Mycologia Iranica 2025, Vol. 12 (No. 2): ??–??

Original Article

Morphological and molecular characterization of *Fusarium* species causing root and crown rot of safflower

Fatemeh Mostafaei, Bahram Sharifnabi ✉, Zeinab Esmaeili

Department of Plant Protection, Faculty of Agriculture, Isfahan University of Technology, Iran

https://doi.org/10.22092/mi.2025.370619.1328

ABSTRACT

Safflower (*Carthamus tinctorius*) is an annual plant belonging to the family *Asteraceae*. The cultivation of safflower has gained increasing attention due to its valuable seed oil, vibrant flower color, and tolerance to drought and salinity. However, infections caused by *Fusarium* species pose a significant challenge worldwide. To investigate the morphological and molecular characteristics of *Fusarium* species associated with crown and root rot in safflower, samples were collected from infected crowns and roots across various cultivation areas in Isfahan Province. Based on morphological traits and molecular data obtained from sequencing the ITS-rDNA region and a portion of the *TEF1* gene, two *Fusarium* species—*F. nygamai*, and *F. falciforme* were identified among 36 isolates exhibiting symptoms of crown and root rot disease. Pathogenicity tests conducted under greenhouse conditions confirmed their pathogenic nature according to Koch's postulates. Notably, this study represents the first report of *F. nygamai* and *F. falciforme* as causal agents of crown and root rot in safflower worldwide.

KEYWORDS

Carthamus tinctorius, Disease, *Fusarium nygamai*, *Fusarium falciforme*, Pathogenicity.

Accepted Manuscript

INTRODUCTION

Safflower, scientifically identified as *Carthamus tinctorius* L., is an annual plant classified under the family *Asteraceae* (Emongor et al. 2017). The cultivation of safflower has attracted considerable attention due to its valuable seed oil, vibrant flower color, and tolerance to drought and salinity (Hussain et al. 2016, Li et al. 2023). According to FAO statistics, global safflower production reached nearly 995,000 tons in 2022. Kazakhstan ranks first worldwide, accounting for more than 44% of total production, followed by Russia, the United States of America, Mexico, India, China, and Turkey. In 2022, Iran produced approximately 4,000 tons of safflower, with the major production areas located in Isfahan Province (FAOSTAT 2022). The provinces of Isfahan, Fars, Hamedan, and Zanjan currently represent the main safflower-producing regions in Iran (Agricultural Statistics of Crops 1402).

Safflower is susceptible to several fungal diseases that can significantly reduce both yield and quality (Taware et al. 2014). The major diseases include leaf spot (*Alternaria carthami*), wilt and root rot caused by *Fusarium* spp., *Macrophomina phaseolina*, and *Phytophthora drechsleri*, as well as powdery mildew (*Leveillula taurica* and *Golovinomyces cichoracearum*) and rust (*Puccinia carthami*) (Da Via et al. 1981, Prasad et al. 2011, Taware et al. 2014).

The genus *Fusarium* is a cosmopolitan group of filamentous ascomycete fungi (Sordariomycetes, Hypocreales, Nectriaceae) that includes many agriculturally important, toxin-producing plant pathogens. These species produce a wide range of toxic secondary metabolites (Ma et al. 2013). *Fusarium* species are widely distributed and can be isolated from plants and soil across the world, where they function as pathogens, endophytes, or saprophytes (Brown and Proctor 2013). Previous reports have shown that safflower wilt is caused by several *Fusarium* species, with the first occurrence reported in California in 1962, followed by reports from other countries (Raghuwanshi and Dake 2009). This disease poses a major biotic constraint, leading to significant yield losses and

Received: 06 Sept. 2025 Revised: 10 Nov. 2025 Accepted: 15 Dec. 2025 Published online: 18 Dec. 2025

✉ Corresponding Author: Bahram Sharifnabi; Email: sharifna@iut.ac.ir

Mycologia Iranica is licensed under a "Creative Commons Attribution 4.0 International (CC-BY 4.0)"

Published by Iranian Mycological Society (IrMS)—https://mij.areeo.ac.ir

reduced safflower productivity. It is therefore considered one of the principal agents responsible for damping-off in safflower, both in Iran and globally (Jangir et al. 2023, Garmaroodi et al. 2024).

Several *Fusarium* species have been reported as causal agents of safflower root and crown rot worldwide. For instance, *F. verticillioides* has been associated with root rot in Mexico (Tirado-Ramírez et al. 2023), while *F. oxysporum* f. sp. *carthami* and *F. solani* have been linked to similar diseases in Iran (Garmaroodi et al. 2024). In Korea, *F. proliferatum* has also been reported as a pathogen causing safflower root rot (Kim et al. 2016).

Given the significant role of *Fusarium* species in safflower diseases and the potential influence of climate change on their distribution and pathogenicity, accurate identification of these species is crucial. Because morphological characteristics can be unreliable due to environmental variation (Geiser et al. 2004), molecular phylogenetic approaches provide a more reliable basis for species identification and evolutionary understanding.

In this study, safflower plants exhibiting wilt symptoms were collected from cultivation fields in Isfahan Province, and *Fusarium* species associated with crown and root rot were identified using both morphological and molecular analyses. Pathogenicity tests were also conducted to confirm the pathogenic role of each identified species. The objectives of this study were: (i) to identify *Fusarium* species isolated from root and crown rot of safflower in Isfahan Province, and (ii) to validate morphological identification through DNA sequencing and molecular characterization.

MATERIALS AND METHODS

Sample collection and Fungal isolation

Infected roots and crowns of safflower plants exhibiting symptoms such as damping-off, leaf yellowing, wilting, and reduced growth were collected from various farms in Isfahan Province between September 2022 and September 2023. The infected samples were placed in separate plastic bags, transported to the laboratory, and stored at 4°C for further analysis.

The infected roots were separated and rinsed under running tap water for at least 10 minutes to remove soil particles and saprophytic organisms. They were then cut into small pieces of approximately two centimeters in length. Root pieces were rinsed in sterile distilled water for 20 seconds, surface-disinfected with 70% ethanol for one minute, and rinsed again in sterile distilled water for 20 seconds. Subsequently, they were treated with a 10% sodium hypochlorite solution for two minutes, followed by three consecutive washes in sterile distilled water. Finally, the disinfected pieces were air-dried on sterile filter paper. The dried root segments were cultured on Potato Dextrose Agar (PDA) medium supplemented with 50 ppm of chloramphenicol antibiotic and incubated at 25°C for 5–10 days. Each fungal isolate was purified using the standard hyphal tip method, and the purified isolates were incubated on sterile filter papers at -20°C.

