

## Original Article

# Identification and pathogenic characterization of *Colletotrichum fructicola* causing anthracnose on *Ficus binnendijkii* in southern Iran

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## ABSTRACT

During a survey of ornamental landscape trees in Bushehr Province, southern Iran, dark-brown leaf spot symptoms were observed on *Ficus binnendijkii* trees, a popular cultivated ornamental species. Fungal isolates from symptomatic leaves were identified as *Colletotrichum fructicola* through morphological and molecular analyses of the *tub2* and *gapdh* loci, with phylogenetic evidence confirming their close similarity to reference strains. To confirm pathogenicity, both wounded and unwounded *F. binnendijkii* leaves were inoculated with *C. fructicola* mycelial plugs and spore suspensions, and the isolated fungus was found to induce substantial necrosis, thus fulfilling Koch's postulates. Analysis of variance (ANOVA) indicated highly significant differences in lesion progression metrics (lesion length and width) among the different inoculation methods ( $P < 0.0001$ ). Specifically, inoculation of wounded leaves with mycelial plugs resulted in more severe lesions compared to the other inoculation methods tested. This study represents the first documented global incidence of anthracnose caused by *C. fructicola* on *F. binnendijkii*. These findings highlight the need for future investigations into the epidemiology of this pathogen on other ornamental plants and the development of effective disease management strategies.

## KEYWORDS

Ascomycota, Leaf spot, Moraceae, Ornamental plants, Pathogenicity.

## INTRODUCTION

The genus *Ficus*, commonly known as fig, belongs to the family *Moraceae* and comprises approximately 800 species (Corner 1965). Many *Ficus* species are cultivated for their ornamental appeal, serving as landscape plants in tropical and subtropical regions (Dehgan 1998) or as popular foliage plants for interior decoration. *Ficus binnendijkii* L. is a particularly adaptable species, valued for its indoor resilience and its frequent use in topiary design (Babaie et al. 2014). This species is native to Southeast Asia and is characterized by its long, saber-shaped, and dark-green leaves (Fang et al. 2007).

Ornamental *Ficus* species are vulnerable to a range of diseases, including fungal, bacterial, and viral infections, which can severely compromise their aesthetic appeal and commercial value (Crouch 2012, Ahmadpour et al. 2024). Among these, fungal pathogens pose a particularly significant threat to ornamental plant production and marketing (Ahmadpour et al. 2024). Leaf anthracnose, caused by various fungal agents, is one of the most prevalent and economically damaging diseases affecting ornamental plants. Species within the genus *Colletotrichum* (*Glomerellaceae*) are recognized by the plant pathology community as among the top ten most economically and scientifically important fungal phytopathogens (Crouch 2012). *Colletotrichum* spp. have been responsible for substantial losses across a wide range of crops globally, including nursery and landscape plants cultivated for ornamental purposes (Crouch 2012). Recent studies have highlighted the pathogenicity of *Colletotrichum* spp. on various *Ficus* species. Notable examples include *C. tropicale* E.I. Rojas, S.A. Rehner & Samuels on *F. altissima* L. (Xie et al. 2025), and *F. binnendijkii* L. (Kong et al. 2020); *C. karstii* You L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai and *C. orchidearum* Allesch. on *F. benamina* L. (Abdinezhad et al. 2024, Ahmadpour et al. 2024); *C. fructicola* Prihast., L. Cai & K.D. Hyde, *C. gloeosporioides* (Penz.) Penz. & Sacc., *C. siamense* Prihast., L. Cai & K.D. Hyde, and *C. truncatum* (Schwein.) Andrus & W.D. Moore on *F. carica* L. (Choi et al. 2013, Nur-Shakirah et al. 2023, Ye et al., 2025); *C. fructicola* on *F. hirta* L. (Zheng et al. 2024); *C. siamense* Prihast.,

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L. Cai & K.D. Hyde on *F. macrocarpa* L. (Lin 2023); and *C. fiorinae* R.G. Shivas & Y.P. Tan on *F. virens* L. (Xue et al. 2017).

During a health assessment survey in 2025 of ornamental landscape trees in Bushehr, Bushehr Province, southern Iran, several isolates morphologically resembling *Colletotrichum* species were recovered from *F. binnendijkii* trees exhibiting symptoms of brown leaf spot. The present study was designed to: (i) identify the obtained isolates through detailed morphological and molecular investigations, including phylogenetic analysis; (ii) determine the pathogenicity of the isolates using different inoculation methods; and (iii) assess the efficacy of various leaf inoculation methods by quantifying lesion development and performing statistical analysis.

## MATERIALS AND METHODS

### Sampling and Fungal Isolation

In March 2025, a field survey was conducted in Bushehr, Bushehr Province, to evaluate the health status of ornamental landscape trees. Symptomatic leaves of *F. binnendijkii* exhibiting anthracnose and brown leaf spot lesions surrounded by a yellow halo were collected from multiple landscape sites. To isolate the causal agent, collected symptomatic leaves were rinsed with tap water, then air-dried. Subsequently, sections of leaf tissue (5 × 5 mm) that displayed the characteristic yellow halo around necrotic lesions were excised and surface-disinfected using a 1.5% sodium hypochlorite solution (prepared from 30% (v/v) commercial bleach containing 5% sodium hypochlorite) for one minute, followed by three rinses with sterile distilled water under aseptic conditions. These surface-sterilized leaf tissues were then transferred to potato dextrose agar (PDA; prepared from extract of 300 g/L boiled potato, 20 g/L dextrose, 16 g/L agar, and distilled water) and incubated at 25 ± 0.5 °C in the dark for three days to allow fungal colonies to emerge. Emerging fungal colonies were then transferred to water agar (WA; 20 g/L agar in distilled water) and incubated at 25 ± 0.5 °C for two days. Hyphal tips were taken from the colony margins using a sterile needle and transferred to PDA to obtain pure cultures. The pure isolates were subsequently used for morphological and molecular identification.

### Morphological and Cultural Characterization

Cultures grown on PDA at 25 ± 0.5 °C were used for morphological characterization. Colony color and texture were examined, and colony growth rate was calculated by measuring colony diameter six days after incubation. Cultures were monitored daily for 15 days to observe the formation of conidiomata and the development of perithecia. Conidia and ascospores obtained from mature conidiomata and perithecia were mounted in sterile water, and the length and width of at least 30 randomly selected spores were measured (Prihasuti et al. 2009). Microscopic characteristics were examined using a light microscope (Standard 20, Carl Zeiss, Oberkochen, Germany) equipped with a Dino-Eye Eyepiece Camera (AM423X, Dino-Lite, AnMo Electronics Corporation, Taiwan).

