demonstrating a close relationship with the type strain. To establish pathogenicity, Koch's postulates were fulfilled through controlled inoculation experiments. Inoculation of detached fig shoots with mycelial plugs consistently induced brown necrosis within eight days. Similarly, conidial suspensions applied to detached leaves resulted in necrotic and chlorotic lesions within one week. Inoculation of one-year-old fig saplings using conidial suspensions revealed the pathogen's capability to cause external lesions and internal vascular discoloration, extending both proximally and distally from inoculation sites over three months. Successful re-isolation of the fungus from symptomatic tissues confirmed its etiological role. This research establishes *C. notabilis* as a new fungal species for the funga of Iran and identifies it as an emerging pathogen of fig trees, representing the first documented report of this species as a plant pathogen.

Keywords: β-tubulin gene, Chaetomiaceae, emerging pathogen, molecular phylogenetics, Moraceae

شناسایی *Canariomyces notabilis* همراه با تغییر رنگ پوست شاخه *Ficus carica*

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خلاصه

این مطالعه، نخستین گزارش از جداسازی و شناسایی قارچ *Canariomyces notabilis* از ایران، همراه با علایم نوظهور تغییر رنگ پوست شاخه‌های انجیر خوراکی (*Ficus carica*) است. جداسازی از پوست شاخه‌های علایم‌دار انجام شد و شناسایی جدایه‌های به دست آمده با بررسی‌های ریخت‌شناختی و مولکولی انجام گرفت. واکاوی‌های فیلوژنتیکی براساس توالی‌های ژن بتاتوبولین (*tub2*) با استفاده از روش‌های استنتاجِ بیزی و درست‌نمایی بیشینه جدایه‌ها را در تبار *C. notabilis* در کنار جدایه تیپ این گونه قرار داد. به منظور اثبات بیماری‌زایی، اصول کُخ از طریق آزمون‌های بیماری‌زایی متعددی انجام شد. مایه‌زنی شاخه‌های بریده انجیر با بلوک‌های میسیلیومی، منجر به ظهور علایم بافت‌مردگی و تغییر رنگ چوب در مدت هشت روز پس از مایه‌زنی شد. همچنین، برگ‌های مایه‌زنی‌شده انجیر با سوسپانسیون هاگ، یک هفته پس از مایه‌زنی، علایم بافت‌مردگی و هاله سبزردی را در اطراف محل‌های مایه‌زنی نشان دادند. به علاوه، مایه‌زنی نهال‌های یک‌ساله انجیر با استفاده از سوسپانسیون هاگ، توانایی این بیمارگر را در ایجاد علایم خارجی (شامل لکه‌های قهوه‌ای روی ساقه‌ها) و همچنین علایم داخلی (شامل تغییر رنگ بافت آوندی به سمت بالا و پایین از محل مایه‌زنی)، در یک بازه سه‌ماهه پس از مایه‌زنی نشان داد. جداسازی مجدد جدایه‌های مایه‌زنی‌شده از حاشیه بافت‌های علایم‌دار، نقش سبب‌شناختی این بیمارگر نوظهور را در علایم مشاهده شده، تأیید کرد. تحقیق حاضر، گونه *C. notabilis* را به عنوان یک گونه جدید برای قارچ‌های ایران و یک بیمارگر نوظهور در درختان انجیر معرفی می‌کند. همچنین، این نخستین مورد مستند از فعالیت این گونه به عنوان یک عامل بیمارگر گیاهی محسوب می‌شود.

واژه‌های کلیدی: بیمارگر نوظهور، تیره توت، ژن بتاتوبولین، فیلوژنتیک مولکولی، Chaetomiaceae

Introduction

The edible fig (*Ficus carica* L.; Moraceae), a fruit species domesticated approximately 11,000 years ago in Southwest Asia and the Mediterranean region, stands as one of the earliest cultivated fruit crops and a significant member of the genus *Ficus* (Kislev *et al.* 2006, Sarkhosh *et al.* 2022, Ramadan 2023). Its global importance as a cultivated crop is substantial, with cultivation spanning approximately 301,486 hectares and an annual production of 1,336,540.71 tons (FAO, 2024). Iran plays a pivotal role in global fig production, cultivating the crop across 12,312 hectares and yielding over 75,432 tons, positioning it as the fifth-largest producer worldwide, following Türkiye, Egypt, Morocco, and Algeria (FAO 2024). The Smyrna-type fig, consumed both fresh and dried, holds considerable economic value in Iran, where it is a primary horticultural product (Jafari *et al.* 2018). Within Iran, Fars Province is a key fig-producing region, with Estahban County distinguished as the leading center for the production and export of the dried Smyrna fig cultivar, 'Sabz' (Tadayon *et al.* 2022).

Fig trees, globally, are susceptible to a variety of fungal pathogens, primarily belonging to the *Ascomycota*, which manifest diverse symptoms on trunks and branches. Reported fungal taxa associated with fig branch and trunk diseases include, member(s) of the Atheliaceae (causing black necrosis in Iran; Negahban *et al.* 2025), Bionectriaceae (associated with canker and dieback in Iran; Bolboli *et al.* 2022b), Botryosphaeriaceae (linked to canker, limb dieback, twig blight, and wilting across Australia, California, China, Italy, Korea, Malaysia, Oman, Tunisia, and Türkiye; Ray *et al.* 2010, Çeliker & Michailides 2012, Chen *et al.* 2018, Seo *et al.* 2019, Aiello *et al.* 2020, Gusella *et al.* 2021, Al-Mahmooli *et al.* 2022, Güney *et al.* 2022, Jabnoun-Khiareddine *et al.* 2022, Nur-Shakirah *et al.* 2022, Gusella *et al.* 2024, Ghaedi *et al.* 2025), Ceratocystidaceae (causing canker and wilt in Italy, Greece, and Japan; Kajitani & Masuya 2011, Tsopelas *et al.* 2021, Habib *et al.* 2023, Gusella *et al.* 2024), Diaporthaceae (associated with canker in Bulgaria, California, Canada, and Iran; Hampson 1981, Ferguson *et al.* 1990, Gomes *et al.* 2013, Banihashemi & Javadi 2009, Bolboli *et al.* 2023), Diatrypaceae (associated with canker in Iran; Ghaedi *et al.* 2023), Dothiodotthiaceae (linked to twig dieback and bud necrosis in Iran; Negahban *et al*. 2026), and Nectriaceae (causing canker and wilt; Bolboli *et al.* 2022a, Gusella *et al.* 2024, Sardooie *et al*. 2026).

A recent survey evaluating the health status of commercial fig orchards (*F. carica* 'Sabz') in Fars Province (Iran) revealed a distinct emerging disorder characterized by the symptom of brown lesions on the bark of one-year-old branches. Fungal isolations obtained from symptomatic tissues yielded isolates related to the Chaetomiaceae within the Sordariales. The primary aims of this study were to identify these isolates through both morphological and molecular analyses, determine their pathogenicity on detached shoots and leaves under laboratory conditions, and assess their pathogenicity on fig saplings in a controlled greenhouse environment.

