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Fusarium species associated with apple trees decline in Isfahan, Iran

Z. Esmaili

B. Sharifnabi ✉

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

Abstract: Apple (*Malus domestica*) is considered one of the most important economic products in Iran. Its cultivation is common in various regions and so far, more than 200 different domestic and foreign apple cultivars are identified, which Golden and Red Delicious cultivars are the most important cultivars in terms of cultivated area. Various fungal, bacterial, nematode, and viral agents cause weakness and decline of the apple tree and the quantitative and qualitative reduction of the crop. Apple root and crown rot is one of the important diseases of apple root, due to soil-born fungal pathogens, which lead to the decline of apple trees. The disease has a global spread and can occur at all ages of apple production. Different fungal species have been identified in infected regions so far, and it may be due to climate change. Sampling was performed from June to August 2019 from apple orchards with decline symptoms in Semirom and Padena in Isfahan province, Iran. Fungal species, including *Fusarium solani*, *F. oxysporum*, *F. equiseti*, *F. acuminatum*, and *F. redolens*, were morphologically identified. To confirm morphological identification, EF1/EF2 and ITS1/ITS4 primers were used in the PCR reaction. *In vitro* and greenhouse tests confirmed the pathogenicity of the identified species. As a result, it was proved that *F. solani*, *F. oxysporum*, *F. equiseti*, *F. acuminatum*, and *F. redolens* could be the causal agents of apple trees decline in Isfahan province, Iran, that *F. solani*, and *F. oxysporum* were more abundant.

Keywords: Root and crown rot, *Fusarium*, disease, PCR, pathogenicity

INTRODUCTION

The domestic apple (*Malus domestica*) is a tree of temperate regions, from the family *Rosaceae* and the subfamily *Pomoidae* (Pereira-Lorenzo et al. 2009). Apple is considered one of the important agricultural products due to its high nutritional and economic value (Moniei 2009). According to the FAO statistics, global apple production in 2019 was about 87 million tons, and 56 million tons were produced in Asia. China has the first rank in the world with 42 million tons, then countries of the United States, Turkey, Iran, Poland, Italy, France, India, Russia, and Chile are in the following ranks. Iran is the fourth world apple producer, with a production of 2.24 million tons annually (FAOSTAT 2019).

The most important apple production regions in Iran are the provinces of West and East Azerbaijan, then Fars, Tehran, Ardabil, Isfahan and except for the Southern areas of the country, its cultivation is also common in other regions of the country. Various factors of fungal, bacterial, nematode, and viral pathogens contribute to the weakness and deterioration of the apple tree and the quantitative and qualitative reduction of the product (Jones & Aldwinckle 1990).

Apple root and crown rot is widespread in the world. Root rot often occurs at the same time as crown rot. One of the primary pathogens of this disease is *Phytophthora cactorum*. The disease was often confused with winter injury, since it was more prevalent after harsh winters, or from cankers caused by fire blight disease. It was in 1939, when Baines (1939), conclusively showed that the Grimes collar rot was caused by *Phytophthora* (Turechek 2004). Many fungal species were reported as apple root and crown rot pathogenic factors. Some important reported species are *Phytophthora* spp. (Welsh 1942; Naffaa & Rashid 2017), *Fusarium* spp. (Heidarian et al. 2007; Brown & Proctor 2013), and *Verticillium* spp. (Naffaa & Rashid 2017).

Fusarium is a cosmopolitan genus of filamentous ascomycete fungi (*Sordariomycetes*, *Hypocreales*, *Nectriaceae*). *Fusarium* includes many toxin-producing plant pathogens of agricultural importance and may produce a diverse array of toxic secondary metabolites (Ma et al. 2013). Members of the *Fusarium* genus are numerous that can be recovered from plants and soil worldwide as pathogens, endophytes, and saprophytes. The plant diseases caused by *Fusarium* species are not restricted to any particular region or cropping scenario. They can be as

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✉ Corresponding Author: E-mail: sharifna@iut.ac.ir

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problematic in temperate, commercial agriculture as in subsistence tropical agriculture. In many cases, the causal agents of these diseases may be difficult to determine, as they may occur in plants affected by other factors or that are simultaneously infected by weak pathogens or saprophytes. *Fusarium* species such as *F. pseudograminearum*, *F. culmorum*, and *F. solani* cause diseases in which the roots and crown of the host plant are rotted, resulting in an insufficient or ineffective root system and increased susceptibility to lodging and collapse (Brown & Proctor 2013).

Apple tree decay disease has been studied in different parts of the world, and various pathogens have been reported. *Phytophthora cactorum* has been isolated from active apple wounds in Canada (Welsh 1942). Also, *P. cactorum*, *P. cambivora*, and *Pythium ultimum* were reported as causative agents of root rot of young apple seedlings grown in contaminated potting soil in greenhouse experiments, in 1991 in British Columbia, Canada (Utkhede & Smith 1991). *Rhizoctonia solani* (18.3%), *Verticillium* sp. (17%), *Phialophora* spp. (7.4%), *Rosellinia* sp. (6.3%), *Acremonium* sp. (5.7%), *Cylindrocarpon* sp. (4%), and *Pestalotiopsis* sp. (2.9%) were isolated from some infected apple trees in southern Syria and two species of *Phytophthora* including *P. cactorum* (91.5%), and *P. cambivora* (8.5%) from all infected apple trees (Naffaa & Rashid 2017). Isolates of *P. cactorum*, *P. cryptogea*, *P. megasperma*, *P. gonapodyides*, *P. cambivora*, *P. citricola*, *P. drechsleri*, and *P. parasitica* in Chile and North America (Zakiei & Alavi 1986; Latorre et al. 2001) and *P. cryptogea*, *P. megasperma*, *P. cactorum*, and *P. cambivora* were isolated in apple orchards from trees infected with root and crown rot in Adelaide, Australia (Jeffers & Aldwinckle 1988; Wilcox 1993; Khatami et al. 2014). *Dematophora necatrix*, *Rhizoctonia solani*, and *Sclerotium rolfsii* were introduced as root rot agents in Pakistani apple orchards. The cause of root rot and crown of apple trees in Spain caused by *P. cactorum*, *P. cryptogea*, *P. gonapodyides*, and *P. megasperma* has been reported (Latorre et al. 1997).