Morphological analysis

Fusarium species were identified based on their cultural and morphological characteristics, as outlined by Nelson et al. (1983) and Leslie and Summerell (2006). For macroscopic evaluation, isolates were cultured on Potato Dextrose Agar (PDA) and incubated at 25°C under a 12-hour light/dark cycle. After 14 days, phenotypic characteristics such as colony growth rate, color, pigment diffusion, texture, and margins in the agar were recorded. Isolates were also cultured on Carnation Leaf Agar (CLA) medium (Fisher et al. 1982) and incubated at 20-25°C under light exposure, particularly near-ultraviolet light (NUV) (Summerell et al. 2003), for 14 to 21 days (Nelson et al. 1994). Microscopic characteristics of each isolate– including the shape and size of macroconidia, microconidia, and chlamydospores, the color of the sporodochium, phialide type, and the arrangement of conidia on conidiophore– were examined using an Olympus optical microscope (model BH12) at 40X and 100X magnifications.

Characteristics of the sporodochium and macroconidia, including presence or absence, color, size, shape, and cell type (terminal and primary), were documented. Additionally, the characteristics of microconidia, such as presence or absence, sporulation type, arrangement (single, false head, or chain), number of septa, and phialide type (mono or polyphialide) were assessed. The features of chlamydospores, including presence or absence, site of formation, wall type, and arrangement (single, batch, paired, or in chain), were also measured. For each isolate, the dimensions of approximately 25 spores were measured, and their averages were calculated. Finally, based on these morphological characteristics, *Fusarium* species were identified using the identification keys provided by Leslie and Summerell (2006) and Nelson et al. (1983).

Pathological analysis

To prepare the inoculum, 200 grams of washed wheat seeds were soaked in water for 2 hours, placed in separate Erlenmeyer flasks, and sealed with aluminum foil. The flasks were autoclaved for 20 minutes on three consecutive days. After sterilization, the wheat seeds were inoculated with ten mycelium discs of the fungus and incubated at 25°C for two weeks. Moisture inside each flask was maintained by adding sterile distilled water. For the pathogenicity test, four to six-leaf stage plants of the Kose cultivar were used. Three replicates (three pots, with three plants in each pot) were set up for each fungal isolate. A small scratch was made at the crown and root of each plant, and five inoculated wheat seeds were placed near the crown as inoculum, then

Accepted Manuscript

covered with soil. For the control treatment, sterile wheat seeds were included with ten PDA discs without fungi. Seedling responses were evaluated one month after inoculation (Esmaili and Sharifnabi 2023).

DNA extraction and PCR amplification

Isolates were cultured on Potato Dextrose Agar (PDA) at 25°C in the dark, and DNA was extracted from seven-day-old mycelium using the method of Murray and Thompson (1980). Partial sequences of the translation elongation factor 1-alpha (*TEF1*) gene and the internal transcribed spacer (ITS-rDNA) region of the nuclear ribosomal DNA were amplified using the primers Tef1 (5′-ATGGGTAAGGAGGACAAGAC-3′)/Tef2 (5′-GGAAGTACCAGTGATCATGTT-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)/ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′), respectively (Divakara et al. 2014, White et al. 1990). For *TEF1* amplification, a 10 µL polymerase chain reaction (PCR) mixture was prepared containing 5 µL of Master Mix, 0.5 µL of each primer (10 pmol/µL), 1 µL of extracted DNA (10-20 ng), and 3 µL of sterile distilled water. For ITS amplification, the mixture consisted of 5 µL of Master Mix, 0.5 µL of each primer (10 pmol/µL), 1 µL of DNA extract (10-20 ng), and 3.5 µL of sterile distilled water. The PCR conditions for the *TEF1* consisted of an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds, concluding with a final extension at 72°C for 10 minutes. For ITS amplification, the program consisted of the following steps: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The results of DNA extraction from *Fusarium* isolates, as well as their PCR reactions with ITS4/ITS5 and Tef1/Tef2 primers, were analyzed on a 1% agarose gel using a 100 bp ladder (White et al. 1990, O'Donnell et al. 2000, Arif et al. 2012).

Phylogenetic analyses

Considering that morphological diagnosis is enhanced by the support and confirmation of molecular studies on fungal species, this study performed molecular examinations to validate morphological observations, accurately identify fungal species, and evaluate the phylogenetic relationships among different species. We analyzed the related nucleotide sequences of the *TEF1* gene and the ITS-rDNA region, which were aligned using MEGA version 7.0 software (Kumar et al. 2016) with the multiple sequence alignment option. Phylogenetic trees were constructed based on Kimura's two-parameter distance model and the Maximum Likelihood (ML) method with the Tamura-Nei distance model in MEGA version 7.0 (Gouy et al. 2010, Kumar et al. 2016). The topology of the tree was assessed by bootstrapping with 1000 resamples of the data, with *Fusarium nectrioides* and *Fusarium dimerum* serving as outgroups. The results revealed the phylogenetic relationships among *Fusarium* species isolated from safflower and reference sequences of *Fusarium* species obtained from GenBank.

RESULTS

Sampling and fungal isolation

Sampling of infected safflower plants was carried out in the cities of Kashan (5 samples), Shahreza (3 samples), and Isfahan (3 samples). The samples were transferred to the laboratory for further necessary research.

Morphological characterization

A total of 36 symptomatic samples were collected, from which eleven *Fusarium* isolates were obtained. These comprised eight isolates of *F. nygamai* and three isolates of *F. falciforme*, all of which were identified as causal agents of crown and root rot disease. *F. nygamai* was found to be more abundant in Kashan, Shahreza, and Isfahan. *F. falciforme* was collected in Borkhar, as well as in Kashan and Isfahan, similar to *F. nygamai*. Based on morphological and molecular characteristics, the causal agents of safflower crown and root rot were identified as *F. nygamai* and *F. falciforme*. The morphological features of these species are described as follows:

Fusarium nygamai L.W. Burgess & Trimboli, Mycologia 78(2): 223 (1986). Fig. 1

Conidiophores short, simple, or sparsely branched, bearing both monophialidic and polyphialidic conidiogenous cells. Phialides cylindrical to slightly tapering, 12–18 × 2–3 µm. Macroconidia hyaline, straight to slightly curved, typically 3–4 septate, with bluntly rounded apical cells and foot-shaped basal cells, 15–19 × 3–5 µm. Microconidia are ovoid to oblong, one- or two-celled, measuring 3–5 × 1–2 µm, and are produced abundantly from both monophialidic and polyphialidic conidiogenous cells. They often form characteristic chains of 2–8 conidia. Chlamydospores spherical, abundant, formed singly, in pairs, or in short chains, occurring intercalarily or terminally within hyphae.

