



## *Fusarium* species associated with medicinal plants of Lamiaceae and Asteraceae

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**Abstract:** The purpose of this study was to identify the *Fusarium* species associated with selected medicinal plant species of Lamiaceae and Asteraceae in Kerman, the second-largest province of Iran. The plants showing wilting/rotting symptoms as well as asymptomatic plants were collected from main medicinal plants reservoirs and production areas. *Fusarium* species were identified based on morphological characteristics as well as subsequent sequencing of the translation elongation factor 1-alpha (*TEF1-α*) gene. The following species were identified: *F. oxysporum*, *F. solani* and *F. nygamai* from *Achillea wilhelmsii* (Asteraceae); *F. solani*, *F. proliferatum* and species of *F. tricinctum* species complex from *Teucrium polium* (Lamiaceae); *F. solani*, *F. proliferatum* and species of *F. tricinctum* species complex from *Ziziphora tenuior* (Lamiaceae); *F. solani* and a species of *F. tricinctum* species complex from *Thymus carmanicus* (Lamiaceae); *F. redolens*, *F. brachygibbosum*, *F. proliferatum*, *F. tricinctum* species complex, *F. compactum* and *F. equiseti* from *Mentha piperita* (Lamiaceae); and *F. solani* and *F. proliferatum* from *Ziziphora clinopodiodes* (Lamiaceae). For the first time, the pathogenicity of *Fusarium nygamai* on *Achillea wilhelmsii* as well as *F. redolens* and *F. brachygibbosum* on *Mentha piperita* were confirmed in this study.

**Key words:** Root rot, rhizosphere microorganisms, morphology, *TEF1-a*

### INTRODUCTION

Several plant species in the Lamiaceae (Labiatae) and Asteraceae (Compositae) families are important ornamental, medicinal and aromatic plants which produce essential oils that are used in traditional and modern medicine. A large number of medicinal plants from the Lamiaceae and Asteraceae are grown in Kerman province, Iran. Kerman is the second-largest province of Iran with an area of 180,726 km<sup>2</sup> encompasses nearly 11 percent of the land area of the country with unique climatic conditions which has rangelands with a large variety of medicinal plants (Rajaei & Mohamadi 2012). In the recent years, much interests have been dedicated in medicinal plants and herbal derived products all over the world, including Iran.

The fungal genus *Fusarium* is notorious as a plant pathogen (Vujanovic et al. 2006, Bacon & Yates 2006) while several species are significant mycotoxigenic species or causal agents of human diseases. They are also found as saprophytes in air, water and soil, and as epiphytes or endophytes in living plants (Leslie & Summerell 2006, O'Donnell et al. 2010; Aoki et al. 2014). Members of the genus *Fusarium* are considered among ten globally most important genera of plant pathogenic fungi (Dean et al. 2012). The *Fusarium oxysporum*, is cosmopolitan fungi and several strains are known as sever threads agents for many crops. Furthermore, species of *F. graminearum* as well as *F. proliferatum*, *F. verticillioides* and *F. sporotrichoides*, produce mycotoxins on crops including wheat, maize, barley, rice and other foodstuffs (Mirocha et al. 1989, Bánáti et al. 2017).

*Fusarium* species such as *Fusarium solani* (Mart.) Sacc. (Green & Skotland 1993, Nasr Esfahani & Monazzah 2011) and *F. oxysporum* Schltdl. (Sattar & Husain 1980, Borges et al. 2018, Ortu et al. 2018) are reported as diseases agents on Lamiaceae species worldwide. Furthermore, *F. oxysporum* and *F. solani* are reported to be associated with several hosts from the Asteraceae family (Kourany et al. 1988, Koike 2011, Kim et al. 2016, Garibaldi et al. 2017, Matic' et al. 2018). However, a comprehensive study of