Apple root and crown rot disease is reported in some areas of Iran. Root rot disease caused by *Armillaria* spp. was reported from apple trees in 1956 (Asef et al. 2003). *Armillaria mellea* has been reported in Isfahan, Khorasan, Markazi, and East Azerbaijan provinces (Heidarian et al. 2007). *P. cactorum* was reported as the causal agent of root and crown rot of apple trees in 1987 in Miandoab, West Azerbaijan, and Fars provinces (Peighami 2006). *P. cactorum*, *P. nicotianae*, *P. citrophthora*, and *R. solani* were isolated as root and crown rot agents of apple in the orchards of Khorasan province in 2002-2006 (Afzali 2008). Also, *P. nicotiana* var. *parasitica* was reported as the causal agent of root and crown rot of apple trees in Shahriar (Heidarian et al. 2007). The abundance of *Dematophora necatrix*, *Armillaria mellea*, *P. cactorum*, *P. nicotiana*, and *Fusarium solani*, and *R. solani*, which are other pathogens affecting root and crown rot of apple trees, were isolated in the years 2004-2006 from apple orchards in Semirom, Hana, and Padena. *P. cactorum* was introduced as an important cause of root and crown rot of seedlings and young trees of Golden Delicious and Red Delicious cultivars from West Azerbaijan province orchards in 2006. *P. citricola* has also been isolated from apple trees. The apple trees that infected with root and crown rot in Kohgiluyeh and Boyer-Ahmad province and Ramhormoz, Khuzestan province (Heidarian et al. 2007; Ghaderi et al. 2009). As climate change, may be an agent of apple root and crown rot shifted to *Fusarium* species, this study aims is to find the *Fusarium* species associated with the disease in Iran.

MATERIALS AND METHODS

Sample collection

Infected apple trees root and soil around the root (Red Delicious cultivars) with symptoms of weakness, yellowing of leaves, and growth reduction were randomly collected from Semirom and Padena orchards in Isfahan province from June to November 2019. Infected root samples, along with surrounding soil from each tree were placed separately in plastic bags, then transferred to the laboratory and stored at four °C for further study.

Fungal isolation

The infected roots were separated and rinsed in running tap water for at least ten minute to remove soil particles and saprophytes. The infected roots were cut into small pieces of two cm and the sterilization steps took place. The root pieces were rinsed in sterile distilled water for 20 second and disinfected with 70% ethanol for one minute, then rinsed again in sterile distilled water for 20 second and then in 10% sodium hypochlorite solution for two minutes, after washing in three consecutive steps with sterile distilled water, and air dried on sterile filter paper. Dried samples were cultured on a PDA medium containing 50 ppm of chloramphenicol antibiotic, and the cultures were incubated at 25 °C for five to ten days.

In this study, suspension of infected soil samples used for possible isolation of pathogens. For this purpose, one gram of infected soil was suspended in nine mL sterile distilled water in ten mL tubes, then nine dilutions were prepared from the initial suspension by sequential dilution. One mL of each dilution was added to the individual Petri dishes. They moved to the form of English eight number for approximately ten second, therefore thoroughly mixing the suspension with the culture medium, then the culture media were stored at 25 °C. The isolates were sub-cultured into Water Agar (WA) medium, and a hyphal tip was transferred to PDA medium. The purified isolates were then stored on sterile filter papers at -20°C.

Morphological characterization

Fusarium species were identified according to their cultural and morphological characteristics as

described by Nelson et al. 1983 and Leslie and Summerell 2006. The isolates were grown on a PDA medium to determine their growth rate and colony pigmentation, so the cultures were incubated at 23-25ºC for 7–10 days in the dark. Colony diameter was measured and colony color were recorded with the naked eye. Isolates were also cultured on CLA (Carnation Leaf Agar by placing several sterile carnation leaves pieces in a Petri dish and floating them on 1.5% water agar) medium (Fisher et al. 1982), then incubated at 20-25°C in the presence of light, particularly exposed to NUV (near ultraviolet light) (black) light (Summerell et al. 2003) and kept for 14-21 days (Nelson et al. 1994). A daily (day/night) cycle was used.

DNA extraction

DNA extraction was performed from mycelium grown for seven to ten days in PDB (250 g potato, 20 g dextrose, 500 mL H_2o) (Nelson et al. 1983). The mycelium washed with sterile distilled water, then powdered in a sterile mortar with liquid nitrogen. Two hundred mg of the powder was transferred to 1.5 mL microtubes and 700 µL of extraction buffer which was kept at 65 °C (20 mM, EDTA, pH 8; 100 mM, Tris-HCl, pH 8; 1.4 M, NaCl; (W/V) 3% CTAB) was added to each sample. Six µL of Beta-Mercaptoethanol was added to each microtube and kept at 65 °C for 40 min, and the contents of the microtubes were slowly mixed every five minutes. Seven hundred µL of the chloroform-isoamyl alcohol mixture (24:1) was added to each microtube. The samples were placed on a shaker for 15 min at 100-150 rpm; meanwhile, the microtubes were shaken vigorously every five minutes. The contents of the microtubes were centrifuged for ten minutes at 10000 rpm at four °C after mixing. The supernatant was removed and transferred to new sterile microtubes. To remove the contaminants better, chloroform-isoamyl alcohol was added to each microtube, equal to the volume of each sample and the samples were centrifuged for ten minutes at 10000 rpm. The supernatant of each microtube was removed and transferred to new sterile microtubes. Cold pure isopropanol was added to each sample at the same volume, and the content of each samples were slowly mixed by inverting each microtube. The samples were kept at -20 °C for 30 minutes. The microtubes were centrifuged for ten minutes at 10000 rpm to precipitate DNA at the bottom of the microtube. The supernatant was slowly removed from the microtubes, and the DNA precipitation was washed with 500 µL of 70% ethanol. The samples were centrifuged for five minutes at 10000 rpm. The microtubes were placed upside down on tissue paper to air dry for two hours. Thirty µL of distilled water was added to each microtube and the samples were kept overnight at four °C to dissolve DNA in distilled water (Murray & Thompson 1980).