### DNA Extraction

For DNA extraction, three mycelial plugs (5 × 5 mm) from the representative pure isolate were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of potato broth (PB; extract of 300 g/L boiled potato in distilled water) and incubated for one week at 25 ± 0.5 °C. The resulting mycelial biomass was harvested, freeze-dried, and used for genomic DNA extraction with the DNG™-PLUS kit (CinnaGen, Iran), following the manufacturer's instructions. DNA concentration and purity were assessed using an MD-1000 NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and samples were adjusted to a final concentration of 100 ng/μL for downstream PCR analyses.

### PCR, Sequencing, and Phylogenetic Analysis

The beta-tubulin (*tub2*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) genes were amplified via polymerase chain reaction (PCR) using the primer pairs Btub2Fd/Btub4Rd (Woudenberg et al. 2009) and *gpd1/gpd2* (Berbee et al. 1999), respectively. The thermal cycling conditions in polymerase chain reaction (PCR) consisted of initial denaturing for 2 min at 95 °C; followed by 35 cycles for 1 min at 95 °C, 40 s at 56 °C, 45 s at 72 °C, then 10 min at 72 °C for *tub2* and 5 min at 96 °C; followed by 35 cycles of 1 min at 96 °C, 1 min at 56 °C, and 45 s at 72 °C for *gapdh*. Sequence data were initially identified using BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and subsequently deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank>). For phylogenetic reconstruction, sequences were aligned using the MAFFT v.7 online server (<https://mafft.cbrc.jp/alignment/server/>). Phylogenetic relationships were then inferred using Bayesian inference (BI) and Maximum Likelihood (ML) methods, implemented in TrEase (Mishra et al. 2023). MEGA v.11.0.13 (Tamura et al. 2021) was employed for visualizing phylogenetic trees.

### Pathogenicity Assessments

To ascertain pathogenicity, detached leaves of *F. binnendijkii* underwent surface disinfection with 70% ethanol, followed by three rinses with sterile distilled water, and were subsequently dried on sterile filter paper. These leaves were then subjected to four distinct inoculation treatments. A set of twelve leaves was inoculated with mycelial plugs (2 × 3 mm) excised from five-day-old *C. fructicola* (isolate BFA06) colonies cultured on PDA. Within this set, six leaves were scarified (2 × 3 mm) with a sterile needle prior to inoculation, while the remaining six leaves remained un wounded (Zhang et al. 2018, Bregant et al. 2024). Control leaves were inoculated with sterile PDA plugs. In a separate treatment, twelve

leaves were inoculated with a spore suspension ( $1 \times 10^6$  conidia/mL). Here, six leaves were scarified ( $2 \times 3$  mm) using a sterile needle, while the remaining six leaves were left unwounded before inoculation with 20  $\mu$ L of the spore suspension, which was derived from a five-day-old culture of isolate BFA06. Sterile distilled water served as the control for this treatment (Bhuyan et al. 2024). All inoculated leaves were incubated in a humid chamber maintained at  $25 \pm 1$  °C. The effect of inoculation methods on lesion length and width, as indicators of pathogenicity, was analyzed using a one-way Analysis of Variance (ANOVA) employing a General Linear Model (GLM) in SAS (v.9.4, SAS Institute, Cary, NC, USA). Mean separation was performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Disease Symptoms and Fungal Isolates

*Ficus binnendijkii* trees exhibiting symptoms of brown leaf spot were observed in Bushehr urban landscapes. Initial disease manifestations appeared as small, circular to irregular brown spots on leaf blades. As the disease progressed, these lesions expanded, developing a distinctive chlorotic halo surrounding tan-brown centers, culminating in leaf desiccation (Fig. 1). Thirteen morphologically similar isolates were recovered from symptomatic leaves and grouped into a single morphotype (Table 1).

### Morphological and Cultural Characteristics

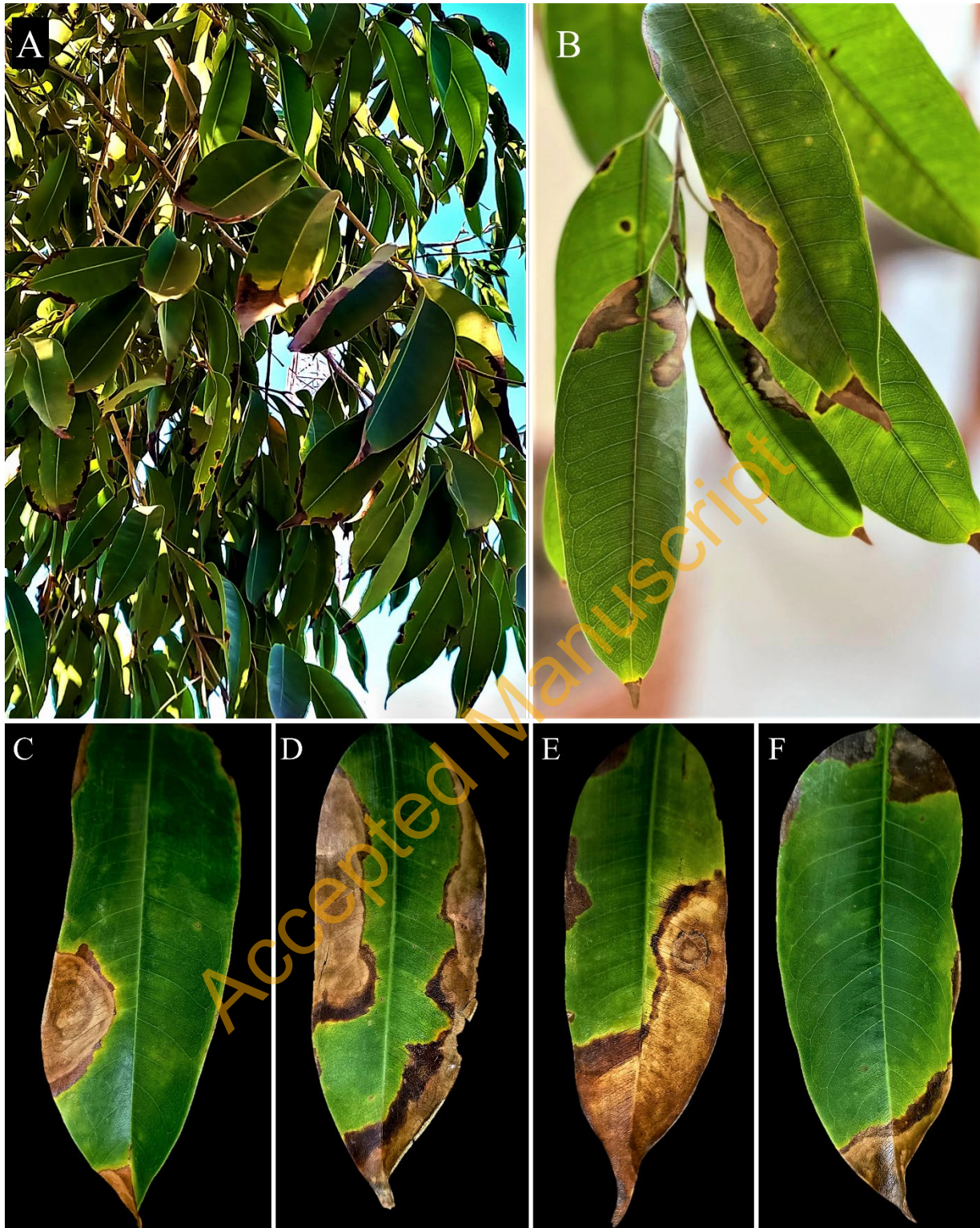
After six days of incubation on PDA at  $25 \pm 0.5$  °C, fungal colonies developed white to slightly gray aerial mycelium with a yellowish to gray reverse, which gradually darkened at the center with age (Fig. 2a, b). After two weeks at  $25 \pm 0.5$  °C, the dense, cottony aerial mycelium produced visible conidia masses (Fig. 2c–f). Microscopic examination revealed hyaline, septate, and branched hyphae, lacking setae. Conidia ( $9.35\text{--}19.11 \times 3.34\text{--}6.98$   $\mu$ m, av.  $14.01 \pm 2.04 \times 4.96 \pm 0.72$   $\mu$ m,  $n = 90$ ) were hyaline, cylindrical, straight, with rounded ends, and abundantly produced (Fig. 2g). Crushed masses of ascomata revealed dark brown, subglobose perithecia, often immersed in the culture medium (Fig. 2h, i). Asci were unitunicate, clavate to cymbiform (Fig. 2j, k), containing eight hyaline, guttulate, fusiform-to-slightly curved ascospores with rounded ends ( $10.50\text{--}19.67 \times 3.51\text{--}6.56$   $\mu$ m, av.  $15.14 \pm 1.89 \times 5.38 \pm 0.65$   $\mu$ m,  $n = 90$ ). The average daily colony growth rate on PDA at  $25 \pm 0.5$  °C was  $11.04 \pm 0.25$  mm. The morphological characterization, encompassing both microscopic and cultural features, revealed a strong resemblance to *Colletotrichum fructicola*, aligning with previous molecularly confirmed descriptions of the species (Prihastuti et al. 2009, Zhang et al. 2015, de Silva et al. 2019, Khodadadi et al. 2020, Zhang et al. 2023).