*Fusarium* species associated with medicinal species in the Lamiaceae and Asteraceae has not been investigated in Iran. To the best of our knowledge, no comprehensive information is available on fungal flora associated with these medicinal species in Iran. One reason is that these plant species are mainly distributed in less accessible areas of rangelands such as high elevations of mountains and rocky areas. Characterization of *Fusarium* species in association with these plant species is of great importance to understand their complex interactions among plant, fungus and habitat. Therefore, this study was designed to isolate and identify *Fusarium* species associated with selected medicinal species of the Lamiaceae and Asteraceae based on the morphological and molecular characteristics and thereafter, to study the pathogenicity of isolates on their hosts. Consequently, studies on pathogenicity of *Fusarium* spp. on these hosts are critical in order to develop management measures for this group of fungi wherever commercial plantations have been started.

## MATERIALS AND METHODS

### Sample collection

In 2017, a survey was conducted across the main medicinal plants reservoirs of northern Kerman province, Iran. The following species including *Teucrium polium*, *Ziziphora tenuior*, *Thymus carmanicus*, *Mentha piperita* and *Ziziphora cliniopodiodes* from Lamiaceae and *Achillea wilhelmsii* and *Artemisia dracuncululus* from Asteraceae, were selected for samplings. Sixty-five samples were collected from the wild plants and the main cultivation sites in northern regions of Kerman, Iran. At each site, three asymptomatic plants as well as three plants showing yellowing or wilting and brown discoloration of vascular tissues were collected 10 m apart in the form of whole plant and transferred to the laboratory in paper bags. Three soil samples (100 g) were collected in proximity of plant roots from a depth of 0–15 cm using a trowel. Soil samples were sieved, air-dried, and stored at 4°C until they were assessed.

### Fusarium isolation and storage conditions

The roots, crown and stem tissues were rinsed under running tap water for 30 minutes to remove excess soil particles and spores of fast-growing contaminant fungi present on tissues surface, surface-disinfected with 70% ethanol for 10 s, rinsed in sterile distilled water (SDW) for 10 s, then submerged for 2 min in 1% NaOCl, rinsed three times in SDW and dried on sterile filter paper. Tissues were cut into the 5–10 mm-long pieces, plated onto PDA (Potato Dextrose Agar) plates supplemented with antibiotic (0.2 g. L<sup>-1</sup> streptomycin sulfate). *Fusarium* species were isolated from soil using dilution method. The amount of 1.0 mL of 10<sup>-2</sup> to 10<sup>-4</sup> fold diluted suspensions was inoculated into a modified Nash/Snyder medium (Nash & Snyder 1962, Burgess

et al. 1994). Plates were incubated for 7 days at 25°C and inspected for *Fusarium*-like colonies. Pure cultures were established using single spore techniques (Summerell et al. 2003) and stored on PDA slants at 4°C. All the isolates were deposited in the KGUT Fungal Culture Collection at Kerman Graduate University of Advanced Technology, Kerman, Iran, stored in 15% glycerol at -80°C.

### Morphological characterization

*Fusarium* cultures were identified based on the approaches described by Nelson et al. (1983), Burgess et al. (1994), and Leslie & Summerell (2006). Isolates were cultured on SNA (Synthetic Nutrient-poor Agar) and CLA (Carnation Leaf Agar) and incubated at 22–25°C in darkness. Microscopic characteristics were observed after 7–14 days of inoculation. Colony morphology (pigmentation and colony growth rate) was assessed on PDA (Leslie & Summerell 2006). Each strain was grown at 15, 20, 25, 30, 35°C in darkness in triplicates. Colony diameter was measured at intervals of 24 h over a period of 96 h.

Microphotographs of fungal features were taken from agar plates and microscopic slides using a Dino-eye microscope camera USB lens (The Microscope Store, LLC., USA). Thirty measurements were taken of the relevant parameters of the conidiophores and conidia.

### DNA extraction, PCR, Sequencing

Genomic DNA was extracted using a CTAB extraction procedure (Doyle & Doyle 1987, Zhang et al. 2010). Approximately, 100 mg of fresh mycelia was scraped off the PDA plate of each isolate and homogenized using liquid nitrogen. Afterward, cells were lysed using CTAB solution and the DNA was extracted using DNG<sup>TM</sup>-plus DNA extraction solution (Sinaclon, Iran) according to the manufacturer's instructions.