Culture characteristics: Colony growth on PDA reached 70–85 mm in diameter after 10 days of incubation at alternating temperatures of 25 °C (day) and 20 °C (night), with a 12 h light/12 h dark photoperiod. Colonies on PDA were purple with well-developed aerial mycelium. On CLA medium, colonies appeared pale purple from the reverse side, and green to cream-colored sporodochia developed under near-ultraviolet (NUV) light.

Specimens examined: Iran, Isfahan Province, Shahreza and Kashan, from infected safflower roots and crowns, August 2022-2023, F. Mostafaei isolates KaR21 (Kashan) and ShR24 (Shahreza) (IRAN 5353C).

Accepted Manuscript

Notes: *Fusarium nygamai* can be distinguished from other *Fusarium* species by its abundant production of microconidial chains and the presence of both short monophialides and polyphialides, which are diagnostic features for this species.

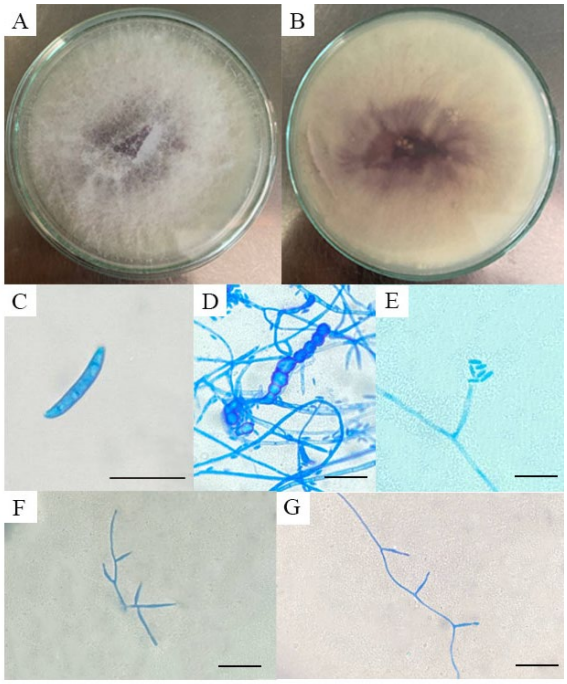


Fig. 1. *Fusarium nygamai*. A. Upper, and B. Reverse of colony on PDA, C. Macroconidia, D. Chlamydospores, E. Monophialide, F. polyphialide, G. Microconidia in chain, and H. false head. Scale bars: C – H = 20 µm.

Fusarium falciforme (Carrión) Summerb. & Schroers, J. Clin. Microbiol. 40(8): 2872 (2002). Fig. 2

Conidiophores elongated, mostly monophialidic, 60–80 µm in length, arising from aerial or substrate hyphae. Phialides cylindrical to slightly tapering, borne singly or in small groups, occasionally producing false heads of conidia. Macroconidia hyaline, straight to slightly curved, with a slightly curved apical cell and a distinctly foot-shaped basal cell, typically 3–5 septate, 30–37 × 4–7 µm. Microconidia clavate to ovoid, usually aseptate or occasionally one-septate, 10–15 × 3–5 µm, produced singly or in small false heads on short monophialides. Chlamydospores abundant, formed singly, in pairs, or in short chains, both terminally and intercalarily within the hyphae, smooth- to rough-walled, 5–9 µm in diameter.

Culture characteristics: Colonies on PDA were white to cream-colored with scattered aerial mycelium as a characteristic feature. After seven days of incubation at alternating temperatures of 25 °C (day) and 20 °C (night) with a 12 h light/12 h dark photoperiod, the colony diameter reached approximately 75–80 mm. On CLA medium under near-ultraviolet (NUV) light, cream-colored sporodochia developed near carnation leaf pieces.

Specimens examined: Iran, Isfahan Province, from infected safflower roots and crowns, August 2022-2023, F. Mostafaei, isolate EsR32 (IRAN 5351C).

Notes: *Fusarium falciforme* is morphologically similar to *F. solani*, sharing comparable conidial dimensions and chlamydospore abundance, but they are separated based on molecular data, particularly the *TEF1* gene and ITS-rDNA region sequences.

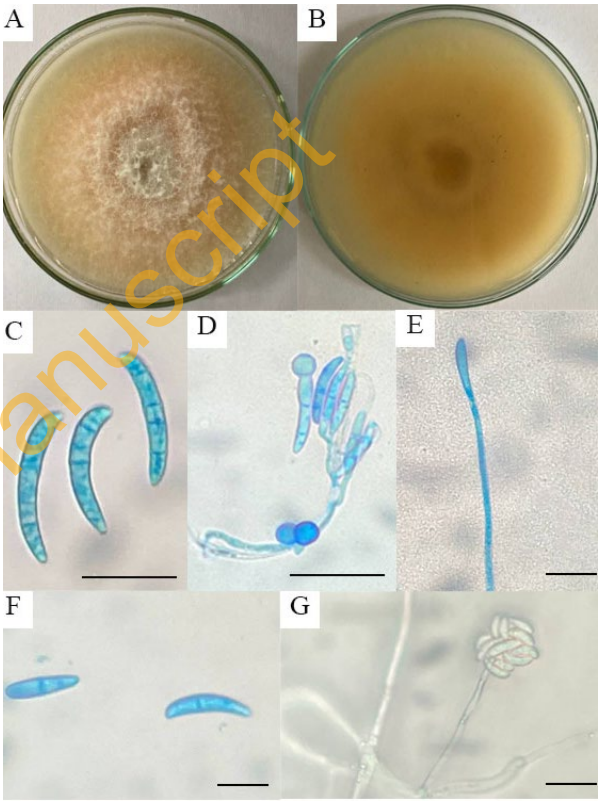


Fig. 2. *Fusarium falciforme*. A. Upper and B. reverse of colony on PDA, C. Macroconidia, D. Monophialide, E. Microconidia, F. Chlamydospore, and G. false head. Scale bars: C – G = 20 µm.

Molecular characterization

The DNA extracted from *Fusarium* isolates and the PCR products obtained using ITS4/ITS5 and Tef1/Tef2 primers were analyzed on a 1% agarose gel with a 100 bp ladder. The amplified fragments were approximately 500 bp for the ITS primer pair and 700 bp for the Tef primer pair. The nucleotide sequences of each species deposited in the NCBI GenBank were evaluated using BLAST software. This evaluation revealed significant similarity with similar species in the GenBank.

Phylogeny

Molecular analysis confirmed the morphological identification of various *Fusarium* species. In this

Accepted Manuscript

Table 1. Root and crown rot pathogenic *Fusarium* isolates used in this study and their accession numbers in GenBank, and the national collection of living fungi of Iran.