Materials and Methods

- Fungal isolation

In June 2025, symptomatic fig branches exhibiting bark discoloration were collected from commercially important *F. carica* 'Sabz' orchards in Meshkan District, located 63 km from Neyriz County, Fars Province. Samples were placed in paper bags, stored at 4 °C, and transported to the laboratory within 24 h for bark scraping, internal symptom examination, and fungal isolation. Small fragments of bark and underlying wood (approximately 5 × 5 mm) were excised from the margin between healthy and necrotic tissues to target actively growing pathogens. The fragments were rinsed under running tap water to remove debris, surface-disinfected in 2% sodium hypochlorite (prepared by diluting commercial bleach) for 1 min, and then washed three times with sterile distilled water. Disinfected tissues were placed on sterile paper towels inside a UV-sterilized laminar-flow hood and allowed to air-dry for 10 min. Dried fragments were transferred onto potato dextrose agar (PDA; extract of 300 g/L boiled potato, 20 g/L dextrose, and 16 g/L agar) amended with tetracycline (100 mg/L) to suppress bacterial growth. Plates were incubated at 25 ± 0.5 °C for three days. Emerging fungal colonies were subcultured onto water agar (WA; 20 g/L agar) and incubated at 25 ± 0.5 °C for two days to facilitate hyphal-tip isolation. Individual hyphal tips were then transferred to fresh PDA plates using a sterile needle (Senanayake *et al.* 2020). All purified isolates were preserved and deposited in the fungal culture collection of the Department of Plant Protection, Shiraz University (Shiraz, Iran), for subsequent morphological, molecular, and pathogenicity analyses.

- Morphological and cultural characterization

For morphological and cultural assessments, a 5 × 5 mm mycelial plug taken from one-week-old PDA cultures was transferred onto four different media to examine colony characteristics and microscopic structures. The media included PDA, malt extract agar (MEA; 20 g/L malt extract, and 16 g/L agar), potato carrot agar (PCA; extracts of 20 g/L boiled potato and 20 g/L boiled carrot , and 16 g/L agar), and cornmeal agar (CMA; extract of 20 g/L boiled cornmeal, and 16 g/L agar). Cultures were incubated at 25 ± 0.5 °C for seven days, after which colony traits, including mycelial surface texture, pigmentation on the obverse and reverse sides, and radial growth, were recorded. Growth measurements were taken using a digital caliper (INSIZE®, China, accuracy 0.01 mm). Each isolate was evaluated on three replicate plates per medium.

To visualize and compare growth-rate distributions across media, kernel density estimates were generated in R (Ver. 4.5.1; http://www.r-project.org) using standard base-R functions for density estimation and plotting. Cultures were further monitored for two months at 25 ± 0.5 °C in darkness to assess the development of sexual and asexual morphs following von Arx (1984). Micromorphological features were examined from temporary slides mounted in sterile distilled water under a light microscope (Carl Zeiss® Axiophot, Germany) equipped with a Dino-Eye eyepiece camera (AM423X, Dino-Lite, AnMo Electronics Corporation, Taiwan). Measurements of diagnostic structures were obtained from at least 30 randomly selected units per isolate.

- DNA extraction, PCR, and phylogenetic analysis

For genomic DNA extraction, isolates were cultivated in 30 mL potato dextrose broth (PDB; extract of 300 g/L boiled potato, and 20 g/L dextrose) and incubated for one week at 25 ± 0.5 °C. Mycelial biomass was harvested, and genomic DNA was extracted using the DNG PLUS extraction kit (CinnaGen, Tehran, Iran) following the manufacturer's instructions. DNA concentration and purity were assessed with a NanoDrop MD 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Approximately 100 ng/µL of genomic DNA was used for PCR amplification.

The β-tubulin (*tub2*) locus was selected for phylogenetic reconstruction (Wang *et al.* 2016, Wang *et al.* 2019). Amplification of *tub2* was performed using primers Btub2Fd and Btub4Rd (Woudenberg *et al.* 2009). PCR reactions (25 µL) contained 1 µL genomic DNA (~100 ng), 1 µL each of forward and reverse primers (10 pM), 12.5 µL *Taq* DNA polymerase 2 × Master Mix RED (Amplicon, Odense, Denmark), and 9.5 µL PCR-grade water. Thermal cycling consisted of an initial denaturation at 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 62 °C for 1 min, and 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. Reactions were conducted on a Peltier Thermal Cycler (Bio-Techne, Minneapolis, MN, USA). PCR products were visualized on 1% agarose gels stained with 0.05% ethidium bromide, and successful amplicons were purified and Sanger-sequenced at the Sangon Biotech Co., Ltd. (Shanghai, China) using the same primer pair.

All newly generated sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank). Phylogenetic analyses included 18 ingroup taxa and *Stolonocarpus gigasporus* (Moustafa & Abdel-Azeem) X. Wei Wang & Houbraken CBS 112062 as the outgroup (Table 1). Sequence alignment was performed using MAFFT Ver. 7 (Katoh *et al.* 2019) and manually refined in BioEdit Ver. 7.0.9.1 (Hall 1999). Phylogenetic inference was performed using Maximum Likelihood (ML) and Bayesian Inference (BI) implemented on the TrEase platform (https://thines-lab.senckenberg.de/trease/). BI analyses were run under the GTR+G+I model for 5,000,000 generations with tree sampling every 1000 generations, discarding the first 30% of samples as burn-in. ML analyses were conducted in RAxML using the GTR+G+I model with 1000 bootstrap replicates. Resulting phylogenetic trees were visualized and edited in MEGA Ver. 11 (Tamura *et al.* 2021). Branch support was considered robust when Bayesian posterior probabilities (BI-PP) exceeded 0.90 and ML bootstrap values (ML-BS) exceeded 90%.