A standard polymerase chain reaction (PCR) protocol was used to amplify a ~700 bp portion of the *TEF1-α* gene (Translation Elongation Factor 1α) region using primers ef1 (forward primer; 5'-ATGGG TAAGGA(A/G)GACAAGAC-3') and ef2 (reverse primer; 5'-GGA(G/A)GTACCAGT(G/C)AT CATGT T-3') (O'Donnell et al. 1998, Geiser et al. 2004). Amplifications were performed in a Biometra TAdvanced Thermal Cycler (Biometra, Göttingen, Germany) with an initial denaturation of 5 min at 95°C followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 54°C, 60 s extension at 72°C and a final extension of 5 min at 72°C. The quantity and quality of PCR products were evaluated on 1% agarose gels and for some of the PCR amplicons, product purification from the gel was conducted using the Kardan gel extraction kit (Kardan co, Iran). The PCR products sequencing was performed by Macrogen (Macrogen Inc., Seoul, South Korea).

### Molecular identification

The obtained sequences were manually edited by Geneious version 7 (Biomatters, USA). The sequencing results were compared with those in MLST *Fusarium* database and NCBI/Genbank database using a basic local alignment search tool (BLAST) (Altschul et al. 1990). All the sequences generated in this study were deposited in GenBank and the accession numbers were obtained (Table 1). Furthermore, the number of 31 DNA sequences was retrieved from GenBank according to the reliable published papers and included in the phylogenetic analyses. The sequences were aligned using Geneious version 7 (Biomatters, USA). Analyses of phylogenetic relatedness among the obtained *Fusarium* isolates and identification of the isolates to the species level were performed using PAUP\* 4.0a133 (Swofford 2002) for parsimony and MrBayes v3.2.2 (Ronquist et al. 2012) for Bayesian analyses. All the alignment gaps were treated as missing data. *Fusarium merismoides* Corda was used as outgroup. The best-fit model (HKY+I+G) was selected using JModeltest v.2.1.4 (Posada 2008) and the Bayesian information criterion (BIC) ratio test.

### Pathogenicity tests

The pathogenicity tests of selected *Fusarium* species being isolated from Lamiaceae and Asteraceae plant hosts were performed to satisfy Koch's postulates. The pathogenicity tests were performed only for the isolates from hosts which had symptoms of *Fusarium* infections in field samplings. Local cultivars of *Achillea wilhelmsii*, *Mentha piperita* and *Ziziphora cliniopodiodes* were included in the pathogenicity tests. The following experiments were designed. The isolates of *F. nygamai*, *F. oxysporum* and *F. solani* were selected to test for pathogenicity on *Achillea wilhelmsii*. The isolates of *F. brachygibbosum*,

*F. equiseti*, *F. proliferatum* and *F. redolens* were selected for pathogenicity test on *Mentha piperita*. The *F. solani* and *F. proliferatum* isolates were also selected to test on *Ziziphora cliniopodiodes* plants.

Five plants for each isolate/plant species combination were inoculated. The pathogenicity of the isolates was tested using root-dip inoculation method (Biles & Martyn 1989). Two isolates from each fungal species were used for inoculum preparation. The inoculum was prepared by growing cultures in potato dextrose broth (PDB) shaking at 180 rpm for 3-5 days. The cultures were filtered through two layers of sterile cheesecloth and then spore concentrations were adjusted to  $5 \times 10^6$  spores ml<sup>-1</sup> using a hemocytometer. For inoculations, few months old plants grown in autoclaved potting mix were removed and washed in running tap water. Afterward, the wounded root tips were dipped into the spore suspension for 30 min, then planted into pots containing sterile potting mix. Sterile PDB was used for controls. Plants were incubated at 20°C for 24 h, then transferred to greenhouse. Plants were inspected daily for symptoms.