Species	Location	Isolate No.	Iran Culture Collection No.[1]	GenBank Accession Numbers	
				TEF1	ITS
F. falciforme	Isfahan	EsR32	IRAN 5351C	PX625948	PX647868
F. nygamai	Shahreza	ShR24	IRAN 5353C	PX625949	PV225560
	Kashan	KaR21	N.A[2]	PX625947	PV225622

[1] *IRAN* Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran. [2] Not available.

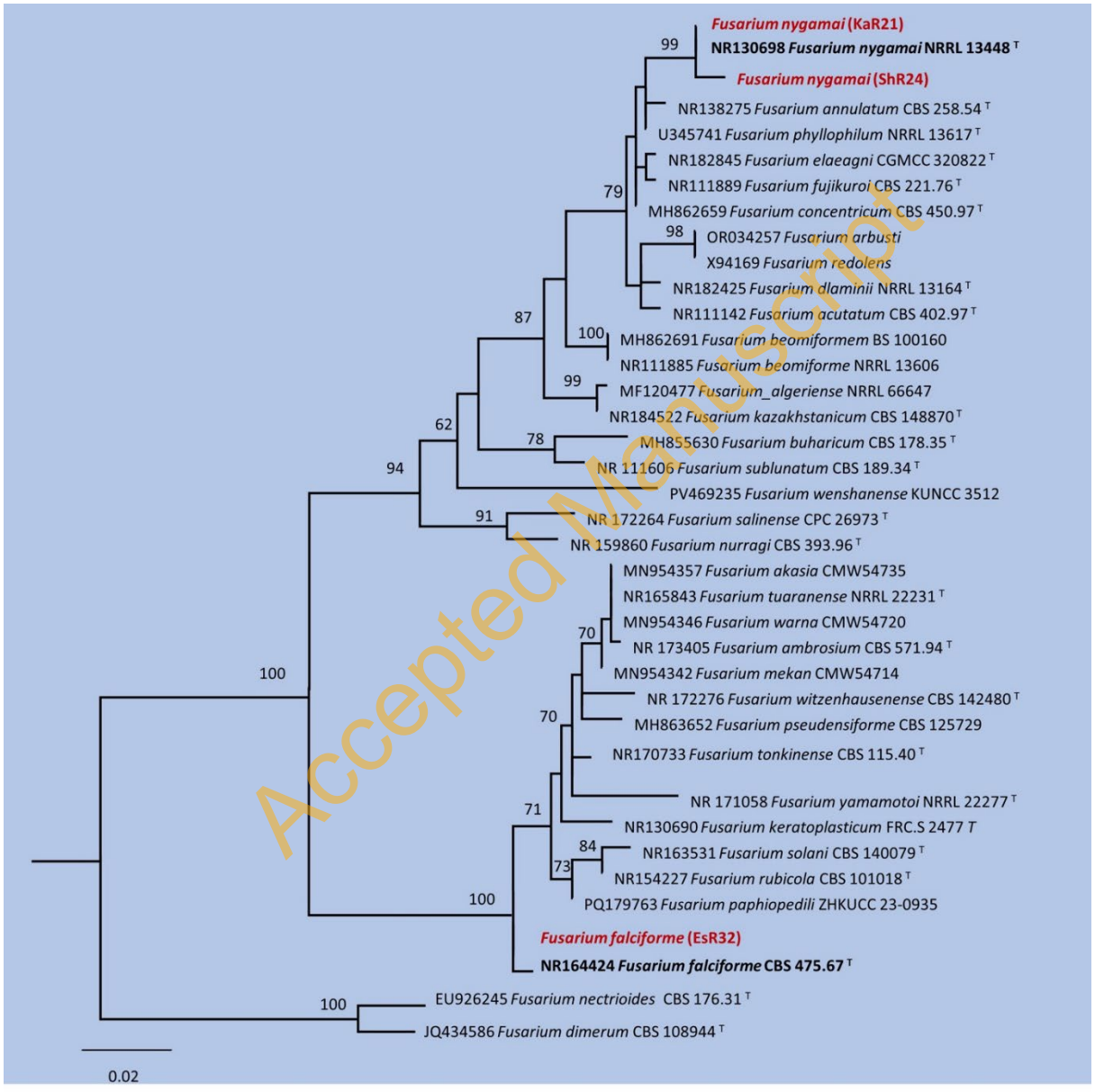
study, four *Fusarium* isolates were selected as representatives for sequencing the ITS-rDNA region. These included isolates collected KaR21 and ShR24 from Kashan and Shahreza (*F. nygamai*), and EsR32 from Isfahan (*F. falciforme*), respectively. For phylogenetic analysis, sequences of different *Fusarium* species were selected from the gene bank for comparison. Additionally, *Fusarium nectrioides* and *Fusarium dimerum* were used as outgroups. The phylogenetic tree was constructed based on the ITS-rDNA region sequences using the Maximum-likelihood method with 1000 bootstrap replicates. The results clearly demonstrate the evolutionary relationships among the *Fusarium* isolates and related species. Sequences obtained from the present study (highlighted in red) were compared with reference sequences retrieved from GenBank.

The tree structure was constructed using a multigene or single-locus dataset, as evident from the GenBank accession numbers associated with each species or isolate. Branches are supported by bootstrap values, which reflect the robustness of the grouping; values above 70% generally indicate strong statistical support for that clade, while values above 90% suggest very reliable relationships.