Molecular characterization

A part of the *EF1-α* gene and the nuclear ribosomal DNA internal transcript spacer (ITS1,4) amplified by PCR using the primers EF1/EF2 (Divakara et al. 2014) and ITS1/ITS4 (White et al. 1990), in a final volume of ten µL made stock from PCR master mix, forward and reverse primers and sterile distilled water, then added eight µL stock (EF1,2: five µL *Taq* DNA polymerase Master, 0.3 µL $MgCl_2$ and 0.3 µL of each primer (ten pmol of each primer) and 2.1 µL sterile distilled water. For ITS1,4: five µL Master-Mix, 0.5 µL of each primer (ten pmol of each primer) and two µL sterile distilled water) to two µL DNA in each sample. Amplification with EF1,2 primers consisted of an initial denaturation at 94°C for two minutes, 30 denaturation cycles at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for ten minutes and amplification with ITS1,4 primers consisted of an initial denaturation at 94°C for two minutes, 35 denaturation cycles at 94°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for one minute; and final extension at 72°C for ten minutes. T100™ Thermal Cycler machine was used for performing PCR reaction. The amplicons were sequenced by Macrogen Co. (South Korea) and the nucleotide sequences identities were determined using BLAST analysis from NCBI available online and most identical nucleotide sequences from each species were recorded together with their information to use in phylogenetic analysis.

The nucleotide sequences were aligned and compared by Kimura's two parameters distance model and the Maximum-likelihood (ML) method with the Tamura-Nei distance model using the program MEGA ver. 7.0 software (Gouy et al. 2010). The topology of the resulting tree was tested by bootstrapping with 100 re-samplings of the data.

Pathological analysis

To perform a pathogenicity test and evaluation of the relationship between decay symptoms of apple trees and *Fusarium* species, a pathogenicity test was performed in laboratory and greenhouse conditions. The pathogenicity test was performed *In vitro* on wheat (Alvand cultivar) at 25 °C. Wheat seeds were disinfected with 0.05% Sodium hypochlorite for 20 minutes under sterile conditions and sterilized with distilled water consecutively. Three replications were considered for each treatment (*Fusarium* species).

One germinated wheat seeds along with a small piece of PDA medium containing the fungal species, and control was placed in each glass tube containing PDA medium. Greenhouse pathogenicity test was also performed at 20-25 °C and 60-70% RH. Wheat seeds were used to inoculate annual grafted apple seedlings (Red Delicious cultivar) with desired fungal species. Five hundred grams of wheat seeds were autoclaved for two hours. Sterile wheat seeds were inoculated with ten mycelial discs (5 mm) of the fungal species that grew on PDA medium for two weeks and were kept at 25 °C. To ensure the colonization of wheat seeds with the desired fungal species, Erlenmeyer flasks containing wheat seeds and fungal species were shaken daily, and the

moisture were kept with sterile distilled water. After sterilizing soil and plastic pots and planting the seedlings, five wheat seeds inoculated with the desired fungal species were placed in the soil around the scraped roots with a sterile scalpel. To provide 60-70% RH, the seedlings were kept under a plastic cover, and to retain moisture, water was sprayed in the space under the plastic cover.After three months, the disease symptoms on inoculated seedlings and controls were evaluated.

RESULTS

In infected orchards, symptoms of apple trees decline were observed in the form of weakness, leaves discoloration from pale green to brown, leaf fall, and deciduous foliage, which was probably due to the attack of fungal agents occurred in the root, followed by reduced crop yield. The crowns were free of contamination.

Based on morphological studies, thirty-four isolates were identified as a species belonging to *Fusarium* and, five species identified as *F. solani*, *F. oxysporum*, *F. equiseti*, *F. acuminatum* and *F. redolens* that *F. solani* and *F. oxysporum* were more abundant among all (Fig. 1,2). *F. redolen*s did not identify by common identification keys; therefore, amplification of the ITS-rDNA was performed.