## RESULTS

### Morphological and Phylogenetic identification of *Fusarium* isolates

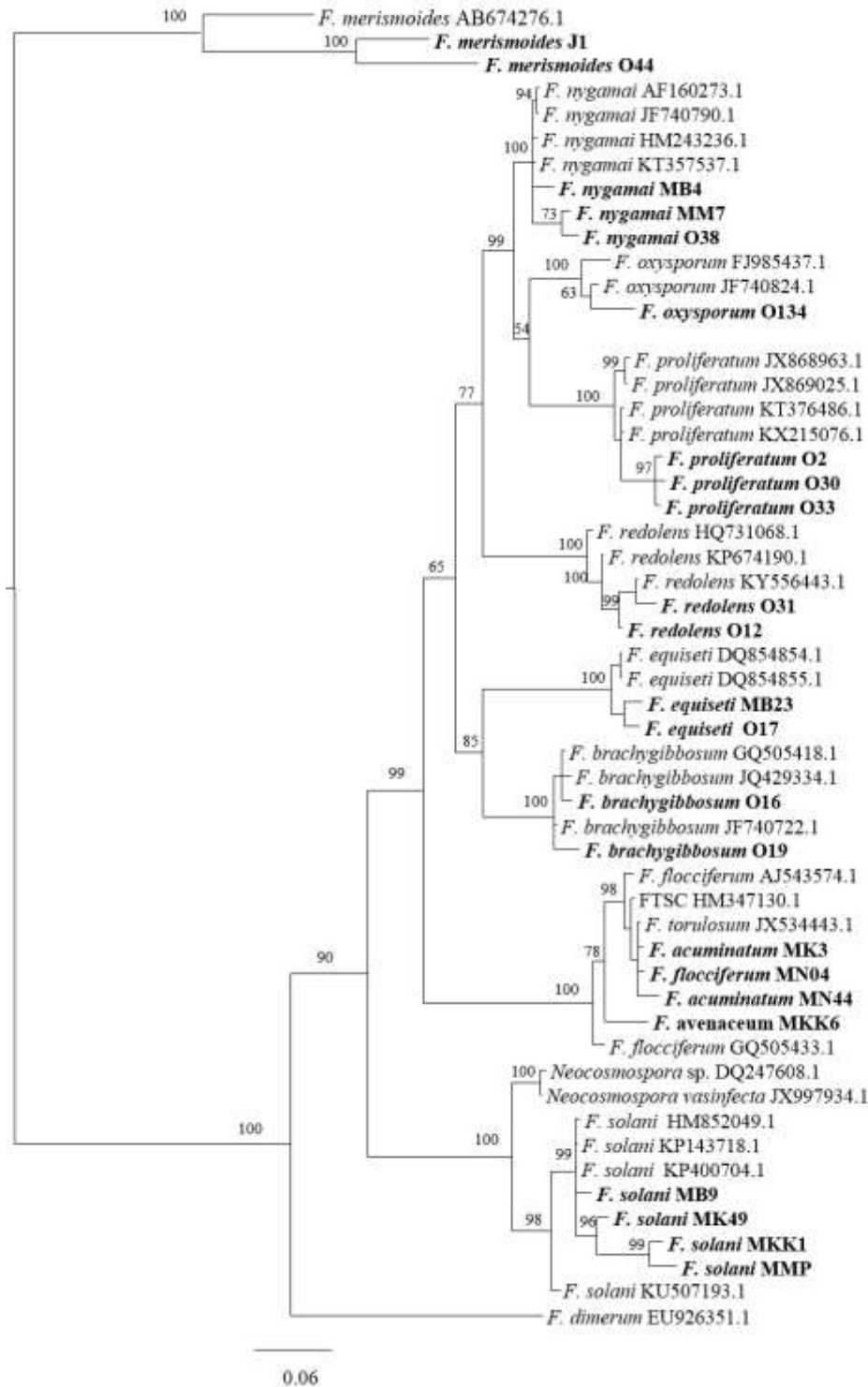
Amplification of the *TEF1-α* gene fragment of approximately 700 bp from all isolates was conducted successfully using primers ef1 and ef2. To elucidate phylogenetic relationships among the isolated *Fusarium* spp. and to aid their accurate identification, a Bayesian tree was constructed using *TEF1-α* sequences (Fig 1).