Fusarium nygamai (KaR21) and *F. nygamai* (ShR24) isolates, shown in red, reveal that they cluster tightly with the reference sequence NR130698. Other isolates highlighted in red, including *F. falciforme* (EsR32), are placed in clades with high bootstrap support, confirming their identity and phylogenetic relationship with reference species, and validating the accuracy of the molecular identification techniques employed. The tree topology demonstrates the separation of different *Fusarium* species into well-supported clades, in line with current taxonomic frameworks. For example, species such as *F. annulatum*, *F. phyllophilum*, and *F. salinense* form distinct branches that are clearly separated from the clusters containing the isolates of interest. The presence of outgroup species (*F. nectrioides* and *F. dimerum*) roots the tree and helps clarify the relationships among the ingroup species, which is a standard methodology in phylogenetic analysis. Scientific significance results confirm that the isolates under study belong to their respective species with high confidence, supporting their molecular identification and differentiation from similar taxa. The diversity visible within the *Fusarium* genus suggests significant levels of evolutionary divergence, with clear boundaries between species complexes. Bootstrap values throughout the tree validate the reliability of these groupings. Placement of isolates within reference clades confirms the utility of DNA-based identification in resolving taxonomic ambiguities among morphologically similar species. Such robust molecular phylogenies are critical for accurate diagnostics and biodiversity assessments. The phylogenetic trees provide scientifically rigorous confirmation of observed *Fusarium* isolates as members of the species with which they cluster. The careful use of bootstrap values, reference sequences, and multiple isolates ensures that the results presented are both credible and aligned with international standards for fungal taxonomy. These findings are vital for the precise identification, ecological study, and management of *Fusarium* species in plant pathology, environmental biology, and agriculture (Figs 3 and 4). The phylogenetic tree based on the *TEF1* gene sequences provides a detailed evolutionary framework for several *Fusarium* species, including the isolates *F. nygamai* (KaR21 and ShR24). It illustrates both species-level relationships and genetic distances within this genus. *F. nygamai* isolates (KaR21 and ShR24), highlighted in red, cluster closely with the reference strain MT011009 *F. nygamai* CBS 74997 with a high bootstrap value of 94%. This strongly supports the species-level identification of these isolates as *F. nygamai*. This clade is positioned near related species such as *F. acutatum*, *F. chinshoyiense*, and *F. foetens*, indicating close evolutionary relationships yet distinct species delimitations within the genus. Other clades with high bootstrap support (99-100%) comprise species such as *F. ramigenum*, *F. prieskaense*, *F. sudanese*, and *F. ficicrescens*, demonstrating well-supported divisions among *Fusarium* species and reinforcing the robustness of the *TEF1* marker in resolving phylogenetic relationships. The outgroup species *F. dimerum* (EU926334) and *F. nectrioides* (EU926312) are appropriately positioned

Accepted Manuscript

to root the tree, providing strong evolutionary context and differentiating the ingroup. Bootstrap values throughout the tree indicate strong statistical confidence in these phylogenetic groupings, underscoring the reliability of *TEF1* sequences for *Fusarium* taxonomy and molecular identification. Overall, the tree effectively confirms the identity of *F. nygamai* isolates within a robust phylogenetic framework, supporting molecular taxonomy and aiding in studies of evolutionary biology, pathogen diagnostics, and biodiversity within *Fusarium*. The *TEF1* gene proves to be a valuable marker for discriminating closely related species and clarifying evolutionary relationships. (Fig. 4).



Accepted Manuscript

Fig. 3. Phylogenetic tree based on ITS-rDNA region sequences of the identified *Fusarium* species using the Maximum-likelihood method. The numbers above each branch indicate the amount of bootstrap support from a 1000-times phylogenetic tree drawing using the Maximum-likelihood method. *Fusarium nectrioides* and *F. dimerum* were used as outgroups. The *Fusarium* isolates from safflower are marked in red.

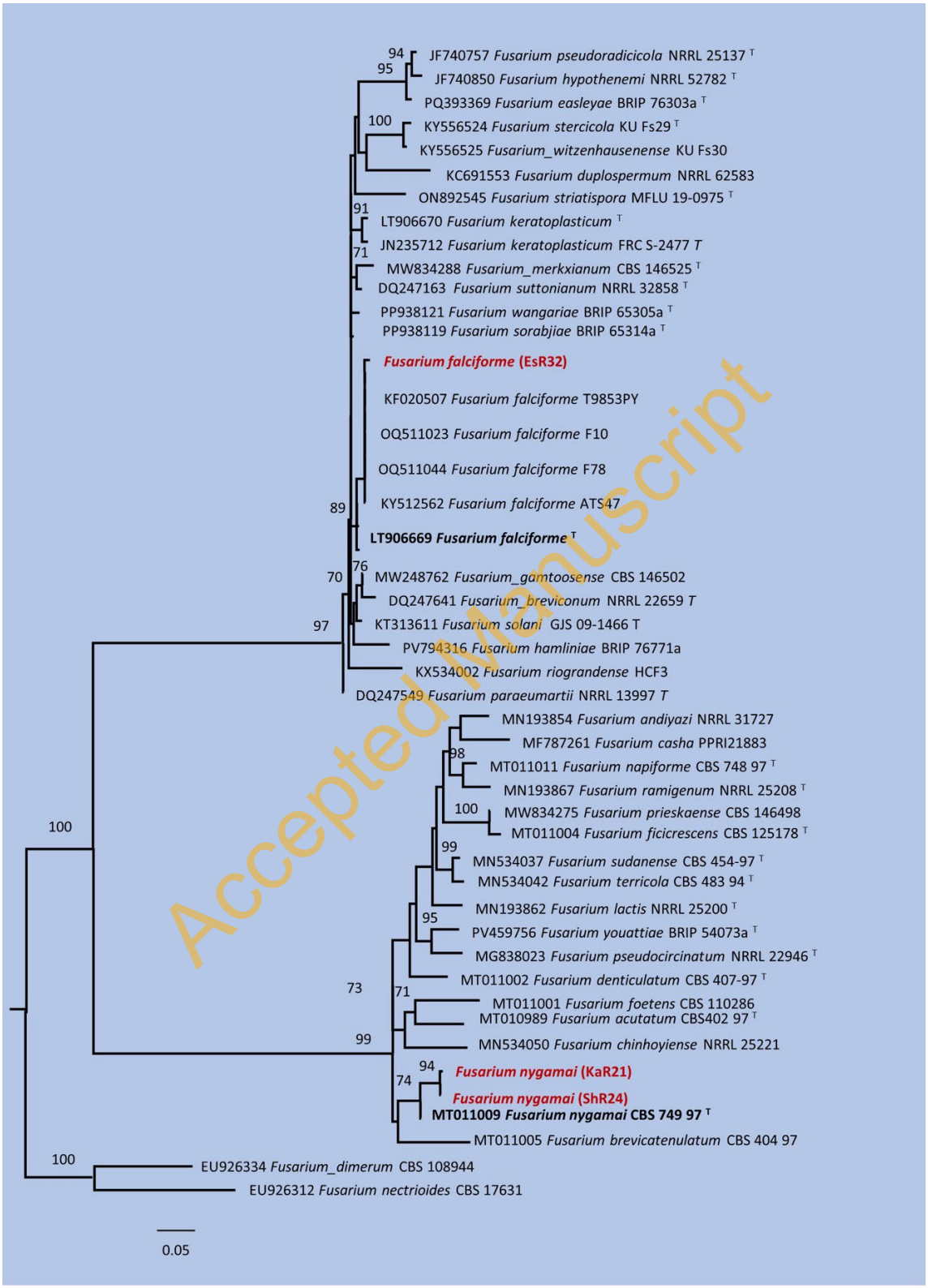


Fig. 4. Phylogenetic tree based on *TEF1* gene sequences of the identified *Fusarium* species using the Maximum-likelihood method. The numbers above each branch indicate the amount of bootstrap support from a 1000-times phylogenetic tree drawing using the Maximum-likelihood method. *Fusarium nectrioides* and *F. dimerum* were used as outgroups. The *Fusarium* isolates from safflower are marked with a red color.