### Molecular Identification

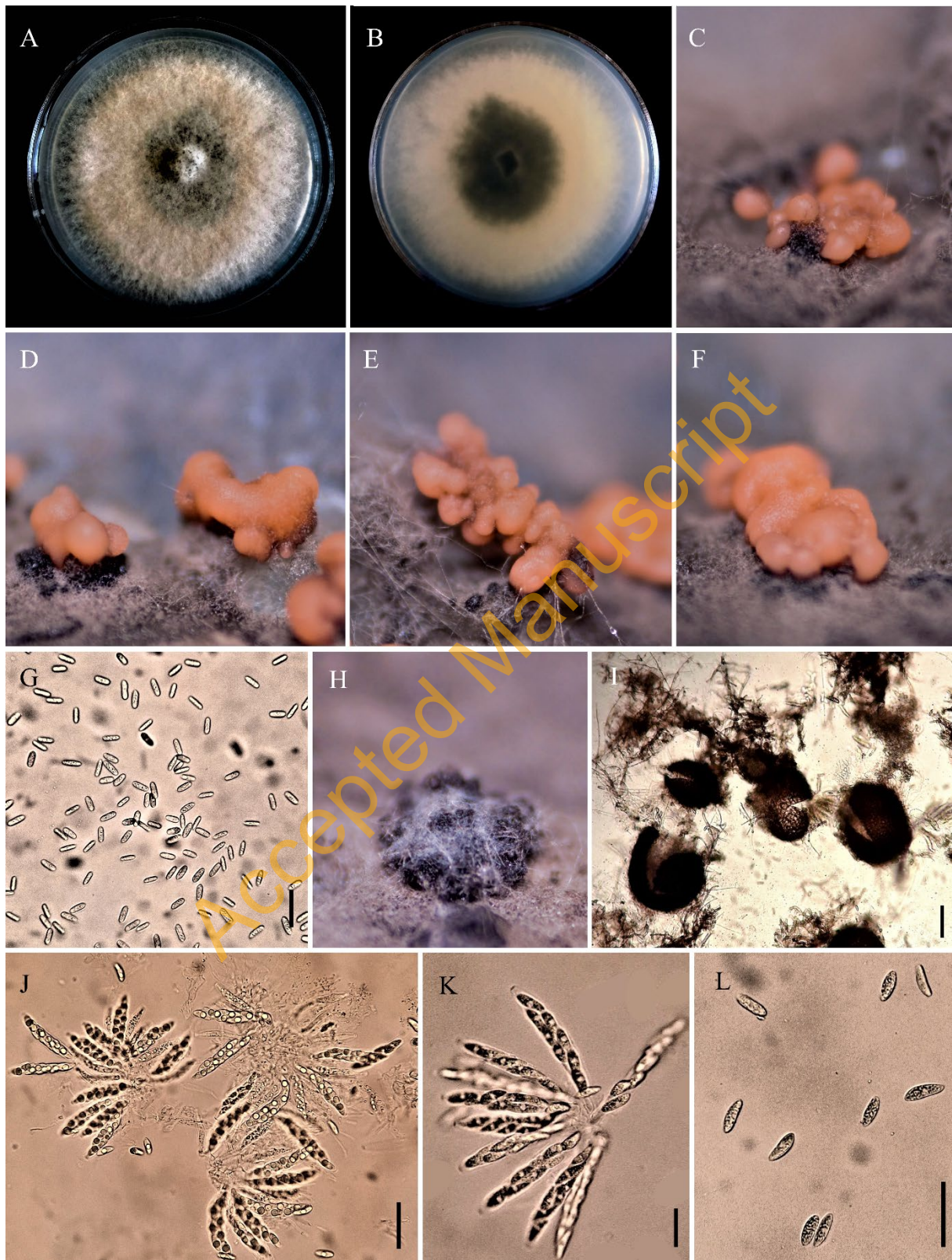
BLASTn analysis of the *tub2* and *gapdh* sequences against the GenBank database revealed high sequence similarity (99–100% identity and 100% coverage) with sequences of *C. fructicola*. Phylogenetic analysis using Maximum Likelihood (ML) and Bayesian Inference (BI) methods, based on individual and concatenated *tub2* and *gapdh* sequences, confirmed that the representative isolate (BFA06) belongs to the genus *Colletotrichum*, clustering within a distinct monophyletic clade corresponding to *C. fructicola* strains CBS 125395, ICMP:18581, CBS 112.14, CBS 197.34, and CBS 132455 with strong statistical support (BI/ML = 0.97/99) in concatenated tree (Table 2; Fig. 3, 4, 5). The *tub2* and *gapdh* sequences obtained in this study were deposited in GenBank under accession numbers PV654524 and PV959504, respectively. Based on phylogenetic placement and detailed morphological characteristics, the species was identified as *C. fructicola*.