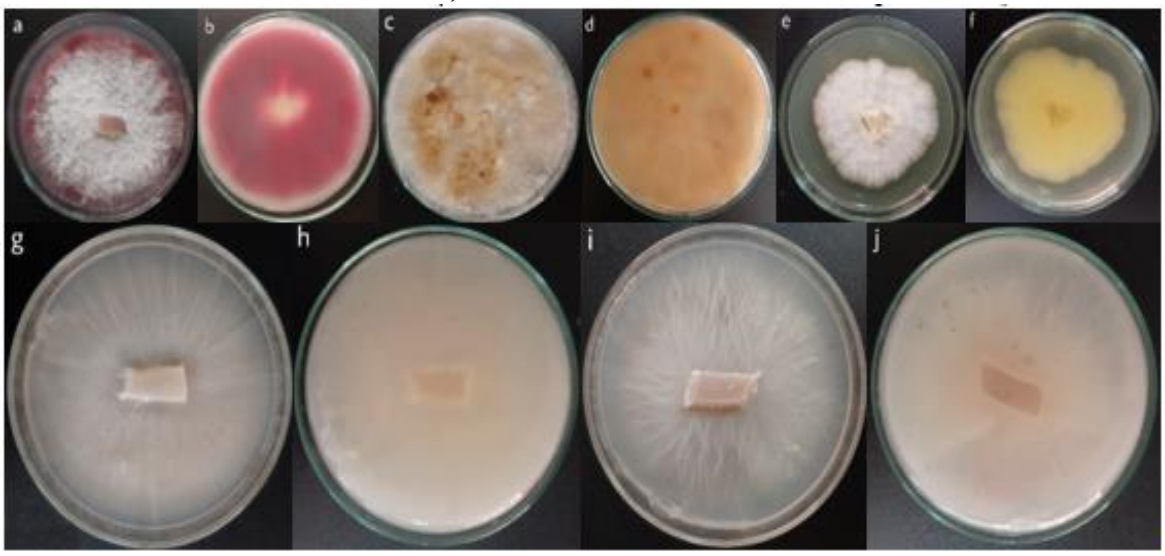

Fig. 1. Colony of *Fusarium* species on PDA medium after 7-10 days. a-b: *F. acuminatum*, c-d: *F. equiseti*, e-f: *F. redolens*, g-h: *F. solani* and i-j: *F. oxysporum.*

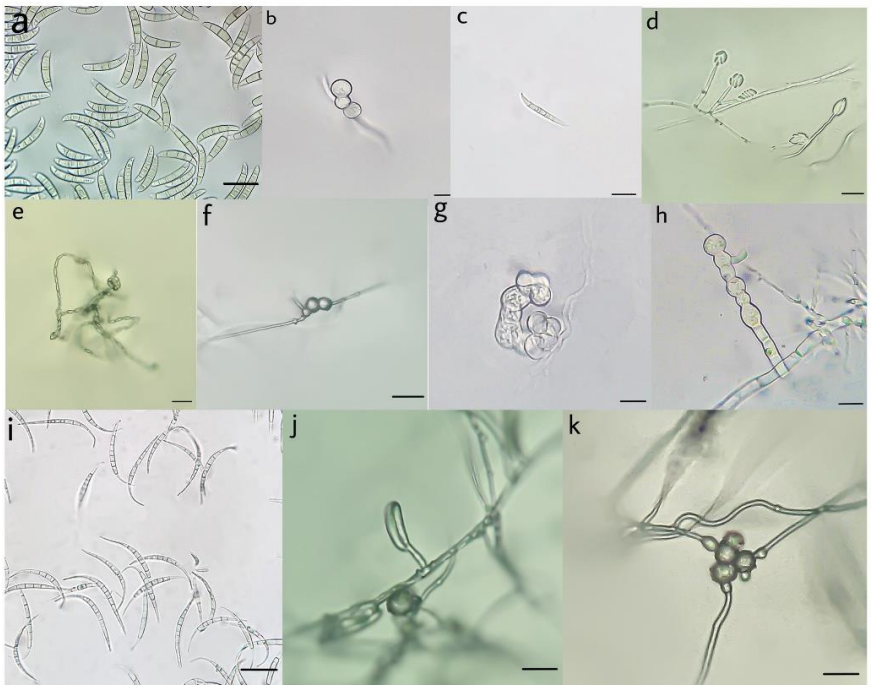

Fig. 2. Morphological characteristics of *Fusarium* species. a-b: macroconidia and chlamydospores in *F. solani*, c: macroconidium in *F. oxysporum*, d-f: microconidiophores, microconidium chain and chlamydospores in *F. acuminatum*, g-i: chlamydospores and microconidiophores in *F. equiseti* and j-k: monophialid and chlamydospores in *F. redolens*. Scale bars: a-k = 10µm.

Table 1. Isolates of *Fusarium* spp. used in this study and their accession number in GenBank.

Species	Isolate	Accession numbers in GenBank	
		ITS	*EF1-α*
F. solani	D_3	-	MZ087788
F. oxysporum	D_{10}	-	MZ087789
F. acuminatum	D_4	-	MZ087791
F. equiseti	E_6	-	MZ087790
F. redolens	G_{14}	MW866632	-

The nucleotide sequence of each species with some of its identical species nucleotide sequences in GenBank were compared and observed high identity. *F. solani* with 100%, *F. oxysporum* with 98%, *F. acuminatum* with 100%, *F. equiseti* with 100%, and *F. redolens* with 100% similarity identified (Table 1).

To know the evolutionary and molecular process of the nucleotide sequence of the *EF1-α* gene and ITS-rDNA in some *Fusarium* species, phylogenetic analysis was performed. The nucleotide sequences were aligned in CLC Sequence software, and phylogenetic trees were drawn by the Maximum-likelihood method, in MEGA software. Bootstrap values obtained after 100-time phylogenetic tree drawing. *Fusarium proliferatum* is considered as an outgroup in drawing both phylogenetic trees. The results of phylogenetic trees indicate different evolutionary pathways of the *EF1-α* gene in *F. solani*, *F. oxysporum*, *F. acuminatum*, and *F. equiseti* species and the ITS-rDNA for *F. redolens*. According to the results for the *EF1-α* gene (Fig. 3) and the ITS-rDNA (Fig. 4) in the phylogenetic tree, it can be concluded that, there is an evolutionary relationship between the *Fusarium* species that were isolated from infected apple trees roots in this research with identical species in different countries. Based on the bootstrap values more than 70%, confidently, we can declare that, these species have evolutionary relationships with each other.

The results of pathogenicity test showed that the isolates of *F. solani*, *F. oxysporum*, *F. equiseti*, *F. acuminatum* and *F. redolens* could infect the root, crown, and stem of wheat and infection was observed in the form of the color changes, it was visible from light to dark (Fig. 5).

The pathogenicity test was also performed in greenhouse conditions. After three months, roots of apple were removed from of the soil and color changes of roots vascular tissues light brown to dark was observed (Fig. 6).