Pathogenicity test

One month after inoculation, seedling reactions to the pathogens were evaluated. The pathogenic isolates caused crown and root necrosis, reduced hairy roots, and leaf yellowing. Following the observation of these symptoms, the infected tissues were transported to the laboratory for further analysis. In order to isolate the pathogen and fulfill Koch's postulates, the infected plant tissues were cultured on PDA medium, which confirmed the pathogenicity of the species (Figs 5 and 6).

Fig. 5. Pathogenicity test under greenhouse conditions on safflower seedlings of the Kuseh cultivar 30 days after inoculation. Symptoms caused by (A) *F. falciforme,* and (B) *F. nygamai* on the root and crown.

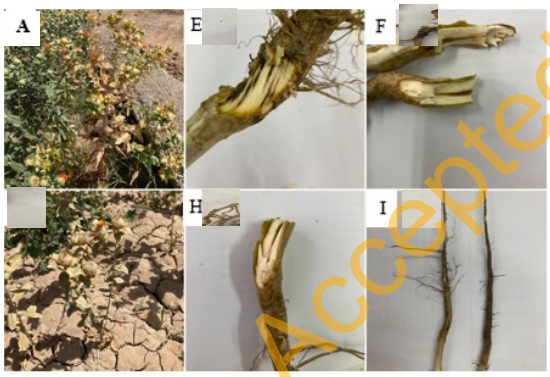


Fig. 6. Symptoms caused by *Fusarium* species. (A-C) Plant dieback, crown discoloration, and vascular necrosis caused by the fungus *F. nygamai*. (D-F) Plant dieback, vascular necrosis, and root discoloration caused by the fungus *F. falciforme*.

DISCUSSION

The species *Fusarium nygamai* and *F. falciforme* identified in this study are the first worldwide reports as causative agents of crown and root rot in safflower. *F. nygamai* causes wilting, crown discoloration, and vascular necrosis, while the host infected with *F. falciforme* shows wilting, vascular necrosis, and discoloration. *F. nygamai* has been isolated from rice in Sardinia (Balmas et al. 2000). Kurmut et al. (2002) noted characteristic symptoms such as black root rot and rotting, and death of the lateral root system. Heavily infected plants showed black neck canker at the soil surface, often accompanied by loss of turgor in the leaves, which turned brown and died. The death of healthy leaves was also recorded. Most isolates were pathogenic for Vicia faba, with disease severity ranging from 28 to 100% (Kurmut et al. 2002). In addition, *F. nygamai* has been reported from lentils (Lens culinaris Medikus) in Pakistan (Rauf et al. 2016), wheat roots and stems (Besharati Fard et al. 2017), and sugar beet in China (Cao et al. 2018). It has also been implicated in wheat root rot in Iraq (Minati 2020) and has been identified for the first time as the cause of *Fusarium* crown rot in wheat in China (Zhang et al. 2023). This study provides valuable information for epidemiological studies of *Fusarium* crown rot (Zhang et al. 2023). Furthermore, this species has been identified as the cause of onion rot in India, particularly in Karnataka, where infected onions show typical base rot symptoms, including root rot, soft rot, white fungal growth on the bulbs, and yellowing, browning, and drying of the leaves (Rajakumara et al. 2024). *F. nygamai* has also been associated with crown and root rot on wheat in Gorgan (Maghsoudlou et al. 2007). Additionally, this species is reported to cause crown and root rot in Kermanshah (Safaee et al. 2012), Khuzestan (Eslahi 2012), and on *Achillea wilhelmsii* in Kerman (Habibi et al. 2018).

Previous studies have reported *F. falciforme* as a causal agent of root and sometimes crown disease in various countries. It has also been identified as a pathogen of tomato root rot (Debbarma et al. 2024). Additionally, Silvia et al. (2023) reported *F. falciforme* on melon in Brazil, where symptoms appeared 15 days after inoculation, including yellowing and wilting, followed by tip dieback and rot. This was the first report of these species causing root rot in muskmelon in Brazil. Their findings underscore the importance of monitoring *Fusarium* agents in melon and highlight implications for breeding programs targeting resistant plant varieties (Silvia et al. 2023).

Fusarium falciforme was isolated and identified as the cause of root rot in chickpeas in Lorestan (Mehdinia et al. 2024) and melons in Iran (Sabahi et al. 2023). Furthermore, *F. falciforme* is reported to cause root and rhizome rot of *Epimedium sagittatum* in China (Chu et al. 2024). Additionally, *F. falciforme* has been reported in Morocco as a causative agent of crown and root rot, producing weak to moderate symptoms, including browning and necrosis at the stem base, crown, and roots, which can extend from the roots to the upper plant parts (Qostal et al. 2025).

Given that safflower is an emerging oilseed and industrial crop of significant economic importance, identifying its pathogens is crucial (Bowles et al. 2010). Recognizing harmful diseases and identifying key safflower pathogens plays a vital role in protecting the crop, mitigating damage, and enhancing its economic value. Importantly, understanding these pathogens can pave the way for further research aimed at developing effective disease management strategies. Among the actionable recommendations arising from

Accepted Manuscript

this research is to investigate the level of resistance to the identified pathogens and assess their impact on both the quantity and quality of oil produced by safflower, which is essential for achieving high-quality crop yields.

ACKNOWLEDGMENT

The authors would like to thank Isfahan University of Technology for its support throughout the duration of this project.

AUTHOR CONTRIBUTION

Bahram Sharifnabi planned, supervised, and supported the research. Fatemeh Mostafaei and Zeinab Esmaeili conducted the laboratory experiments and performed data analysis.

DATA AVAILABILITY

The datasets used during the current study are available from the corresponding author upon request.

DECLARATION

The authors declare no conflicts of interest.

FUNDING

Please refer to acknowledgments.

ETHICS APPROVAL

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

REFERENCES

Anonymous. 1402. Agricultural Statistics of Crops, Center for Statistics and Information and Communication Technology, Ministry of Agricultural Jihad, Tehran. https://doi.org/10.1080/03235408.2016.1275423.

Arif M, Chawla S, Zaidi M, Rayar J, Variar M, Singh U. 2012. Development of specific primers for genus *Fusarium* and *F. solani* using rDNA sub-unit and transcription elongation factor (TEF-1α) gene. African Journal of Biotechnology. 11(2): 444 –447. doi:10.5897/AJB10.489.