**Table 1.** The list of *Colletotrichum fructicola* isolates recovered from infected *Ficus binnendijkii* leaves in Bushehr, Bushehr Province, Iran.

Isolate	Latitude	Longitude	Isolate	Latitude	Longitude
BFAL01	28.9825760	50.8333020	BFAL08	28.9891285	50.8277016
BFAL02	28.9825760	50.8333020	BFAL09	28.9823019	50.8320310
BFAL03	28.9825760	50.8333020	BFAL10	28.9823019	50.8320310
BFAL04	28.9825760	50.8333020	BFAL11	28.9825760	50.8333020
BFAL05	28.9891285	50.8277016	BFAL12	28.9825760	50.8333020
BFAL06	28.9891285	50.8277016	BFAL13	28.9825760	50.8333020
BFAL07	28.9891285	50.8277016			



**Fig. 1.** Symptoms of anthracnose on *Ficus binnendijkii*, caused by *Colletotrichum fructicola* in Bushehr, Bushehr Province, southern Iran. Symptomatic leaves on infected trees (A and B). Leaf spots on detached leaves are characteristically surrounded by a yellow halo (C–F).

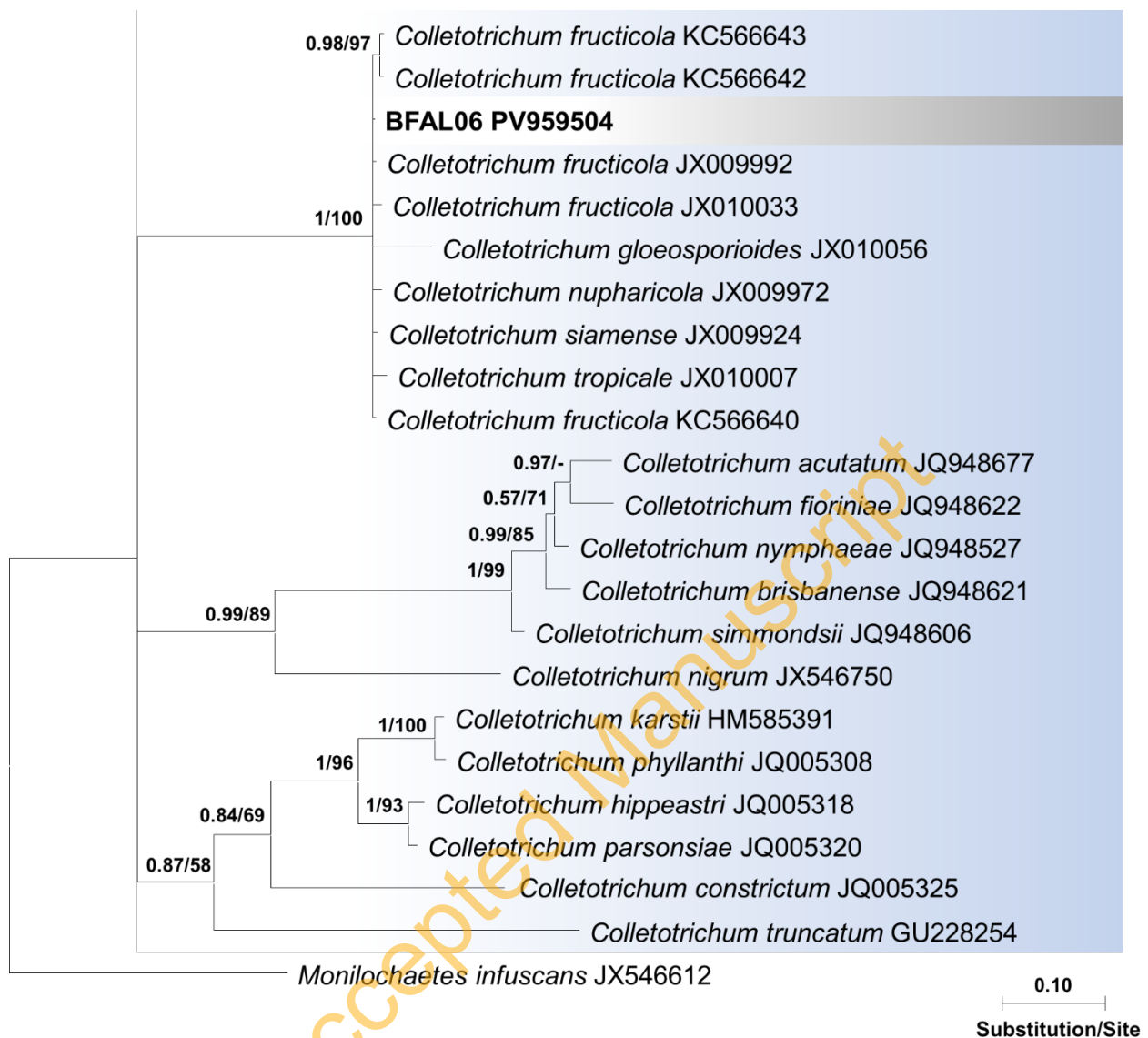


**Fig. 2.** Colony morphology and microscopical structures of *Colletotrichum fructicola* (isolate BFA06), recovered from infected *Ficus binnendijkii* leaves, cultured on potato dextrose agar (PDA). (A, B) Front and reverse of the colony, (C–F) Conidiomata and conidial masses, (G) Conidia, (H) Ascomatal mass, (I) Perithecia, (J, K) Asci, ascospores, and paraphyses, (L) Ascospores. Scale bars: (G, K, L) 30 μm, (I, J) 50 μm.