**Table 1.** *Fusarium* spp. isolates used in phylogenetic analysis, isolated from medicinal plants of Lamiaceae and Asteraceae in Kerman, Iran.

Isolate	Species	Host/substrate	GenBank accession EF-1α
MKK1	<i>F. solani</i>	<i>Ziziphora tenuior</i>	MH684600
MMP	<i>F. solani</i>	<i>Mentha</i> -soil	MH684601
MK49	<i>F. solani</i>	<i>Thymus carmanicus</i>	MH684602
MB9	<i>F. solani</i>	<i>Achillea wilhelmsii</i>	MH684603
J1	<i>F. merismoides</i>	Soil	MH684604
O44	<i>F. merismoides</i>	<i>Mentha</i> -soil	MH684605
O134	<i>F. oxysporum</i>	<i>Achillea wilhelmsii</i>	MH684606
MB4	<i>F. nygamai</i>	<i>Achillea wilhelmsii</i>	MH684607
MM7	<i>F. nygamai</i>	<i>Rosmarinus officinalis</i>	MH684608
O38	<i>F. nygamai</i>	<i>Achillea wilhelmsii</i>	MH684609
O2	<i>F. proliferatum</i>	<i>Mentha piperita</i>	MH684610
O30	<i>F. proliferatum</i>	<i>Teucrium polium</i>	MH684612
O33	<i>F. proliferatum</i>	<i>Ziziphora cliniopodiodes</i>	MH684611
O31	<i>F. redolens</i>	<i>Mentha piperita</i>	MH684613
O12	<i>F. redolens</i>	<i>Mentha piperita</i>	MH684614
O17	<i>F. equiseti</i>	<i>Mentha piperita</i>	MH684615
MB23	<i>F. equiseti</i>	<i>Ziziphora tenuior</i>	MH684616
O16	<i>F. brachygibbosum</i>	<i>Mentha piperita</i>	MH684617
O19	<i>F. brachygibbosum</i>	<i>Mentha piperita</i>	MH684618
MK3	<i>Fusarium tritinctum</i> species complex	<i>Teucrium polium</i>	MH684619
MN44	<i>F. reticulatum</i>	<i>Mentha piperita</i>	MH684620
MN04	<i>F. flocciferum</i>	<i>Achillea wilhelmsii</i>	MH684621
MKK6	<i>Fusarium tritinctum</i> species complex	<i>Ziziphora tenuior</i>	MH684622

The phylogenetic reconstruction revealed eight species complexes namely *Fusarium solani* species complex (FSSC), *Gibberella fujikuroi* species complex (GFSC), *Fusarium redolens* species complex (FRSC), *Fusarium incarnatum-equiseti* species complex (FEISC), *Fusarium sambucinum* species complex (FSSC), *Fusarium tricinctum* species

complex (FTSC), *Fusarium oxysporum* species complex (FOSC) and *F. merismoides* species complex (FMSC). Maximum parsimony analysis yielded a tree with a topology similar to Bayesian tree. The analysis of tree constructed based on the Bayesian inference (BI), is presented here because of the slightly better clade support.



**Fig. 1.** Bayesian tree generated from the nuclear *TEF1-α* sequences. Numbers on the branches are posterior probabilities. The tree was rooted to *Fusarium merismoides*. Isolates being sequenced in this study are shown in bold.