Transverse slices of infected roots were isolated of the border between healthy and infected tissues and cultured in a PDA medium. After seven to ten days of growth of samples in PDA medium and their morphological identification, it was shown that, all tested species in the greenhouse are pathogenic to the root of apple trees. Koch's principles were also performed. In this study, the results of the pathogenicity test in greenhouse conditions on annual apple seedlings showed that *F. solani*, *F. oxysporum*, *F.acuminatum*, *F. equiseti*, and *F. redolens* caused a change from light brown to dark brown of the root vessels and eventually root rot.

DISCUSSION

Based on morphological and molecular characterizations, *F. solani*, *F. oxysporum*, *F. acuminatum*, *F. equiseti*, and *F. redolens* were identified. Infected trees may decay slowly, or may die suddenly in the last growing season (Naffaa & Rashid 2017). *F. solani* has been reported in Iran on many plants such as kiwi, cherry, Enshio tangerine, berry, olive, onion, garlic, peanut, beet, and silver cypress, and *F. oxysporum* has been reported on peach, banana, berry, strawberry, melon, olive, bean, sesame, saffron and cypress (Ershad 2022). *F. acuminatum* has been reported on peach, Enshio tangerine, melon, cucumber, onion, potato, beet, wheat, barley, lentil, sunflower, and safflower (Ershad 2022).

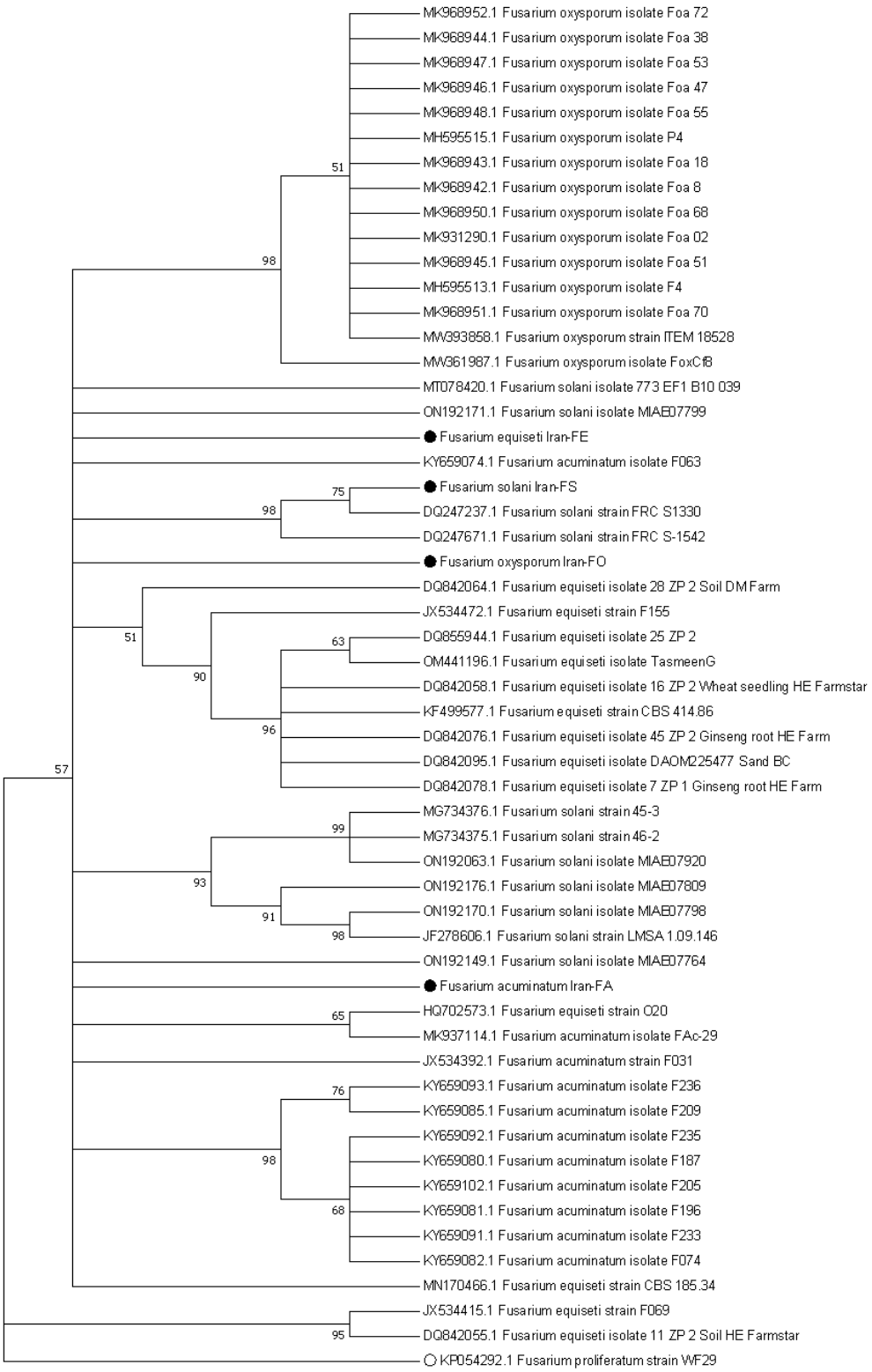

Fig. 3. Phylogenetic tree based on *EF1-α* gene sequences of fifty-five species of *Fusarium* using the Maximum-likelihood method. The numbers above each branch indicate the amount of bootstrap support from 100-time phylogenetic tree drawing using the Maximum-likelihood method. *Fusarium proliferatum* (KP054292) is an outgroup sample. The tested species are marked with black circles.