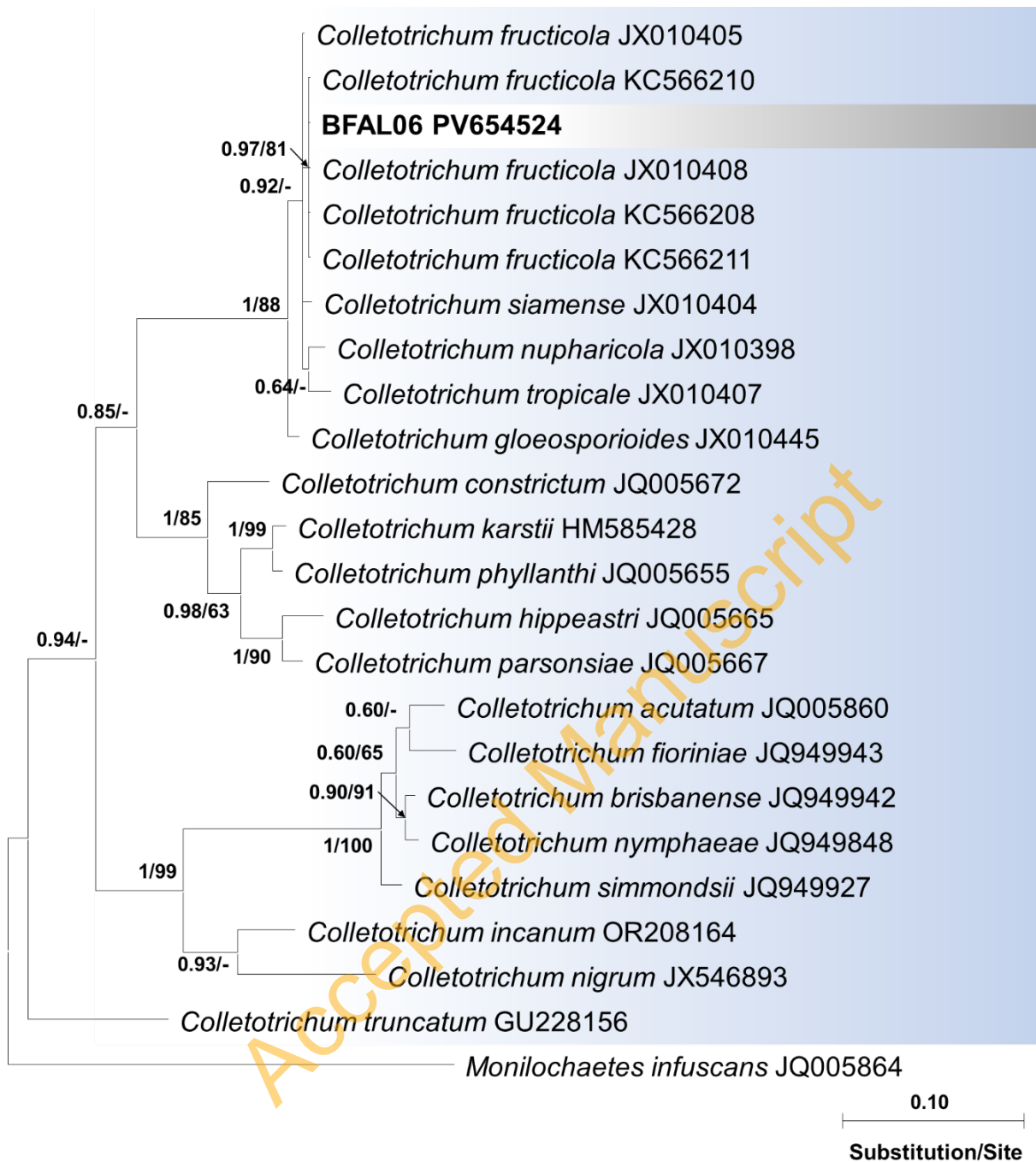
**Table 2.** Information on *Colletotrichum* species included in the phylogenetic analysis.

Species	Culture No. <sup>a</sup>	Host	Country	GenBank accession number <sup>b</sup>	
				<i>tub2</i>	<i>gapdh</i>
<i>C. acutatum</i>	CBS 112996 <sup>c</sup>	<i>Carica papaya</i>	Australia	JQ005860	JQ948677
<i>C. brisbanense</i>	CBS 29267 <sup>c</sup>	<i>Capsicum annuum</i>	Australia	JQ949942	JQ948621
<i>C. constrictum</i>	CBS 128504 <sup>c</sup>	<i>Citrus limon</i>	New Zealand	JQ005672	JQ005325
<i>C. fioriniae</i>	CBS 128517 <sup>c</sup>	<i>Fiorinia</i> sp.	USA	JQ949943	JQ948622
<i>C. fructicola</i>	CBS 125395	<i>Theobroma cacao</i>	Panama	JX010408	JX009992
<i>C. fructicola</i>	ICMP:18581 <sup>c</sup>	<i>Coffea arabica</i>	Thailand	JX010405	JX010033
<i>C. fructicola</i>	BFA06	<i>Ficus binnendijkii</i>	Iran	<b>PV654524</b>	<b>PV959504</b>
<i>C. fructicola</i>	CBS 112.14	-	-	KC566208	KC566640
<i>C. fructicola</i>	CBS 197.34	-	-	KC566211	KC566643
<i>C. fructicola</i>	CBS 132455	-	-	KC566210	KC566642
<i>C. gloeosporioides</i>	CBS 112999 <sup>c</sup>	<i>Citrus sinensis</i>	Italy	JX010445	JX010056
<i>C. hippeastri</i>	CBS 125376 <sup>c</sup>	<i>Hippeastrum vittatum</i>	China	JQ005665	JQ005318
<i>C. incanum</i>	CBS 150848	-	-	OR208164	-
<i>C. karstii</i>	CBS 132134 <sup>c</sup>	<i>Vanda</i> sp.	China	HM585428	HM585391
<i>C. nigrum</i>	CBS 132451	-	-	JX546893	JX546750
<i>C. nupharicola</i>	CBS 470.96 <sup>c</sup>	<i>Nuphar lutea</i> subsp. <i>polysepala</i>	USA	JX010398	JX009972
<i>C. nymphaeae</i>	CBS 515.78 <sup>c</sup>	<i>Nymphaea alba</i>	Netherlands	JQ949848	JQ948527
<i>C. parsonsiae</i>	CBS 128525 <sup>c</sup>	<i>Parsonsia capsularis</i>	New Zealand	JQ005667	JQ005320
<i>C. phyllanthi</i>	CBS 175.67 <sup>c</sup>	<i>Phyllanthus acidus</i>	India	JQ005655	JQ005308
<i>C. siamense</i>	CBS 130417 <sup>c</sup>	<i>Coffea arabica</i>	Thailand	JX010404	JX009924
<i>C. simmondsii</i>	CBS 122122 <sup>c</sup>	<i>Carica papaya</i>	Australia	JQ949927	JQ948606
<i>C. tropicale</i>	CBS 124949 <sup>c</sup>	<i>Theobroma cacao</i>	Panama	JX010407	JX010007
<i>C. truncatum</i>	CBS 151.35	<i>Phaseolus lunatus</i>	USA	GU228156	GU228254
<i>Monilochaetes infuscans</i>	CBS 86996 <sup>c</sup>	-	-	JQ005864	JX546612