Table 1. Isolates included in the phylogenetic analysis in this study

Taxon	Isolate	Origin	*tub2* accession No.
Canariomyces arenarius	CBS 507.74^{T}	Desert soil, Egypt	MK926898
C. microsporus	CBS 276.74^{T}	Desert soil, Egypt	MK926899
C. microsporus	CBS 161.80	Leaf of *Thymus*, Japan	MK926900
C. microsporus	CBS 808.73	Saline desert soil, Kuwait	MK926901
C. notabilis	CBS 508.74	Desert soil, Egypt	MK926903
C. notabilis	CBS 548.83^{T}	Litter of *Phoenix canariensis*, Spain	MK926902
C. notabilis	**YF05**	***Ficus carica*, Iran**	**PZ277504**
C. notabilis	**YF06**	***F. carica*, Iran**	**PZ277505**
C. subthermophilus	CBS 509.74^{T}	Desert soil, Egypt	MK926904
C. vonarxii	CBS 251.85	Substrate unknown, Nigeria	MK926906
C. vonarxii	CBS 160.80^{T}	Dried flower of *Hibiscus*, Sudan	MK926905
Madurella fahalii	CBS 129176^{T}	Mycetoma of a man's foot, Sudan	MK926919
M. mycetomatis	CBS 109801^{T}	Foot mycetoma of a woman, Sudan	MK926920
M. pseudomycetomatis	CBS 129177^{T}	Mycetoma of a man's lower jaw, China	MK926921
M. pseudomycetomatis	CBS 217.55	Man hand, Argentina	MK926922
M. pseudomycetomatis	CBS 124574	Black grains discharged by a case of mycetoma, China	MK926923
M. tropicana	CBS 201.38^{T}	Man foot, Indonesia	MK926924
M. tropicana	CBS206.47	Man foot, Netherlands	MK926925
Stolonocarpus gigasporus[a]	CBS 112062^{T}	Dung of *Camelus dromedarius*, Egypt	MK926936

Note: The isolates generated in this study are in bold. $^{T.}$ Ex-type isolates; [a.] Outgroup

- Impact of *Canariomyces notabilis* on *Ficus carica*

The pathogenic impact of *C. notabilis* isolates associated with fig bark discoloration was evaluated using detached shoots, detached leaves, and one-year-old saplings of *F. carica* 'Sabz'. For detached shoot assays, symptomless shoots (~25 cm × 10 mm) were surface sterilized with 0.5% sodium hypochlorite for 5 min, wounded at the center using a sterile 5 mm cork borer, and inoculated with a 5 × 5 × 2 mm mycelial plug from one-week-old PDA cultures then wounds were sealed with Parafilm (Roux *et al.* 2007, Soltaninejad *et al.* 2017). Control shoots received sterile PDA plugs. Four shoots per isolate were used as biological replicates. Shoots were incubated at 25 ± 0.5 °C under a 16 h light/8 h dark photoperiod. After 8 days, upward lesion length (ULL), downward lesion length (DLL), and lesion width (LW) were measured using a digital caliper. Koch's postulates were confirmed by re-isolation on PDA amended with tetracycline (100 mg/L). Detached leaf assays were conducted using symptomless leaves surface disinfected in 0.5% sodium hypochlorite for 3 min and rinsed with sterile distilled water. Three 2 × 2 mm wounds were made on each leaf, and 20 µL of a conidial suspension (10^6 conidia/mL) was applied to each wound, while controls received sterile distilled water. Four leaves per isolate were incubated at 25 ± 0.5 °C with a 12 h photoperiod on moist sterile cotton wool in sterile plastic dishes. Lesion length and width on adaxial and abaxial surfaces were measured using a digital caliper, and mean values were visualized as radar charts in R graphics (Ver. 4.5.1). Koch's postulates were verified through fungal re-isolation on PDA.

The most aggressive isolate identified in detached shoot assays was further tested on one-year-old fig saplings maintained under greenhouse conditions (20–35 °C). Stems were wounded using a sterilized 2 mm drill bit (3 mm depth), inoculated with 60 µL of a conidial suspension (10^6 conidia/mL), and sealed with Parafilm. Control saplings received sterile distilled water. Three saplings per isolate served as biological replicates. Three months after inoculation, lesion length (LL), lesion width (LW), upward vascular progression (UVP), and downward vascular progression (DVP) were measured after longitudinal stem sectioning. Mean values were used to generate radar charts in R graphics (Ver. 4.5.1). Koch's postulates were confirmed by re-isolation on PDA supplemented with tetracycline (100 mg/L).

Results

- Disease symptoms and fungal isolates

Field surveys conducted in June 2025 within fig orchards of Fars Province (Iran), revealed the presence of dieback symptoms affecting both one-year-old and mature branches of the commercially important dry fig cultivar, 'Sabz'. Meticulous examination of diseased trees identified emerging symptoms of bark discoloration on one-year-old branches (Fig. 1). Internal examination of symptomatic branches, following bark removal and both longitudinal and cross-sectioning, revealed dark brown lesions extending beneath the bark, while the internal woody tissues remained asymptomatic. Fungal isolation from these distinct symptoms yielded three morphologically uniform isolates (YF05, YF06, YF07) from a total of eight isolates collected. These three isolates, exhibiting identical cultural characteristics, were grouped as a single morphotype and subsequently used for detailed morphological, cultural, molecular, and pathogenicity analyses.

Fig. 1. Symptoms of bark discoloration on one-year-old *Ficus carica* 'Sabz' branches infected with *Canariomyces notabilis* from Meshkan District, Neyriz County, Fars Province (Iran). a & c. Discoloration and necrosis on the bark, b & d. Symptom of browning after superficial bark abrasion.

- Morphological and cultural features

Colony of isolates covered four culture media within two weeks. Colonies initially presented with white to gray aerial mycelia on MEA, PCA, and PDA, which later developed into a dark gray to olivaceous gray, floccose texture. In contrast, colonies grown on CMA lacked aerial mycelia, and the mycelial color progressing from gray to dark gray. The reverse side of the colonies varied: on MEA, it was initially white to pale gray, later developing into pale orange to buff at the center with white to pale gray margins. On PDA and PCA, the reverse side transitioned from white to pale gray to brown and olivaceous gray. Distinctly, the reverse side of colonies on CMA appeared dark gray (Fig. 2). Quantitative analysis of growth rates, visualized through a density plot, indicated a higher average growth rate on MEA, with a pronounced peak around 3.4 mm/day. PCA also supported robust growth, with a distribution centered near 3.1 mm/day. The density curves for CMA and PDA were closely aligned, both centered around 3.0 mm/day (Fig. 3).