Balmas V, Corda P, Marcello A, Bottalico A. 2000. *Fusarium nygamai* associated with *Fusarium* foot rot of rice in Sardinia. Plant Disease 84(7): 807. https://doi.org/10.1094/PDIS.2000.84.7.807B.

Besharati Fard M, Mohammadi A, Drvishnia M. 2017. *Fusarium* species associated with wheat crown and root tissues in the eastern Iran. Archives of Phytopathology and Plant Protection. 50: 123–133. https://doi.org/10.1080/03235408.2016.1275423.

Bowles VG, Mayerhofer R, Davis C, Good AG, Hall JC. 2010. A phylogenetic investigation of *Carthamus* combining sequence and microsatellite data. Plant Systematics and Evolution. 287: 85–97. https://doi.org/10.1007/s00606-010-0292-3.

Brown DW, Proctor RH. 2013. *Fusarium*: genomics, molecular and cellular biology. Caister Academic Press, Norfolk, UK.

Cao S, Yang N, Zhao C. Liu J, Han C, Wu X. 2018. Diversity of *Fusarium* species associated with root rot of sugar beet in China. Journal of General Plant Pathology. 84: 321–329. https://doi.org/10.1007/s10327-018-0792-5.

Chu L, Li D, Hou W, Dan Z, Wang X, Dong C. 2024. First report of *Fusarium falciforme* causing root and rhizome rot of *Epimedium sagittatum* in China. Plant Disease. 108(5): 1400. https://doi.org/10.1094/PDIS-01-24-0185-PDN.

Da Via D, Knowles, P, Klisiewicz J. 1981. Evaluation of the world safflower collection for resistance to *Phytophthora*. Crop Science. 21: 226–229. https://doi.org/10.2135/cropsci1981.0011183X002100020005x.

Debbarma R, Maya B, Ashwini JH, Kamil D. 2024. Oligonucleotide-based detection of three plant pathogenic species of *Fusarium solani* species complex (FSSC) causing tomato root rot and wilt disease. Physiological and Molecular Plant Pathology. 134: 102478. https://doi.org/10.1016/j.pmpp.2024.102478.

Divakara ST, Santosh P, Aiyaz M, Ramana MV, Hariprasad P, Nayaka SC, Niranjana SR. 2014. Molecular identification and characterization of *Fusarium* spp. Associated with sorghum seeds. Journal of the Science of Food and Agriculture. 94(6): 1132-1139. https://doi.org/10.1002/jsfa.6380.

Ekwomadu TI, Mwanza, M. 2023. *Fusarium* fungi pathogens, identification, adverse effects, disease management, and global food security: a review of the latest research. Agriculture. 13(9): 1810. https://doi.org/10.3390/agriculture13091810.

Emongor V, Oagile O, Phuduhudu D, Oarabile P. 2017. Safflower production. Botswana University of Agriculture and Natural Resources. Gaborone.

Eslahi MR. 2012. Fungi associated with root and crown rot of wheat in Khuzestan province, Iran. Journal of Crop Protection. 1(2):107–113.

Esmaili Z, Sharifnabi, B. 2023. *Fusarium* species associated with apple trees decline in Isfahan, Iran. Mycologia Iranica. 10(1): 23–34. 10.22092/MI.2022.360249.1235.

FAOSTAT 2022. Food and Agriculture Organization of the United Nations. Internet resource: http://Faostat.Fao.org.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE, 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology. 72(1): 151.

Garmaroodi HS, Jabbari H, Nazari M, Valipour M. 2024. Assessment of genetic resistance of safflower (*Carthamus tinctorius*) to *Fusarium* damping off in greenhouse conditions. Journal of Iranian Plant Protection Research. 38(1): 35–47. https://doi.org/10.22067/jpp.2024.86136.1172.

Accepted Manuscript

Geiser, DM, del Mar Jimenez-Gasco M, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, O'Donnell K. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. European Journal of Plant Pathology. 110: 473–479. https://doi.org/10.1023/B:EJPP.0000032386.75915.a0.

Gouy M, Guindon Ph, Gascuel O. 2010. SeaView Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution. 27: 221–224.

Habibi A, Mansouri SM, Sadeghi B. 2018. *Fusarium* species associated with medicinal plants of Lamiaceae and Asteraceae. Mycologia Iranica. 5(2): 91–101. doi: 10.22043/mi.2019.120383.

Hussain MI, Lyra DA, Farooq M, Nikoloudakis N, Khalid N. 2016. Salt and drought stresses in safflower: a review. Agronomy for Sustainable Development. 36(4):1–31. https://doi.org/10.1007/s13593-015-0344-8.

Jangir P, Singh N, Goel S, Kapoor R. 2023. Emergence of wilt disease caused by *Fusarium proliferatum* in safflower-growing fields in India. Journal of Plant Pathology. 105: 1103–1108. https://doi.org/10.1007/s42161-023-01452-8.

Li W, Yoo E, Sung J, Lee S, Hwang S, Lee GA. 2023. Distinct effects of seed coat and flower colors on metabolite contents and antioxidant activities in safflower seeds. Antioxidants. 12(4): 961. https://doi.org/10.3390/antiox12040961.

Kim SG, Ko HC, Hur OS, Luitel BP, Rhee JH, Yoon MS, Baek HJ, Ryu KY, Sung JS. 2016. First report of *Fusarium* wilt caused by *Fusarium proliferatum* on safflower. Research in Plant Disease. 22(2): 111–115. https://doi.org/10.5423/RPD.2016.22.2.111.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 33(7): 1870–1874. https://doi.org/10.1093/ molbev/msw054.

Kurmut AM, Nirenberg HI, Bochow H, Buttner C. 2002. *Fusarium nygamai*, a casual agent of root rot of *Vicia faba* L. in the Sudan. Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen. Universiteit Gent 67(2): 269–274.

Leslie J, Summerell B. 2006. The *Fusarium* Laboratory Manual. Blackwell, USA.

Ma LJ, Geiser DM, Proctor RH, Rooney AP, O'Donnell K, Trail F, Gardiner DM, Manners JM, Kazan K. 2013. *Fusarium Pathogenomics*. Annual Review of Microbiology. 67: 399–416. https://doi.org/10.1146/annurev-micro-092412-155650.

Maghsoudlou R, Taheri AAH, Rahnema K. 2007. Identification and pathogenicity of *Fusarium* spp. Isolated from root and crown of wheat in Gorgan area. Journal of Agricultural Sciences and Natural Resources. 14(2): 175–189.

Mehdinia F, Darvishnia M, Mirzaei Najafgholi H, Nazarian-Firouzabadi F. 2024. Study of *Fusarium* species causing wilt and root rot in chickpeas in Lorestan province. Plant Protection. 47(3): 101–116. 10.22055/ppr.2024.48286.1774.