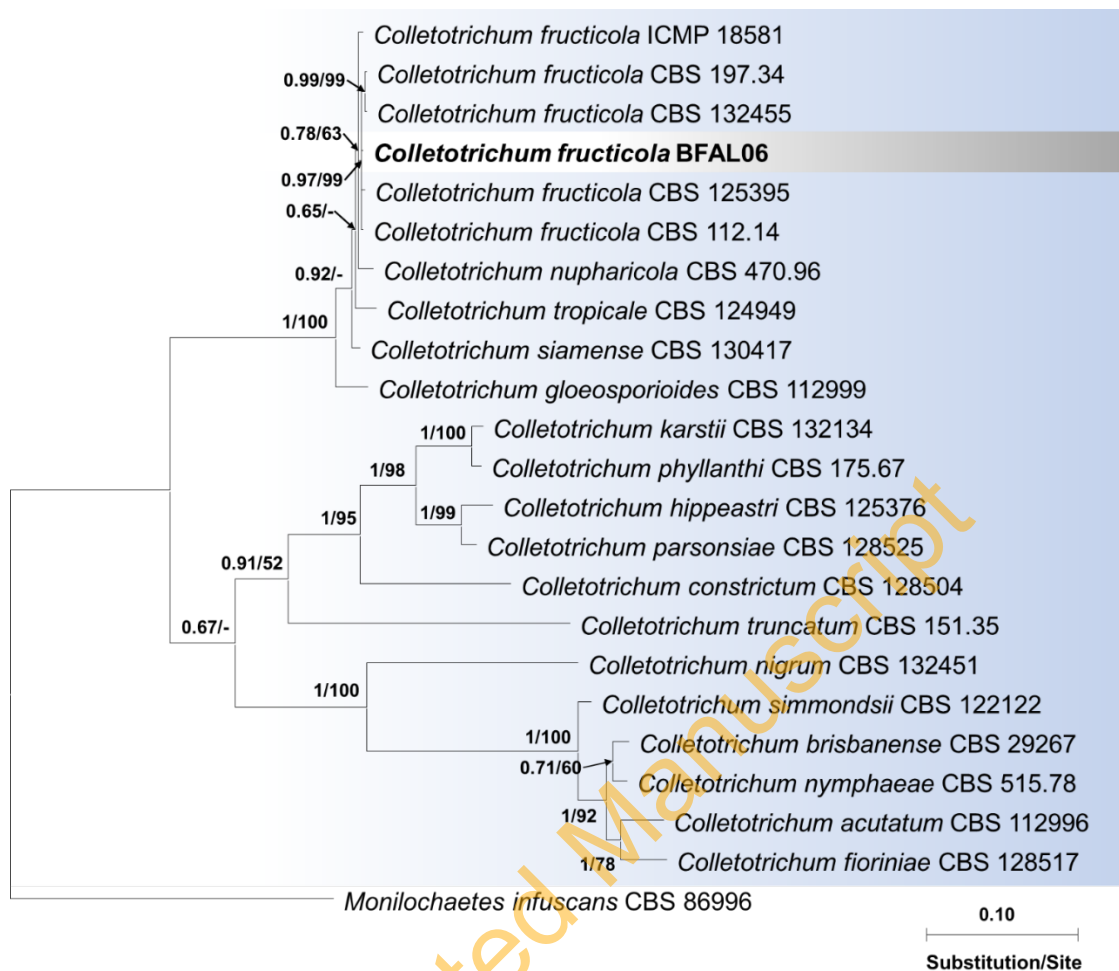
<sup>a</sup> CBS: Westerdijk Fungal Biodiversity Institute. <sup>b</sup> Beta-tubulin (*tub2*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*). The accession number in bold represents the isolates used in this study. <sup>c</sup> Type strain.



**Fig. 3.** Phylogenetic tree, based on glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene sequences, showing the taxonomic placement of *Colletotrichum fructicola* isolate BFA06. The tree illustrates the phylogenetic relationships among 17 *Colletotrichum* species, rooted with *Monilochaetes infuscans* (CBS 86996) using Bayesian inference. Node support values (Posterior probability from Bayesian Inference/ bootstrap percentages from Maximum Likelihood) when exceeding 0.50 or 50%, or both, with arrows indicating specific support values within the tree.



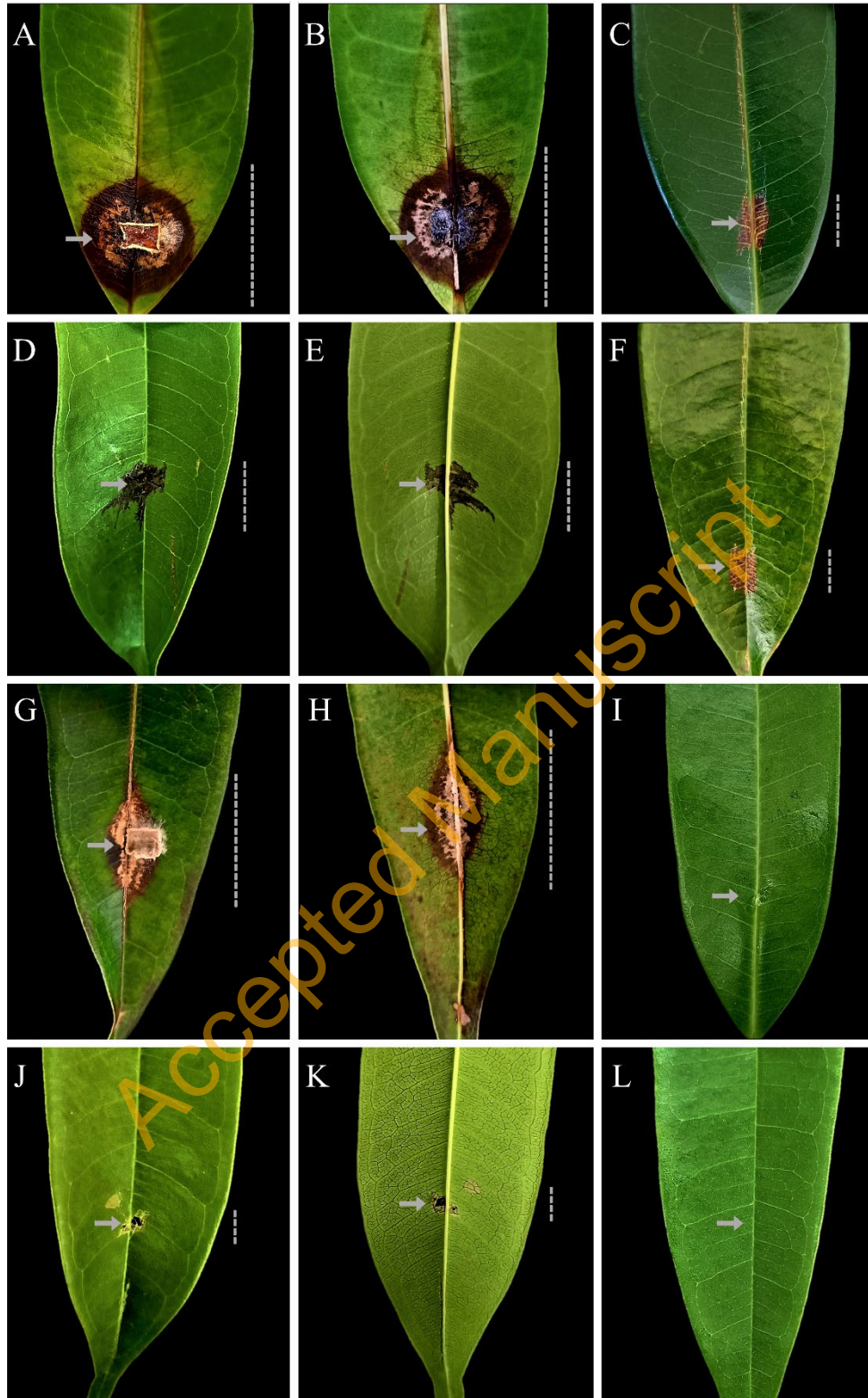
**Fig. 4.** Phylogenetic tree, based on  $\beta$ -tubulin (*tub2*) gene sequences, showing the taxonomic placement of *Colletotrichum fructicola* isolate BFA06. The tree illustrates the phylogenetic relationships among 18 *Colletotrichum* species, rooted with *Monilochaetes infuscans* (CBS 86996) using Bayesian inference. Node support values (Posterior probability from Bayesian Inference/ bootstrap percentages from Maximum Likelihood) when exceeding 0.50 or 50%, or both, with arrows indicating specific support values within the tree.