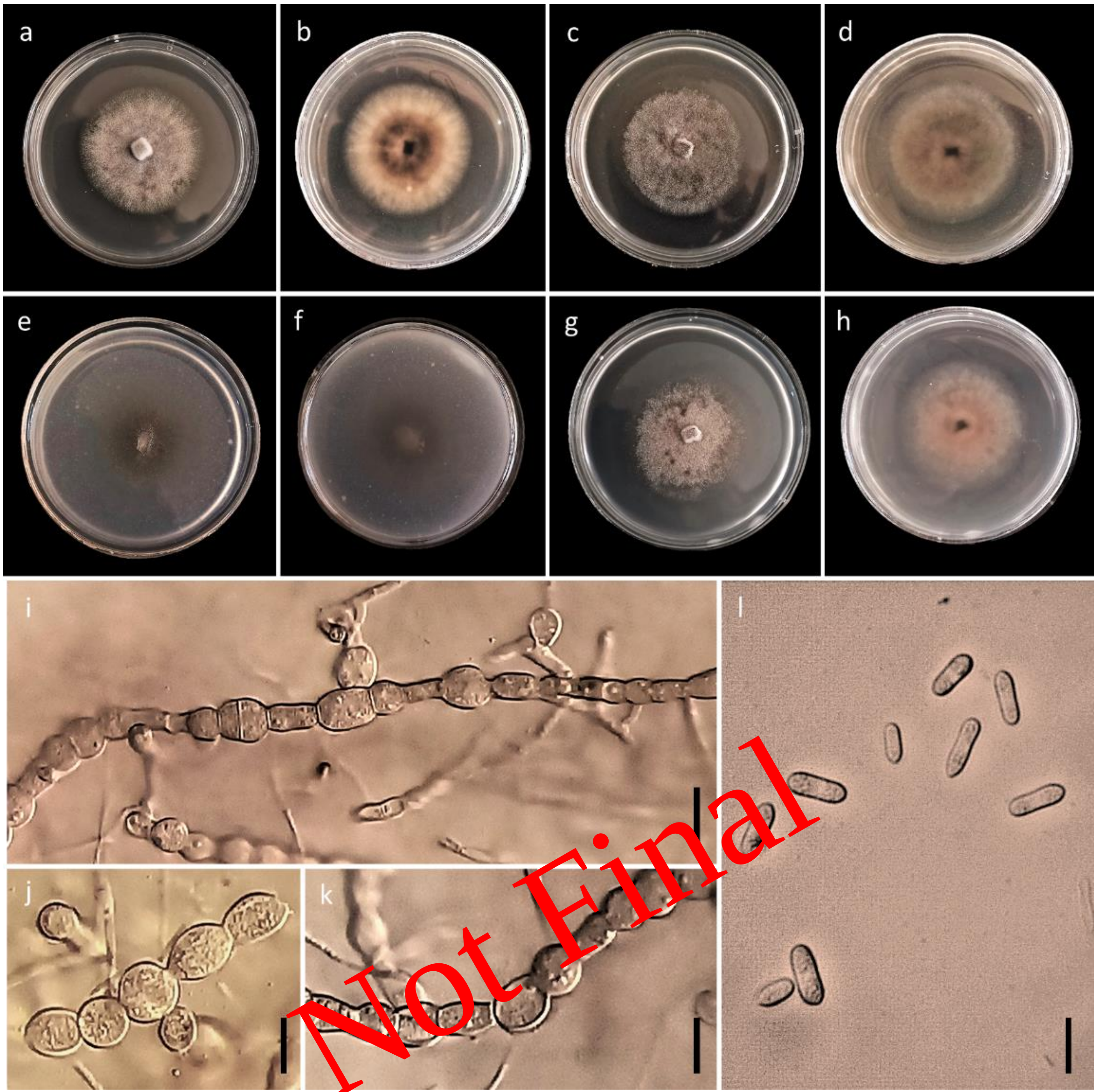

Fig. 2. Colony morphology and micromorphological characteristics of *Canariomyces notabilis* (isolate YF05) on various media after 7 days of incubation at 25 ± 0.5 °C: a & b. Potato dextrose agar (PDA), c & d. Potato carrot agar (PCA), e & f. Corn meal agar (CMA), g & h. Malt extract agar (MEA). Images display both the obverse (a, c, e & g) and reverse (b, d, f & h) colony views, i-k. Catenulate chlamydospore-like structures, l. Conidia (Bars = 10 µm).

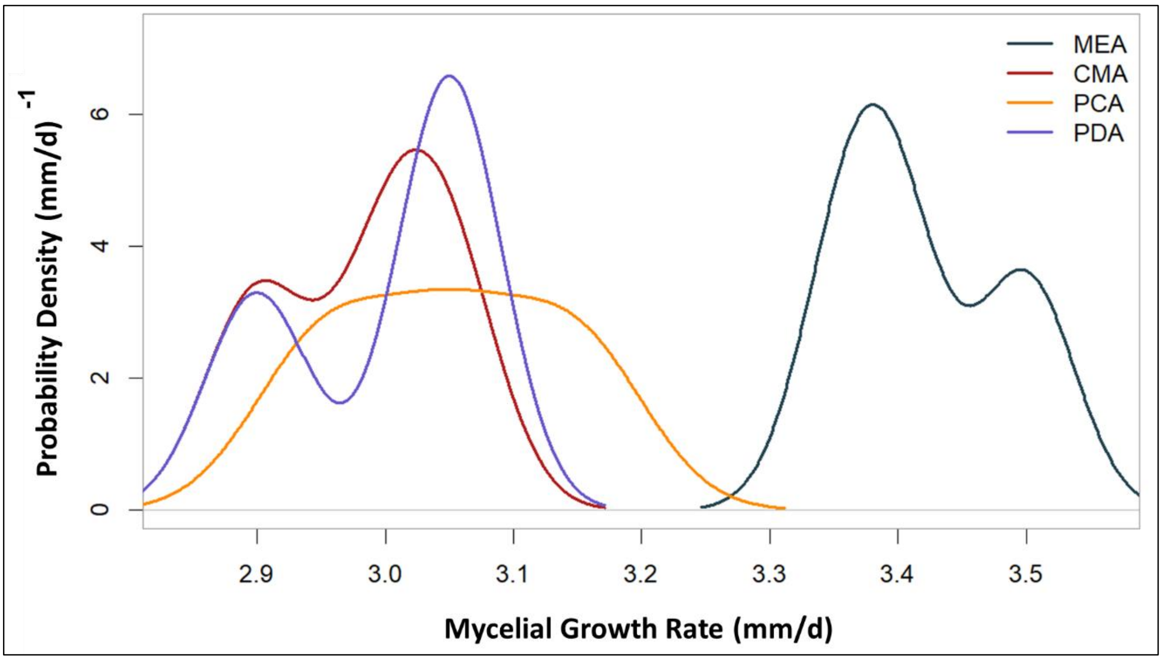

Fig. 3. Comparative analysis of mycelial growth rates (mm/d) for *Canariomyces notabilis* (isolate YF05) across four nutrient media at 25 ± 0.5 °C. The density plot illustrates the distribution of growth rates on Malt Extract Agar (MEA), Cornmeal Agar (CMA), Potato Carrot Agar (PCA), and Potato Dextrose Agar (PDA) (n = 3).

Not Final

Microscopic examination revealed that, hyphae were initially hyaline, later becoming brown to olivaceous gray. Conidiogenous cells were observed as reduced hyphal cells, exhibiting monoblastic development and producing conidia laterally. Conidia were predominantly cylindrical to ellipsoidal, hyaline or pale brown, occasionally truncated at the base, and consistently unicellular, measuring an average of 12.6 ± 1.3 × 4.8 ± 0.7 µm (von Arx 1984, Wang *et al.* 2019). Chlamydospore-like structures were observed after three weeks of incubation on PDA, with average dimensions of 8.7 ± 1.7 × 8.8 ± 1.7 µm (Fig. 2). No sexual structures were observed during the two-month evaluation period across the four culture media.