In the present study, the number of 121 *Fusarium* spp. isolates was characterized from selected medicinal plants of Lamiaceae and Asteraceae families (*Teucrium polium*, *Ziziphora tenuior*, *Thymus carmanicus*, *Mentha piperita*, *Ziziphora cliniopodiodes*, *Achillea wilhelmsii* and *Artemisia dracunculus*) collected in Kerman, Iran, based on morphological and molecular criteria. The following species were isolated: *Fusarium oxysporum*, *F. solani*, *F. nygamai* and *F. redolens*, *F. proliferatum*, *F. brachygibbosum*, *F. equiseti*, *F. merismoides* and species of *F. tricinctum* species complex (Table 2).

*Fusarium solani* was the most dominant isolated species (24.8%) across all the sampled plant species, followed by *F. proliferatum* (19%). Other most frequently isolated species recovered from collected samples that were outside the scope of this study was *Rhizoctonia solani*. Species belonging to *F. tricinctum* and *F. solani* species complexes were recovered almost from rhizosphere soil of most plant species.

Twenty-six isolates were obtained from *Achillea wilhelmsii* plants showing stem base yellowing and leaf necrosis. Eleven isolates belonged to *F. nygamai* and the remaining isolates were members of *F. solani* as well as isolates of *F. tricinctum* species complex and isolates of *F. oxysporum*. Thirty eight isolates which were associated with *Mentha piperita* were identified as *F. brachygibbosum*, *F. redolens*, *F. equiseti*, *F. proliferatum* and species of *F. tricinctum* species. Twelve isolates of *F. solani* and *F. proliferatum* were associated with *Ziziphora cliniopodiodes*. Besides, *Ziziphora tenuior* was associated with 17 *Fusarium* isolates belonging to *F. solani*, *F. proliferatum* and few isolates of *F. tricinctum* species complex (Table 2). *Thymus carmanicus* and *Artemisia dracunculus* did not associate with any *Fusarium* species except for the few isolates belonging to *F. tricinctum* species complex being isolated from rhizosphere soil dilutions. Most *Fusarium* isolates being nonpathogenic to their hosts were grouped in the same clade with the pathogenic isolates from other hosts (Fig. 1).

### Pathogenicity tests

Among the examined fungal species, *Fusarium*

*nygamai* isolates (MB4 and MB8, Fig 2) were pathogenic to *Achillea wilhelmsii* and foliar symptoms were observed 15 days after inoculation while non-inoculated plants remained asymptomatic. To complete Koch's postulates, the tested isolates were consistently re-isolated from symptomatic tissues. The inoculated plants showed wilt symptoms similar to those observed in the field. Foliar yellowing symptoms began at the stem base and progressed throughout the whole plants, occasionally resulted in necrotic leaves. Examination of the roots showed necrotic lesions in tap roots. No symptoms were observed in *Achillea wilhelmsii* plants inoculated with *F. solani*, *F. oxysporum* and *F. tricinctum* species complex isolates. However, in the plants inoculated with *F. solani*, mild yellowing at the stem base was observed after 18 days of inoculation and the tested isolates (MB9 and MB12) were recovered from *Achillea wilhelmsii* tissues on solid media. No vascular discoloration in the tissues were observed.