**Fig. 5.** Phylogenetic tree, based on concatenated  $\beta$ -tubulin (*tub2*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene sequences, showing the taxonomic placement of *Colletotrichum fructicola* isolate BFA06. The tree illustrates the phylogenetic relationships among 17 *Colletotrichum* species, rooted with *Monilochaetes infuscans* (CBS 86996) using Bayesian inference. Node support values (Posterior probability from Bayesian Inference/ bootstrap percentages from Maximum Likelihood) when exceeding 0.50 or 50%, or both, with arrows indicating specific support values within the tree.

### Pathogenicity Assessments

Pathogenicity of *C. fructicola* on *F. binnendijkii* leaves was confirmed through inoculation assays, resulting in the development of leaf necrosis and discoloration (Fig. 6). The method of inoculation significantly influenced lesion development. Necrotic lesions appeared on both wounded and unwounded leaves inoculated with mycelial plugs, emerging within two and four days post-inoculation, respectively. Spore suspension inoculations resulted in mild necrosis symptoms developing three and four days post-inoculation on wounded and unwounded leaves, respectively. Statistical analysis using one-way ANOVA demonstrated significant differences in lesion progression (length and width) among the various inoculation methods ( $P < 0.0001$ ; Table 3). Post-hoc analysis using Tukey's HSD test revealed that inoculation of wounded leaves with mycelial plugs resulted in the most extensive lesion development, with lesion lengths averaging  $29.12 \pm 3.86$  mm and lesion widths averaging  $19.39 \pm 1.37$  mm. These lesion dimensions were significantly larger than those observed with all other inoculation methods. Conversely, inoculation of unwounded leaves with spore suspension resulted in the smallest lesions, with an average lesion length of  $5.25 \pm 0.96$  mm and an average lesion width of  $3.25 \pm 0.29$  mm (Fig. 7). Negative controls, consisting of unwounded leaves inoculated with sterile PDA or water, did not exhibit any symptoms. *Colletotrichum fructicola* was successfully re-isolated from symptomatic leaf tissue, and the re-isolated pathogen exhibited morphological characteristics consistent with the original inoculated isolate, thereby fulfilling Koch's postulates.