- Molecular identification and phylogenetic placement

Phylogenetic analyses employing both Bayesian inference (BI) and Maximum Likelihood (ML) methods, based on *tub2* sequences, demonstrated that, the isolates (YF05 and YF07) of the present study, obtained from one-year-old fig branches exhibiting bark discoloration symptoms, clustered within a well-supported monophyletic lineage within the *Canariomyces* clade (BI posterior probability [PP] = 1.00; ML bootstrap support [BS] = 100%). This lineage was closely related to *C. notabilis* Arx isolates, including the ex-type strain CBS 548.83 (Fig. 4, Table 1). The *C. notabilis* clade itself demonstrated robust support in both ML and BI phylograms (BI-PP = 0.99; ML-BS = 93%). The congruent phylogenetic placement of the isolates from this study with their distinct morphological characteristics provides compelling evidence for their identification as *C. notabilis*.

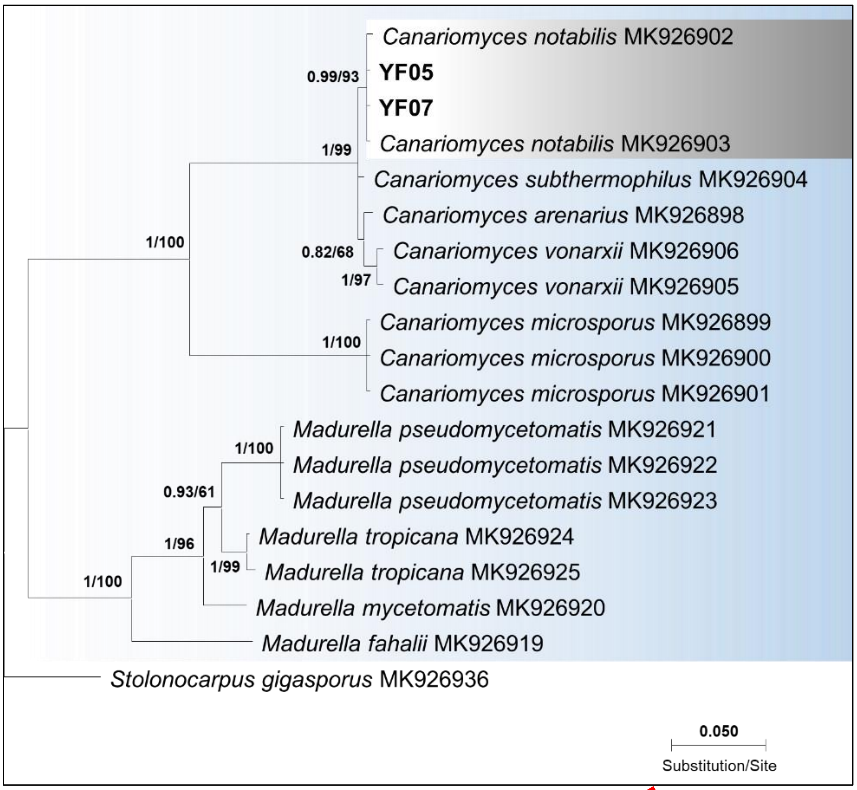

Fig. 4. Phylogenetic position of *Canariomyces notabilis* isolates recovered from dark discoloration of fig (*Ficus carica*) trees sampled in Fars Province (Iran), inferred from Bayesian Inference (BI) of *tub2* (β-tubulin) sequences among *Canariomyces* species. Numbers at nodes represent Bayesian posterior probability values (BI-PP) followed by Maximum Likelihood bootstrap values (ML-BS). Branches with BI-PP = 1 and ML-BS = 100 are considered fully supported. The tree is rooted to *Stolonocarpus gigasporus*. Isolates obtained in this study are shown in bold.

- Impact of *Canariomyces notabilis* on *Ficus carica*

Inoculation of detached fig shoots with mycelial plugs of *C. notabilis* isolates YF05, YF06, and YF07 induced brown necrotic lesions around the inoculation sites within eight days post-inoculation (Fig. 5a–f, u–x), whereas control shoots inoculated with sterile agar plugs remained asymptomatic. Similarly, detached fig leaves inoculated with a conidial suspension of isolate YF05 developed distinct dark brown lesions with chlorotic margins within one week (Fig. 5o, p), while control leaves treated with sterile distilled water showed no symptoms. Mean lesion dimensions on the adaxial and abaxial leaf surfaces were 5.5 ± 0.6 × 7.5 ± 2.6 mm and 4.5 ± 0.5 × 5.0 ± 0.5 mm, respectively (Fig. 5t). Pathogenicity assays on one-year-old fig saplings further confirmed the virulence of isolate YF05 under greenhouse conditions. Three months after stem inoculation with a conidial suspension, dark brown lesions developed around the injection sites, and longitudinal stem sections revealed vascular discoloration extending both upward and downward from the inoculation points (Fig. 5h–m). Control saplings treated with sterile distilled water exhibited no disease symptoms except slight discoloration at the inoculation site. Mean external lesion length and width were 10.7 ± 1.5 mm and 4.7 ± 0.6 mm, respectively, while vascular discoloration extended upward by 10.3 ± 1.5 mm and downward by 13.3 ± 4.9 mm (Fig. 5s). In all pathogenicity assays, the inoculated isolates were successfully re-isolated from symptomatic tissues, thereby fulfilling Koch’s postulates.