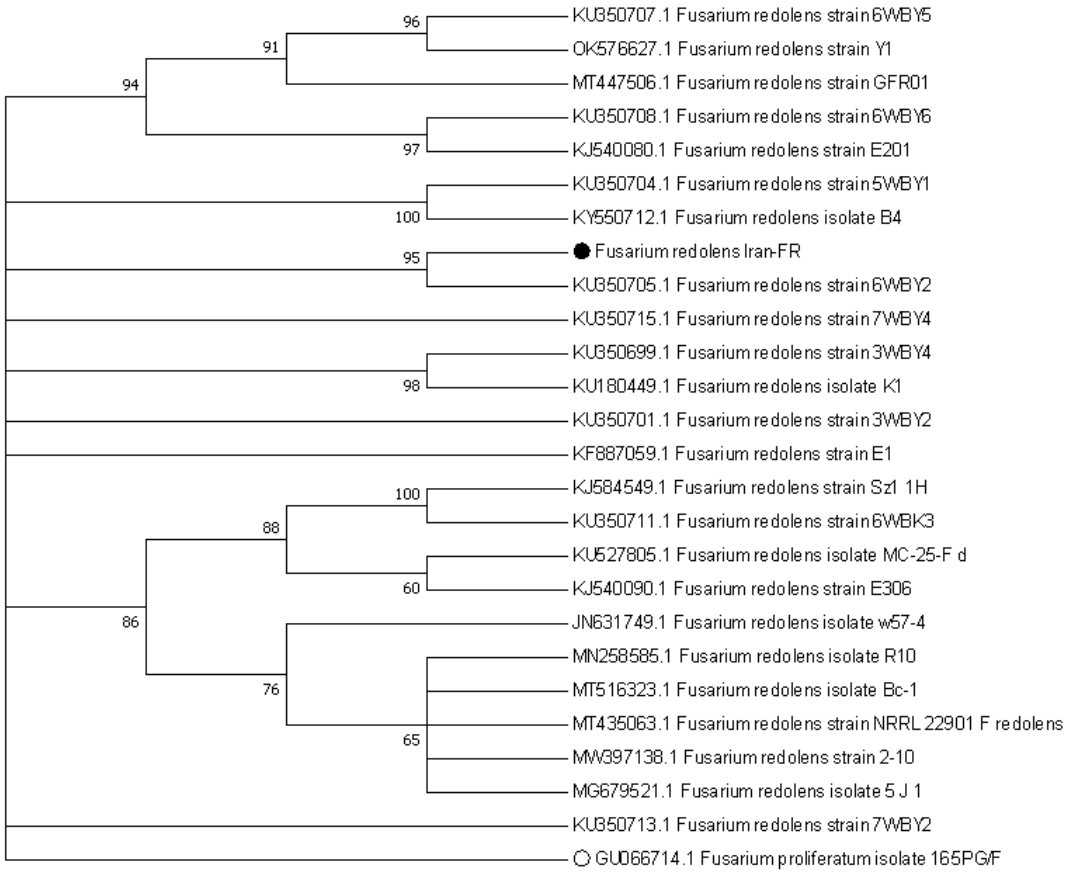

Fig. 4. Phylogenetic tree based on ITS-rDNA region sequences of twenty-six species of *Fusarium* using the Maximum-likelihood method. The numbers above each branch indicate the amount of bootstrap support from 100-time phylogenetic tree drawing using the Maximum-likelihood method. *Fusarium proliferatum* (GU066714) species is an outgroup sample. The tested species are marked with black circles.

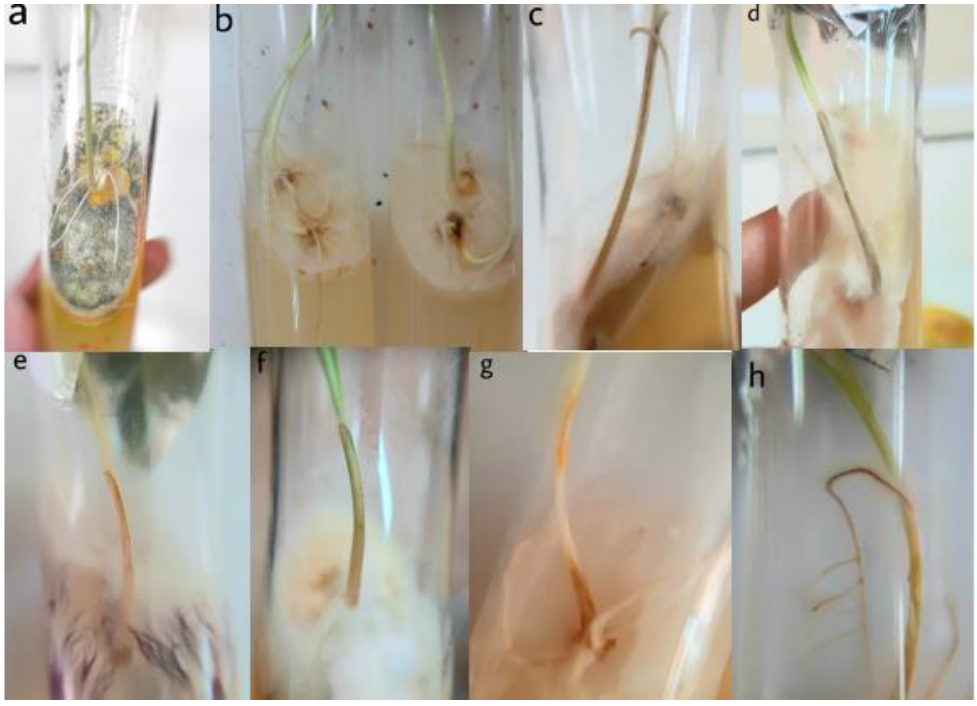

Fig. 5. *In vitro* pathogenicity test on wheat. Treatment samples are, a: *Penicillium* spp. (control), b-c: *F. solani*, d: *F. equiseti*, e: *F: oxysporum*, f-g: *F. redolens*, h: *F: acuminatum*.