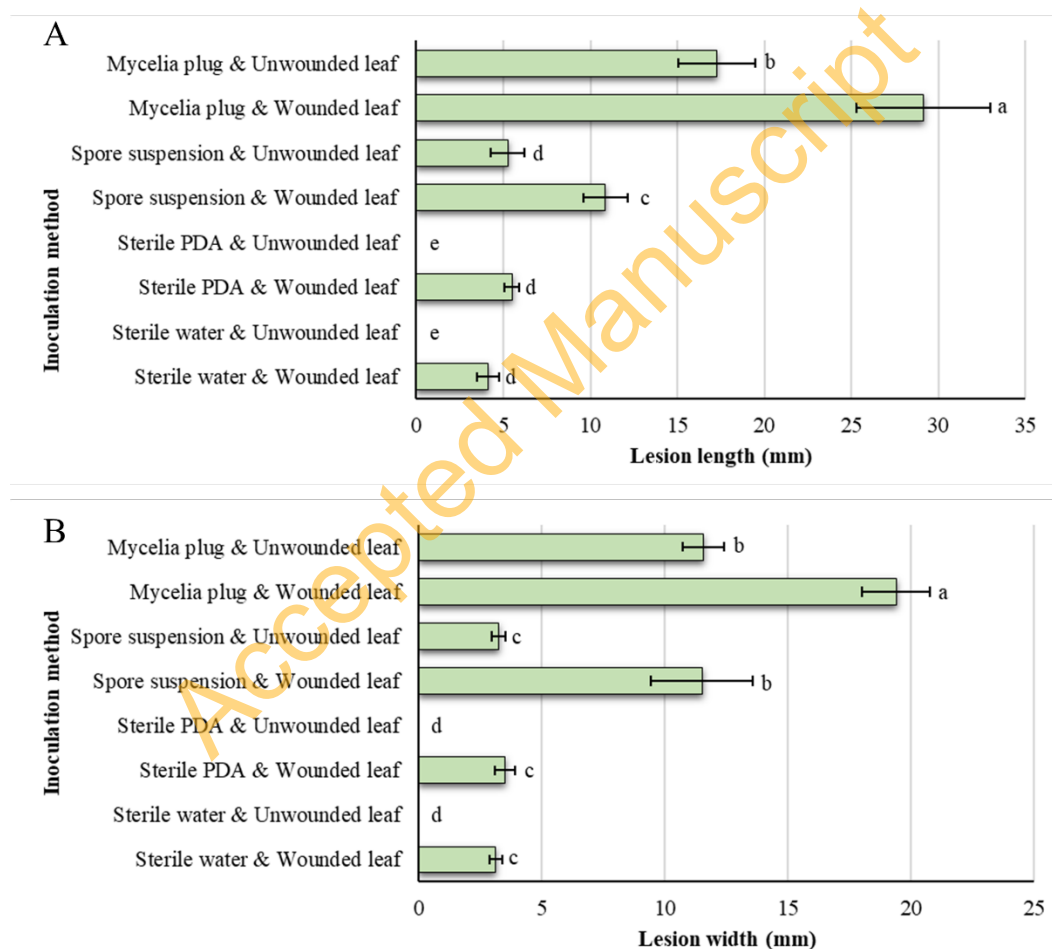


**Fig. 6.** Leaf spot symptoms on inoculated *Ficus binnendijkii* leaves five days after artificial inoculation with *Colletotrichum fructicola* (isolate BFAL06). Panels show leaves wounded (A–F) and unwounded (G–L) before inoculation. Inoculated leaves received either a mycelia plug (A, B, G, H) or spore suspension (D, E, J, K). Negative controls inoculated with sterile PDA plugs (C, I) or sterile water (F, L). Adaxial surface of inoculated leaves (A, D, G, J) and abaxial surface of inoculated leaves (B, E, H, K). Arrows indicate inoculation sites, and dashed lines illustrate the progression of disease symptoms.

**Table 3.** One-way ANOVA results for two pathogenicity traits (lesion length and width) of inoculated *Ficus binnendijkii* leaves with *Colletotrichum fructicola*.

S.O.V.	df	Lesion length			Lesion width		
		MS	F-value	P-value	MS	F-value	P-value
<b>Inoculation method</b>	7	393.76	138	<0.0001	189.80	209.73	<0.0001
<b>Error</b>	24	2.85			0.90		
<b>CV (%)</b>		18.72966			14.5469		

CV: Coefficient of variation; df: Degrees of freedom; MS: Mean Square; S.O.V.: Source of variation.



**Fig. 7.** Lesion development on *Ficus binnendijkii* leaves following inoculation with *Colletotrichum fructicola* (isolate BFAL06) using different inoculation methods, five days post-inoculation at  $25 \pm 1$  °C. (A) Average lesion length and (B) average lesion width on wounded and unwounded leaves inoculated with either a mycelia or spore suspension of *C. fructicola*. Negative controls included leaves inoculated with sterile PDA plugs or water. Values represent the mean of four replicates; error bars indicate standard deviation. Different letters indicate statistically significant differences ( $P \leq 0.05$ ).

The conducted pathogenicity assays revealed a significant difference in symptom severity between inoculation methods, with mycelial plugs consistently inducing more pronounced disease symptoms compared to spore suspensions. This disparity suggests that mycelial plug inoculation facilitates the delivery of a concentrated and viable pathogen inoculum, offering a highly reproducible method for introducing a pre-established fungal colony directly onto the leaf surface.

By bypassing the initial spore germination phase, this method accelerates the infection process. In contrast, the use of spore suspensions more closely mimics natural infection events, where airborne spores land on the plant surface and must undergo germination and penetration to initiate the infection cycle (Seassau et al. 2010; Zhang et al. 2018). The observed differences underscore the importance of selecting an appropriate inoculation method in pathogenicity studies, as each method presents distinct advantages and limitations in replicating natural disease development.

## CONCLUSION

To the best of our knowledge, this study provides the first documented evidence of *C. fructicola* causing anthracnose on *F. binnendijkii* both in Iran and globally. Anthracnose, incited by *C. fructicola*, is a disease of significant concern due to its expansive host range, encompassing numerous ornamental species (Zhang et al. 2023). Considering the pathogen's broad host range, coupled with variations in symptom expression, the potential ramifications for other urban landscape trees warrant careful consideration. Consequently, the implementation of effective management strategies is paramount for controlling leaf spot disease on ornamental *F. binnendijkii*. A key component of these strategies should emphasize accurate and rapid molecular detection of *C. fructicola* to enable early intervention and effectively mitigate its potential impact.

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Not implicated.

## AUTHOR CONTRIBUTION

Hamed Negahban: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Reza Mostowfizadeh-Ghalamfarsa: Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & 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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## شناسایی و ویژگی‌های بیماری‌زایی *Colletotrichum fructicola*، عامل آنتراکنوز در *Ficus binnendijkii* در جنوب ایران

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### چکیده

در طی یک پایش از درختان زینتی فضای سبز در شهر بوشهر، استان بوشهر، واقع در جنوب ایران، علائم لکه‌برگی قهوه‌ای تیره روی درختان *Ficus binnendijkii*، یکی از گونه‌های زینتی محبوب، مشاهده شد. جدایه‌های قارچی به‌دست‌آمده از برگ‌های دارای علائم، بر اساس ویژگی‌های ریخت‌شناختی و مولکولی با استفاده از ژن‌های بتاتوبولین (*tub2*) و گلیسرآلدئید-۳-فسفات دهیدروژناز (*gapdh*)، به‌عنوان *Colletotrichum fructicola* شناسایی شدند. واکاو‌های فیلوژنتیکی نیز شباهت بالایی جدایه حاصل در این مطالعه را با جدایه‌های مرجع و معتبر این گونه تأیید کرد. برای اثبات بیماری‌زایی، برگ‌های خراش‌داده‌شده و بدون خراش *F. binnendijkii* با بلوک‌های میسلیومی و سوسپانسیون اسپور *C. fructicola* مایه‌زنی شدند. نتایج آزمون بیماری‌زایی نشان داد که قارچ جداسازی شده دارای توانایی ایجاد بافت‌مردگی و تغییر رنگ قابل‌توجهی در برگ‌های مایه‌زنی‌شده است. جداسازی مجدد جدایه‌های مایه‌زنی‌شده از علائم حاصل، موجب تأیید اصول کخ شد. تجزیه واریانس (ANOVA) اختلافات بسیار معنی‌داری در شاخص‌های پیشرفت لکه (طول و عرض لکه) بین روش‌های مختلف مایه‌زنی نشان داد ( $P < 0.0001$ ). به‌طور خاص، مایه‌زنی برگ‌های خراش‌داده‌شده با بلوک‌های میسلیومی شدیدترین لکه را نسبت به سایر روش‌ها ایجاد کرد. این پژوهش نخستین گزارش جهانی از بروز آنتراکنوز ناشی از آلودگی *C. fructicola* روی *F. binnendijkii* است. یافته‌های این مطالعه بر ضرورت بررسی‌های بیشتر در زمینه همه‌گیری‌شناسی این بیمارگر در سایر گیاهان زینتی و ابداع راهبردهای مؤثر مدیریت بیماری تأکید می‌کند.

**واژگان کلیدی:** آسکومیست، بیماری‌زایی، توت‌سانان، گیاهان زینتی، لکه‌برگی