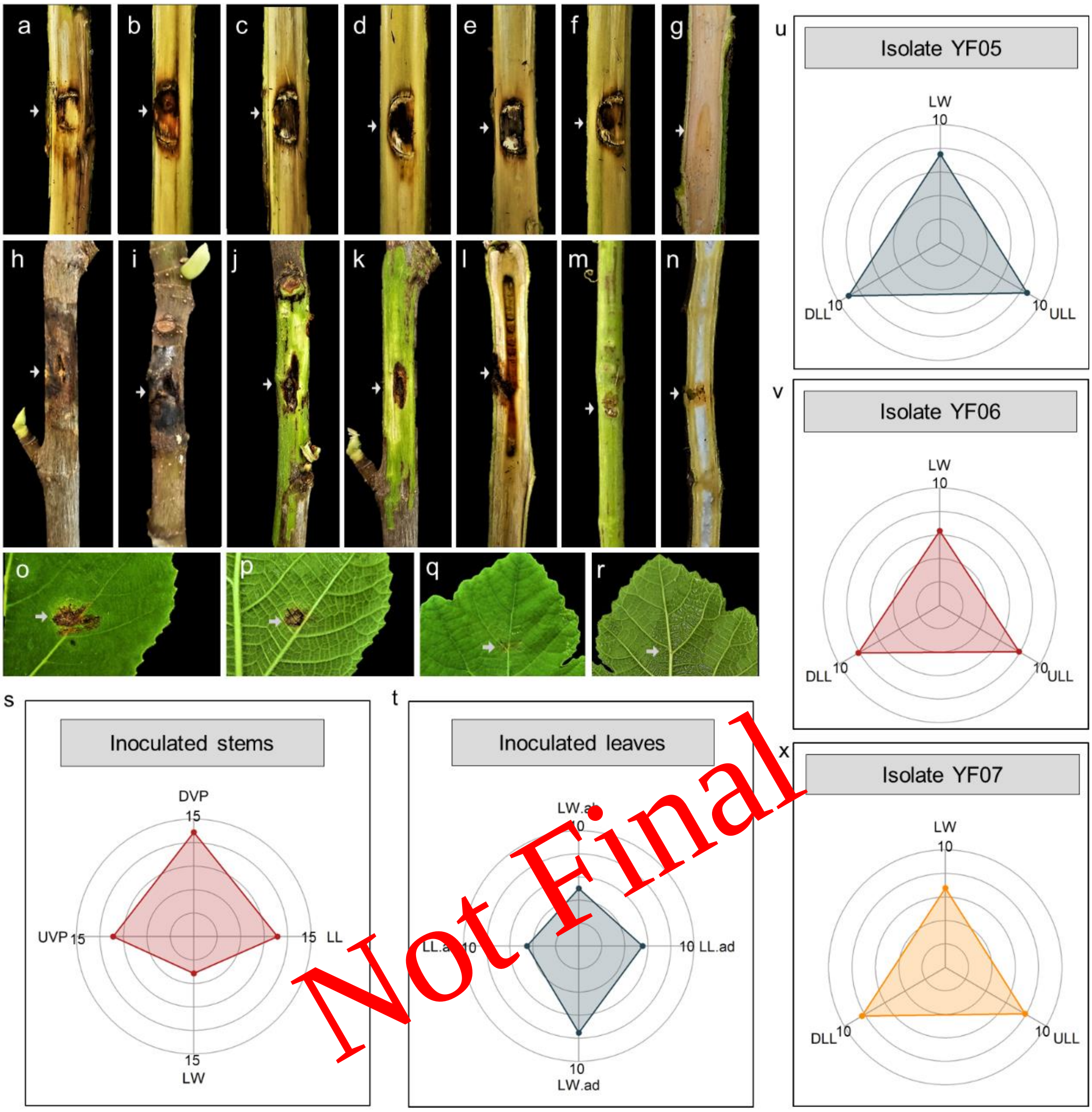

Fig. 5. The effect of *Canariomyces notabilis* isolates on detached fig shoots, one-year-old saplings, and detached leaves, along with radar charts illustrating pathogenicity traits: a–f. Detached fig shoots inoculated with mycelial plugs of isolates YF05 (a & b), YF06 (c & d), and YF07 (e & f), showing brown lesions eight days post-inoculation. Two detached shoots are presented as independent biological replicates per isolate; g. Control shoot inoculated with sterile PDA plugs; h–l. One-year-old saplings inoculated with a conidial suspension of isolate YF05, shown 90 days post-inoculation. Two stems are presented as independent biological replicates. Symptoms include visible lesions on inoculated stems (h & i), brown lesions following bark scratching (j & k), and internal discoloration after longitudinal stem sectioning (l); m–n. Control saplings inoculated with sterile distilled water; o–p. Detached leaves inoculated with a conidial suspension of isolate YF05, after one week, showing the adaxial (o) and abaxial (p) surfaces; q–r. Control leaves inoculated with sterile distilled water. Arrows indicate inoculation sites; s. Mean pathogenicity traits (mm) in inoculated stems, including lesion length (LL), lesion width (LW), upward vascular progression (UVP), and downward vascular progression (DVP) of discoloration, assessed 90 days post-inoculation (n = 3); t. Mean pathogenicity traits (mm) in inoculated leaves, including adaxial lesion length (LL.ad), abaxial lesion length (LL.ab), adaxial lesion width (LW.ad), and abaxial lesion width (LW.ab), assessed one week post-inoculation (n = 4); u–x. Radar charts summarizing mean pathogenicity traits (mm) in inoculated detached shoots for isolates YF05, YF06, and YF07, including upward lesion length (ULL), downward lesion length (DLL), and lesion width (LW), measured eight days post-inoculation (n = 4).

Discussion

This study presents the identification of *Canariomyces notabilis* associated with bark discoloration symptoms on fig trees in southern Iran. To the authors knowledge, this research constitutes the first documentation of this species in

Iran and the first report of its pathogenicity on fig trees, thereby providing the first characterization of *C. notabilis* as a plant pathogen. Investigations into branch diseases affecting fig trees in southern Iran derived from the present investigation, revealed emerging symptoms of bark discoloration, accompanied by discoloration beneath the bark with limited involvement of the woody tissues. In cases of severe disease progression, these symptoms led to branch dieback in both one-year-old and mature branches. Fungal isolates recovered from these symptomatic tissues were subsequently identified as belonging to *C. notabilis* through morphological, cultural, and phylogenetic analyses.

The phylogenetic analysis using both Bayesian Inference and Maximum Likelihood methods based on the *tub2* gene sequences demonstrated the locus's strong discriminatory power for distinguishing *Canariomyces* species. Fig-derived isolates of the present study clustered within a monophyletic clade alongside the type strain CBS 548.83 of *C. notabilis*, confirming their identity. The *tub2* locus has previously been established as a reliable marker for species delimitation within the Chaetomiaceae (Wang *et al.* 2016, Wang *et al.* 2019). Earlier taxonomic studies, employing multilocus phylogenetic analyses, have reclassified the genus *Canariomyces* from the Microascaceae (Microascales) to the Chaetomiaceae, noting its close relationship to genera such as *Madurella* and *Stolonocarpus*, and identifying distinct morphological differences (Wang *et al.* 2019).

Morphological and cultural characterizations of the Iranian *C. notabilis* isolates from fig trees derived fom the present study align with the original species description provided by von Arx (1984), particularly concerning cultural and asexual characteristics. Despite efforts to induce sexual structures on various media over two months, none were observed. This finding is consistent with the observations of Wang *et al.* (2019) on the ex-type isolate *C. notabilis* CBS 548.83, which also predominantly exhibited asexual structures and lacked sexual reproduction under their evaluation conditions. Notably, investigations derived from the present study, revealed the presence of catenulate chlamydospore-like structures in isolates of the present study, a feature not previously recorded in the species description by von Arx (1984). This observation may represent a novel morphological characteristic or variation within the species.

Canariomyces species have previously been described in association with soil and plant substrates in various global locations, including Egypt, Japan, Kuwait, Nigeria, Spain, and Sudan (Mouchacca 1973, von Arx 1975, von Arx 1984, von Arx *et al*. 1988, Wang *et al.* 2019). Prior to this study, no *Canariomyces* species had been investigated as plant pathogens. Here, pathogenicity assays demonstrated that, *C. notabilis* isolates from discolored fig bark induce necrosis and wood discoloration in detached shoots and one-year-old fig saplings, fulfilling Koch's postulates. The fungus also caused lesions on fig leaves, indicating a broader pathogenic potential.