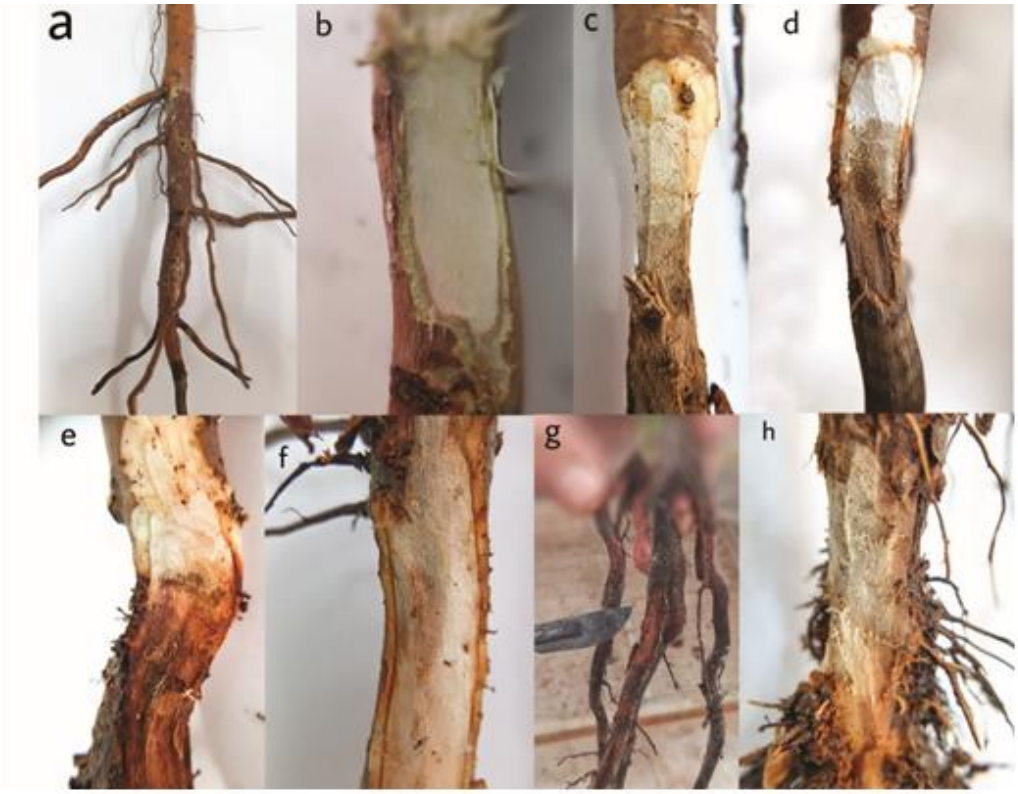

Fig. 6. Pathogenicity test in greenhouse conditions on annual apple seedlings. a-b: Control, c: *Fusarium oxysporum*, d: *F. equiseti* , e: *F. redolens*, f: *F. acuminatum* , g-h: *F. solani*

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F. equiseti has been reported on Enshio tangerine, tangerine, orange, sour orange, pistachio, corn, wheat, sesame, canola, onion, bean, tomato, and satin (Ershad 2022).*F. redolens* is a synonym of *Fusarium oxysporum* var. *redolens* and is generally a soil-born fungus that is common in temperate regions. Due to its morphological similarities, this species is supposed to be a variety of *F. oxysporum*. It isn't easy to distinguish *F. redolens* from *F. oxysporum* due to the intermediate forms between the two species. Unambiguous identification of these two species from each other has only recently been made possible by molecular methods such as PCR-RFLP in the ITS region of rDNA and gene sequencing. *F. redolens* is pathogenic on many host plants such as peas, carnations, bulbous plants, and spinach and causes wilting, seedling death, and root rot (Gerlach et al. 1982; Esmaeili Taheri et al. 2011). *F. redolens* has been isolated from necrotic and discolored root, and crown tissues of chickpea, pea, lentil and durum wheat in Saskatchewan, Canada and has been reported to cause root, and crown disease in a wide range of host plants. This species causes vascular rot on lentils and root rot on asparagus and soybeans in Europe and the United States. This species, as a variety of *F. oxysporum*, was isolated from oat, barley, and wheat seeds in Canada and has been reported as one of the most abundant fungal isolates from soil, in Manitoba, Canada, and caused peas and lentils root rot in Alberta, Canada (Esmaeili Taheri et al. 2011). *F. redolens* was reported in East Azerbaijan as the pathogen of onion bulb and seedling rot (Ghanbarzadeh et al. 2014) also, was reported as the pathogen of tomato root rot disease (Chehri 2016). The first report of *F. redolens* was on chickpeas in the west of Iran (Saeedi & Jamali 2021) as the pathogen of root rot disease.

The evolutionary relationships of some identified species were confirmed by drawing phylogenetic trees. A phylogenetic tree based on the nucleotide sequences of the *EF1-α* gene and ITS-rDNA was drawn to prove of evolutionary relationships among some *Fusarium* pathogenic species. The analysis included 22 *EF1-α* reference sequences of different *Fusarium* species and ten *EF1-α* reference sequences of *F. oxysporum* isolates, which were associated with tomato crop. This analysis showed that the isolates were not grouped according to geographical location. Six of the ninety-five fungal isolates analyzed could be related to the *F. solani* clade, while three isolates were grouped close to *Fusarium* species different from *F. oxysporum*. The remaining fungal isolates were grouped with reference sequences of the *F. oxysporum* species complex (pathogenicity factors of tomato Fusarium wilt disease) (Carmona et al. 2020). Also, a phylogenetic tree was created by concatenated DNA sequences of *EF1-α* and *β-tubulin* gene for pathogenicity factors of chickpea Fusarium wilt disease. Based on phylogenetic analysis, the isolates belonged to five different species. *F. oxysporum* f. sp. *ciceris*, and *F. redolens* had the most frequent in all of sampling areas. *F. hostae*, *F. equiseti*, and *F. acuminatum* had less frequent.