Minati MH. 2020. First record of nine *Fusarium* spp. causing root rot on wheat (*Triticum aestivum* L.) in Iraq. AIP Conference Proceedings 2290: 020009. https://doi.org/10.1063/5.0027398.

Murray M, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 8(19): 4321–4325. https://doi.org/10.1093/ nar/8.19.4321.

Nelson PE, Toussoun TA, Marasas W. 1983. *Fusarium* species: an illustrated manual for identification. Penn State University Press. United States of America.

Nelson PE, Dignani MC, Anaissie EJ. 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. Clinical Microbiology Reviews. 7(4): 479–504. https://doi.org/10.1128/cmr.7.4.479.

O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience. 41(1): 61–78. https://doi.org/10.1007/BF02464387.

Prasad R, Navaneetha T, Nageswara Rao N. 2011. Cultural, morphological, pathogenic, and molecular diversity in *Macrophomina phaseolina* isolates of safflower from southern India. Indian Phytopathological Society 64(3): 247–253.

Qostal S, Kribel S, Mouden N. Chliyeh M, El Alaoui MA, Selmaoui K, Benkirane R, Touhami AO, Douira A, 2025. *Fusarium falciforme,* another causal agent of root rot of wheat and barley varieties in Morocco. 3 Biotech 15: 165. https://doi.org/10.1007/s13205-025-04333-2.

Raghuwanshi K, Dake G. 2009. *Fusarium* wilt of safflower (*Carthamus tictorius* L.): A review. Journal of Plant Disease Sciences. 4: 110–117.

Rajakumara A, Bhat G, Shivakumar G, Madalageri BB, Noojibail P, Anandalakshmi R. 2024. *Fusarium brachygibbosum* and *Fusarium nygamai* cause basal rot of onion (*Allium cepa*) in India. New Disease Reports. 50(2): e12311. https://doi.org/10.1002/ndr2.12311.

Rauf CA, Rafique K, Naz F, Kang S. 2016. First report of lentil wilt caused by *Fusarium nygamai* in Pakistan. Journal of Plant Pathology 98(3): 696.

Sabahi F, Banihashemi Z, Mirtalebi M, Rep M, Cacciola SO. 2023. Molecular variability of the *Fusarium solani* species complex associated with *Fusarium* wilt of melon in Iran. Journal of Fungi. 9(4): 486. https://doi.org/10.3390/jof9040486.

Accepted Manuscript

Safaee DH, Younesi H, Sheikholeslami M. 2012. *Fusarium* species that cause root and crown rot of wheat in Kermanshah province. Iranian Journal of Plant Patholog.y 48(2): 265–268.

Silvia SGA, Costa MM, Cardoso AMA, Nascimento LV, Barroso KA, Nunes GHS, Pfenning LH, Ambrósio MMQ. 2023. *Fusarium falciforme* and *Fusarium suttonianum* cause root rot of melon in Brazil. Plant Pathology. 72(4): 721–730. https://doi.org/10.1111/ppa.13701.

Summerell BA, Salleh B, Leslie JF. 2003. A Utilitarian Approach to *Fusarium* Identification. Plant Disease 87(2): 117–128. https://doi.org/10.1094/PDIS. 2003. 87.2.117.

Taware M, Gholve V, Dey U. 2014. Bio-efficacy of fungicides, bioagents, and plant extracts/botanicals against *Alternaria carthami*, the causal agent of *Alternaria* blight of Safflower (*Carthamus tinctorius* L.). African Journal of Microbiology Research. 8(13): 1400–1412. https://doi.org/ 10.5897/ AJMR 2013

Tirado-Ramírez M, López-Orona C, López-Billard J, Molina-Cárdenas L, Sánchez-Rangel J, Amarillas-Bueno L, Vega-Gutiérrez T. 2023. First report of *Fusarium verticillioides* causing safflower root rot in Sinaloa, Mexico. Plant Disease. 107(9): 2851. https://doi.org/10.1094/PDIS-02-23-0257-PDN.

White TL, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Innis, MA, Gelfand, DH, Sninsky, JJ, White, TJ (Eds.). Academic Press, pp 315–322.

Zhang N, Zhu YN, Wei ZX, Gou JJ, Gao HJ, Yang WX. 2023. First Report of *Fusarium nygamai* causing crown rot of wheat in China. Plant Disease. 107(7): 2262. https://doi.org/10.1094/PDIS-12-22-2844-PDN.

Accepted Manuscript

خصوصیات ریخت‌شناسی و مولکولی گونه‌های *Fusarium* عامل بیماری پوسیدگی طوقه و ریشه گلرنگ

فاطمه مصطفایی، بهرام شریف‌نبی✉، زینب اسماعیلی

گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، ایران

چکیده

گلرنگ گیاهی یکساله متعلق به خانواده *Asteraceae* می‌باشد. با این حال، آلودگی به گونه‌های *Fusarium* یک چالش جهانی قابل توجه به شمار می‌رود. به منظور انجام مطالعات ریخت‌شناسی و مولکولی روی گونه‌های *Fusarium* مرتبط با بیماری پوسیدگی طوقه و ریشه در گلرنگ، نمونه‌هایی از طوقه و ریشه گیاهان آلوده از مناطق مختلف کشت در استان اصفهان جمع‌آوری گردید. با استفاده از تجزیه و تحلیل ریخت‌شناسی و داده‌های مولکولی حاصل از توالی‌یابی ناحیه ژنومی ITS-rDNA و بخشی از ژن *TEF1*، دو گونه *Fusarium* شامل *F. nygamai* و *F. falciforme* از میان ۳۶ جدایه دارای علائم این بیماری شناسایی شدند. آزمون بیماری‌زایی جدایه‌ها در شرایط گلخانه‌ای براساس فرضیات کخ تایید شد. شایان ذکر است که این مطالعه اولین گزارش از *F. nygamai* و *F. falciforme* به عنوان عوامل بیماری‌زای پوسیدگی طوقه و ریشه در گلرنگ در سراسر جهان می‌باشد.

کلمات کلیدی: بیماری، بیماری‌زایی، *Carthamus tinctorius*، *Fusarium nygamai*، *Fusarium falciforme*.

Accepted Manuscript

مکاتبه کننده: بهرام شریف نبی sharifna@iut.ac.ir

تاریخ دریافت: ۱۴۰۴/۰۶/۱۵، تاریخ بازنگری: ۱۴۰۴/۰۸/۱۹، تاریخ پذیرش: ۱۴۰۴/۰۹/۲۴، تاریخ انتشار برخط: ۱۴۰۴/۰۹/۲۷