This study, therefore provides the first report of *C. notabilis* in Iran and the first evidence of its pathogenicity on fig worldwide, identifying it as an emerging pathogen of this economically important crop. Given ongoing climate change, bark discoloration symptoms in fig trees may intensify under environmental stress, potentially facilitating colonization by weakly pathogenic or endophytic fungi such as *C. notabilis* (Garbelotto *et al*. 2025). Future studies should investigate its occurrence in asymptomatic fig tissues and neighboring plant species to clarify its latent behavior, host range, and epidemiology, thereby supporting the development of effective disease management strategies.

References

Aiello, D., Gusella, G., Fiorenza, A., Guarnaccia, V. & Polizzi, G. 2020. Identification of *Neofusicoccum parvum* causing canker and twig blight on *Ficus carica* in Italy. Phytopathologia Mediterranea 59(1): 213–218. DOI: 10.14601/PHYTO-10798.

Al-Mahmooli, I.H., Al-Maqbaly, Y.M., Al-Sadi, A.M., Al-Badi, R.S., Velazhahan, R. & Hussain, S. 2022. First report of dieback on fig (*Ficus carica*) caused by *Lasiodiplodia theobromae* in north Al-Batinah governorate of Oman. Plant Disease 106(9): 2522. DOI: 10.1094/PDIS-10-21-2165-PDN.

Banihashemi, Z. & Javadi, A.R. 2009. Further investigations on the biology of *Phomopsis cinerascens*, the cause of fig canker in Iran. Phytopathologia Mediterranea 48: 454–460.

Bolboli, Z., MostowfizadehGhalamfarsa, R., Jafari, M. & Sarkhosh, A. 2023. Susceptibility of fig cultivars to Diaporthe canker in Iran. Plant Pathology 72(3): 507–520. DOI: 10.1111/ppa.13687.

Bolboli, Z., Mostowfizadeh-Ghalamfarsa, R., Sandoval-Denis, M., Jafari, M. & Crous, P.W. 2022a. *Neocosmospora caricae* sp. nov. and *N. metavorans*, two new stem and trunk canker pathogens on *Ficus carica* in Iran. Mycological Progress 21(10): 89. DOI: 10.1007/s11557-022-01834-9.

Bolboli, Z., Tavakolian, B., Mostowfizadeh-Ghalamfarsa, R., Jafari, M. & Cacciola, S.O. 2022b. *Stilbocrea banihashemiana* sp. nov. a new fungal pathogen causing stem cankers and twig dieback of fruit trees. Journal of Fungi 8(7): 694. DOI: 10.3390/jof8070694.

Çeliker, N.M. & Michailides, T.J. 2012. First report of *Lasiodiplodia theobromae* causing canker and shoot blight of fig in Turkey. New Disease Reports 25(12): 2044–0588. DOI: 10.5197/j.2044-0588.2012.025.012.

Chen, Y., Wei, H., Du, G., Zhu, L., Song, Q., Hu, Y., Wang, E., Wang, M. & Fan, X. 2018. First report of *Lasiodiplodia theobromae* causing stem canker on common fig (*Ficus carica*) in Zhejiang Province of China. Plant Disease 102: 2656–2657. DOI: 10.1094/PDIS-05-18-0887-PDN.

FAO. 2024. Food and agriculture organization of the United Nations. FAOSTAT http://www.fao.org/faostat (accessed on 06 April 2026).

Ferguson, L., Michailides, T.J. & Shorey, H.H. 1990. The California fig industry. DOI: 10.1002/9781118060858.ch9.

Garbelotto, M., Scali, E., Swain, S., Macon, D., Lacan, I., Schmidt, D., Popenuck, T., Martino, I. & Guarnaccia, V. 2025. *In*: California's changing climate, latent pathogens drive novel woody plant diebacks on a large geographic scale. Plant Pathology 74(9): 2697–2714. DOI: 10.1111/ppa.70041.

Ghaedi, M., Bolboli, Z. & Mostowfizadeh-Ghalamfarsa, R. 2023. First report of *Peroneutypa scoparia* associated with canker disease on *Ficus carica* in northern Iran. New Disease Reports 48(1). DOI: 10.1002/ndr2.12201.

Ghaedi, M., Bolboli, Z., Salami, M., Jafari, M. & Mostowfizadeh-Ghalamfarsa, R. 2025. Susceptibility of fig cultivars to three fungal pathogens associated with Botryosphaeria canker. Physiological and Molecular Plant Pathology 138: 102685. DOI: 10.1016/j.pmpp.2025.102685.

Gomes, R.R., Glienke, C., Videira, S.I.R., Lombard, L., Groenewald, J.Z. & Crous, P.W. 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31: 1–41. DOI: 10.3767/003158513X666844.

Güney, İ.G., Bozoğlu, T., Özer, G., Türkölmez, Ş. & Derviş, S. 2022. First report of *Neoscytalidium dimidiatum* associated with dieback and canker of common fig (*Ficus carica* L.) in Turkey. Journal of Plant Diseases and Protection 129(3): 701–705. DOI: 10.1007/s41348-022-00586-8.

Gusella, G., Gugliuzzo, A., Guarnaccia, V., Martino, I., Aiello, D., Costanzo, M.B., Russo, A., Groenewald, J.Z., Crous, P.W. & Polizzi, G. 2024. Fungal species causing canker and wilt of *Ficus carica* and evidence of their association by bark beetles in Italy. Plant Disease 108(7): 2136–2147. DOI: 10.1094/PDIS-01-24-0251-RE.

Gusella, G., Morgan, D.P. & Michailides, T.J. 2021. Further investigation on limb dieback of fig (*Ficus carica*) caused by *Neoscytalidium dimidiatum* in California. Plant Disease 105(2): 324–330. DOI: 10.1094/PDIS-06-20-1226-RE.

Habib, W., Carlucci, M., Manco, L., Altamura, G., Delle Donne, A.G. & Nigro, F. 2023. First report of *Ceratocystis ficicola* causing canker and wilt disease on common fig (*Ficus carica*) in Italy. Plant Disease 107(10): 3287. DOI: 10.1094/PDIS-03-23-0464-PDN.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hampson, M.C. 1981. Phomopsis canker on weeping fig in Newfoundland. Canadian Plant Disease Survey 61: 3–5.

JabnounKhiareddine, H., Aydi Ben Abdallah, R., Mars, M. & Daami-Remadi, M. 2022. First report of fig tree dieback caused by *Lasiodiplodia theobromae* in Tunisia. Journal of Phytopathology 170(7–8): 546–556. DOI: 10.1111/jph.13104.