The results confirmed the usefulness of molecular identification of *Fusarium* spp. based on at least the two gene loci. In the present study, the isolates of *F. oxysporum* f. sp. *ciceris* formed a distinct clade that highly supported previous reports on the *F. oxysporum* complex as a monophyletic group independent from *F. redolens*. The phylogenetic tree was drawn based on the *EF1-α* and *β-tubulin* sequences and showed a distinct clade among *F. redolens* isolates. Also, *F. equiseti*, and *F. acuminatum* species with similar samples from GenBank were in one clade in the phylogeny tree (Younesi et al. 2021). The pathogenicity test of all identified species was proved *In vitro* and greenhouse conditions and vascular systems also were infected. Based on the pathogenicity test of several *Fusarium* species on wheat, *F. acuminatum*, *F. oxysporum*, and *F. strillhyphosume* did not cause symptoms in wheat, but some *Fusarium* isolates such as *F. fujikuroi*, *F. crookwellense*, *F. solani*, *F. circinatum*, *F. pseudocircinatum*, *F. culmorum*, *F. nygamai*, *F. verticillioides*, *F. avenaceum*, *F. proliferatum*, and some other *Fusarium* isolates also caused infection of the wheat root, crown and leaves and identified as pathogenic isolates on wheat (Rahimi Tamandegani & Zafari 2019). Also, based on the pathogenicity test of five *Fusarium* species on wheat, the percentage of growth delay was calculated in the growth of wheat pods *In vitro* at temperatures of 10, 15, 20, 25, and 30 °C in Europe, *F. avenaceum*, *F. culmorum*, and *F. graminearum* at 20-25 °C caused more than 83.3% delay in pod growth compared to the control sample and among the five species of *Fusarium*, *F. graminearum* had the highest pathogenicity and 96% reduction of pod growth at 25 °C and at least 44% reduction at the other four temperatures and *Michrodochium nivale* had the lowest pathogenicity after six days of storage at 15 °C and 55% reduction in pod growth at 10 °C (Brennan et al. 2003). Based on the pathogenicity test of several *Fusarium* species on one-month seedlings in South Africa, only *F. avenaceum* STEU7206 caused significant root rot and *F. solani* STE-U7214 significantly reduced seedling height (Tewoldemedhin et al. 2011). In wild apple forests at Tian Shan Mountains in Northwest China, symptoms of xylem browning and branches withering, were observed and *F. poliferatum*, *F. sporotrichioides*, *F. solani*, and *F. avenaceum* were isolated (Cheng et al. 2019). In another study based on the pathogenicity test on forty-day-old apple seedlings in South Tyrol, Italy, *F. oxysporum* was reported as non-pathogenic and *F. solani* had shown low pathogenicity (Manici et al. 2003).

In this research, based on morphological and molecular studies, *F. solani*, *F. oxysporum*, *F. acuminatum*, *F. equiseti*, and *F. redolens* species were identified. In addition, the pathogenicity tests *In vitro* and greenhouse conditions, showed that, the identified species are pathogenic to apple trees in Isfahan, Iran, and need to be monitored and controlled.

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گونه‌های فوزاریوم مرتبط با زوال درختان سیب در استان اصفهان

زینب اسماعیلی، بهرام شریف‌نبی*
گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، ایران

چکیده: سیب (*Malus domestica*) به عنوان یکی از ده محصول مهم اقتصادی کشور ایران شناخته شده است. کشت آن در مناطق مختلف رایج است و تاکنون ۲۰۰ رقم اهلی و خارجی سیب شناسایی شده است که رقم‌های رد دلیشز و گلدن دلیشز از مهمترین رقم‌های کشت شده در مناطق کشت سیب هستند. عوامل مختلف قارچی، باکتریایی، نماتدی و ویروسی در ضعف و زوال درخت سیب و کاهش کمی و کیفی محصول نقش دارند. بیماری پوسیدگی ریشه و طوقه سیب به عنوان یکی از بیماری‌های مهم درختان سیب در اثر عوامل بیماری‌زای قارچی خاکزاد که منجر به زوال درختان سیب می‌شود، انتشار جهانی داشته و در همه سنین درخت سیب می‌تواند رخ دهد. تاکنون گونه‌های مختلف قارچی در مناطق آلوده شناسایی شدند که ممکن است به علت تغییر اقلیم، تفاوت هایی را نشان دهد. نمونه‌برداری از خرداد تا آبان ماه ۱۳۹۸ از باغ‌های سیب آلوده به علائم زوال در شهرستان سمیرم و بخش پادنا در استان اصفهان انجام گرفت و گونه‌های قارچی *Fusarium solani*، *F. oxysporum*، *F. equiseti*، *F. acuminatum* و *F. redolens* شناسایی شدند. به منظور شناسایی مولکولی گونه‌های جداسازی شده، از جفت آغازگرهای EF1/EF2 و ITS1/ITS4 در واکنش PCR استفاده شد. آزمون بیماری‌زایی در شرایط آزمایشگاهی و گلخانه‌ای، بیماری‌زایی گونه‌های شناسایی شده را اثبات کرد. گونه‌های *F. solani*، *F. oxysporum*، *F. equiseti*، *F. acuminatum* و *F. redolens*موجب بیماری پوسیدگی ریشه درختان سیب در استان اصفهان شدند که گونه‌های *F. solani* و *F. oxysporum* بیشترین فراوانی را داشتند.

کلمات کلیدی: پوسیدگی ریشه و طوقه، *Fusarium*، بیماری، PCR، بیماری‌زایی

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