Jafari, M., Rahemi, M. & Haghighi, A.A.K. 2018. Role of fig rootstock on changes of water status and nutrient concentrations in 'Sabz' cultivar under drought stress condition. Scientia Horticulturae 230: 56–61. DOI: 10.1016/j.scienta.2017.10.014.

Kajitani, Y. & Masuya, H. 2011. *Ceratocystis ficicola* sp. nov., a causal fungus of fig canker in Japan. Mycoscience 52(5): 349–353. DOI: 10.1007/S10267-011-0116-5.

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. DOI: 10.1093/molbev/mst010.

Kislev, M.E., Hartmann, A. & Bar-Yosef, O. 2006. Early domesticated fig in the Jordan Valley. Science 312(5778): 1372–1374. DOI: 10.1126/science.1125910.

Mouchacca, J. 1973. Les *Thielavia* des sols arides: epseces nouvelles et analyse generique. Bulletin trimestriel de la Societe Mycologique de France 89: 295–311.

Negahban, H., Bolboli, Z., Jafari, M. & Mostowfizadeh-Ghalamfarsa, R. 2026. *Thyrostroma parviniae* sp. nov., causing bud necrosis and branch dieback in fig trees from Iran. In Press. DOI: 10.1371/journal.pone.0341992.

Negahban, H., Bolboli, Z. & Mostowfizadeh-Ghalamfarsa, R. 2025. Emerging Fig Black Necrosis incited by *Butlerelfia eustacei* and its potential impact on various fig cultivars. Journal of Plant Pathology 107(1): 335–350. DOI: 10.1007/s42161-024-01739-4.

Nur-Shakirah, A.O., Khadijah, M.S., Kee, Y.J., Huda-Shakirah, A.R., Mohd Hafifi, A.B., Nurul-Aliyaa, Y.A., Chew, B.L., Zakaria, L., Nor, N.M.I.M., Sreeramanan, S., Leong, Y. & Mohd, M.H. 2022. Characterization of *Lasiodiplodia* species causing leaf blight, stem rot and fruit rot of fig (*Ficus carica*) in Malaysia. Plant Pathology 71(7): 1594–1605. DOI: 10.1111/ppa.13580.

R Core Team. 2023. *R:* A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org.

Ramadan, M.F. (ed.). 2023. Fig (*Ficus carica*): Production, processing, and properties. Springer Nature. DOI: 10.1007/978-3-031-16493-4.

Ray, J.D., Burgess, T. & Lanoiselet, V.M. 2010. First record of *Neoscytalidium dimidiatum* and *N. novaehollandiae* on Mangifera indica and *N. dimidiatum* on *Ficus carica* in Australia. Australasian Plant Disease Notes 5(1): 48–50. DOI: 10.1071/DN10018.

Roux, J., Eisenberg, B., Kanzler, A., Nel, A., Coetzee, V., Kietzka, E. & Wingfield, M.J. 2007. Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. New Forests 33(2): 109–123. DOI: 10.1007/s11056-006-9017-4.

Sardooei, S., Bolboli, Z., Jafari, M. & Mostowfizadeh-Ghalamfarsa, R. 2026. *Fusarium decemcellulare* and *F. annulatum*, two emerging canker pathogens of fig trees and their potential pathogenicity on some fruit trees. Journal of Plant Diseases and Protection 133(2): 45. DOI: 10.1007/s41348-026-01243-0.

Sarkhosh, A., Yavari, A. & Ferguson, L. 2022. The fig: botany, production and uses. CABI, Wallingford. DOI: 10.1079/9781789242881.0000.

Senanayake, I.C., Rathnayaka, A.R., Marasinghe, D.S., Calabon, M. S., Gentekaki, E., Lee, H.B., Hurdeal, V.G., Pem, D., Dissanayake, L.S., Wijesinghe, S.N., Bundhun, D., Nguyen, T.T., Goonasekara, I.D., Abeywickrama, P.D., Bhunjun, C.S., Jayawardena, R.S., Wanasinghe, D.N., Jeewon, R., Bhat, D.J. & Xiang, M.M. 2020. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere 11(1): 2678–2754. DOI: 10.5943/mycosphere/11/1/20.

Seo, Y., Back, C.G., Park, M.J. & Park, J.H. 2019. First report of *Lasiodiplodia theobromae* causing canker and dieback of common fig in Korea. Plant Disease 103(5): 1023–1023. DOI: 10.1094/PDIS-09-18-1613-PDN.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52(3–4): 591–611. DOI: 10.1093/biomet/52.3-4.591.

Soltaninejad, N., Mohammadi, H. & Massumi, H. 2017. Isolation, identification and pathogenicity of Botryosphaeriaceae and Phaeoacremonnium species associated with decline of *Prunus* species in Iran. Journal of Plant Pathology 99: 571–581.

Tadayon, M.S. & Hosseini, S.M. 2022. Shade net and mulching measures for improving soil and plant water status of fig trees under rainfed conditions. Agricultural Water Management 271: 107796. DOI: 10.1016/j.agwat.2022.107796.

Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. DOI: 10.1093/molbev/msab120.

Tsopelas, P., Soulioti, N., Wingfiield, M.J., Barnes, I., Marincowitz, S., Tjamos, E.C. & Paplomatas, E.J. 2021. *Ceratocystis ficicola* causing a serious disease of *Ficus carica* in Greece. Phytopathologia Mediterranea 60(2): 337–349. DOI: 10.36253/phyto-12794.

von Arx, J.A. 1975. On *Thielavia* and some similar genera of ascomycetes. Studies in Mycology 8: 1–32.

von Arx, J.A. 1984. *Canariomyces notabilis*, a peculiar ascomycete from the Canary Isolands. Persoonia 12(2): 185–187.

von Arx, J.A., Figueras, M.J. & Guarro, J. 1988. Sordariaceous ascomycetes without ascospore ejaculation. Beiheft zur Nova Hedwigia 94: 1–104.

Wang, X.W., Lombard, L., Groenewald, J.Z., Li, J., Videira, S.I.R., Samson, R.A., Liu, X.Z. & Crous, P.W. 2016. Phylogenetic reassessment of the *Chaetomium globosum* species complex. Persoonia 36: 83–133. DOI: 10.3767/003158516X689657.

Wang, X.W., Bai, F.Y., Bensch, K., Meijer, M., Sun, B.D., Han, Y.F., Crous, P.W., Samson R.A., Yang, F.Y. & Houbraken, J. 2019. Phylogenetic re-evaluation of *Thielavia* with the introduction of a new family Podosporaceae. Studies in Mycology 93: 155–252. DOI: 10.1016/j.simyco.2019.08.002.

Woudenberg, J.H.C., Aveskamp, M.M., De Gruyter, J., Spiers, A.G. & Crous, P.W. 2009. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. Persoonia. Molecular Phylogeny and Evolution of Fungi 22(1): 56–62. DOI: 10.3767/003158509X427808.