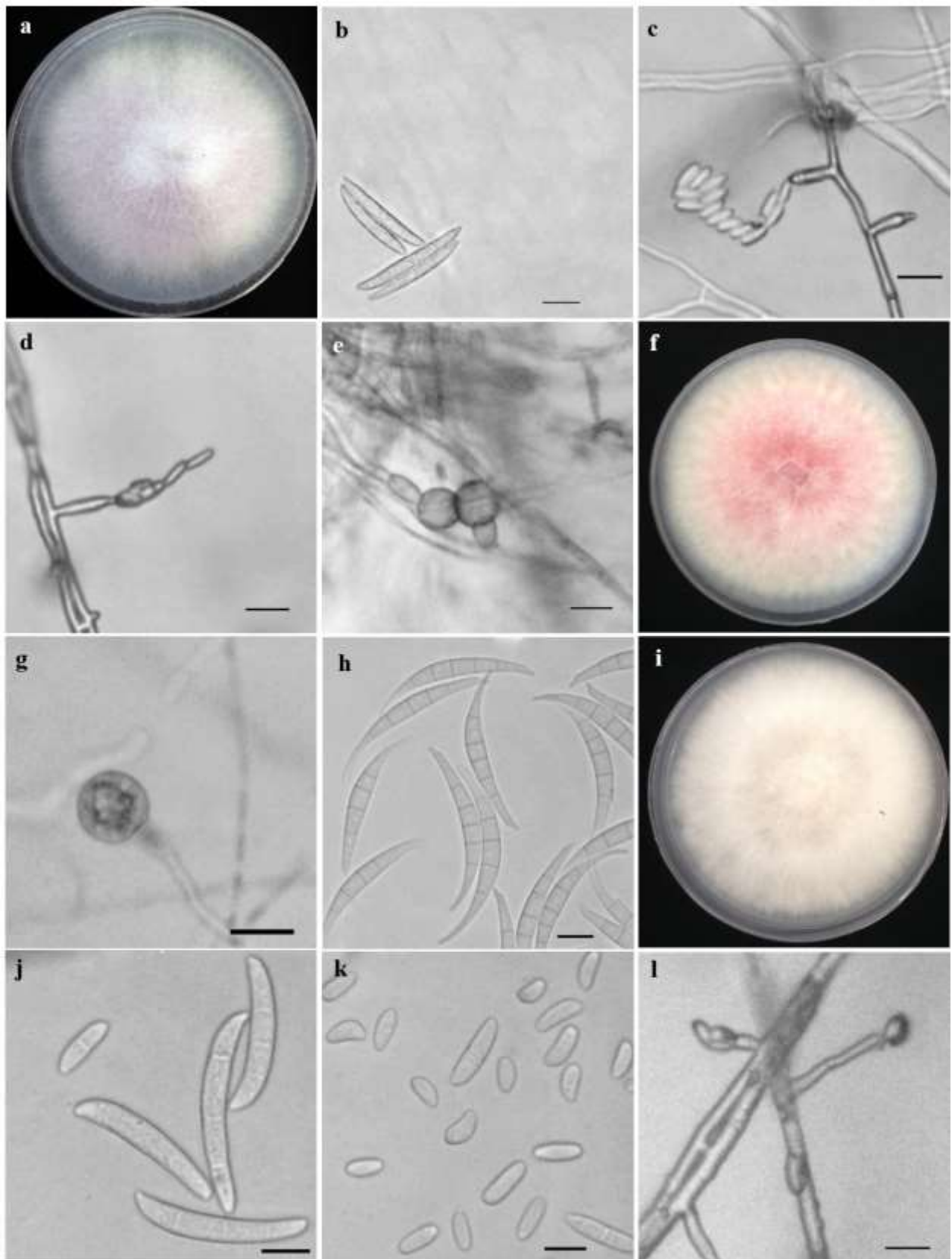
The *Fusarium redolens* and *F. brachygibbosum* isolates were pathogenic to *Mentha piperita*. *Fusarium brachygibbosum* isolates (O16 and O19, Fig 2) caused whole plant decline 12 days after inoculation, whereas no symptoms were observed in the control plants. In one case, the plant showed stem lesions up to few cm long as well as stem dieback. The fungus was re-isolated from the symptomatic tissues but not from the control samples. In pathogenicity tests of *F. redolens*, yellowing and few necrotic root lesions were observed on *Mentha piperita* 15 days after inoculation with *F. redolens* isolates (O31 and O46, Fig 2), whereas no symptoms were observed in the control plants. In addition, no symptoms were observed in the control plants and in the plants inoculated with isolates of *F. proliferatum* and *F. equiseti* having been proved to be nonpathogenic on *M. piperita*.

Isolates of *F. solani* (MKK1) and *F. proliferatum* (O30) being obtained from *Ziziphora cliniopodiodes* plants with foliar yellowing and *Fusarium*-like symptoms did not show any symptoms in inoculated plants. No *Fusarium* isolates were recovered from inoculated *Z. cliniopodiodes* tissues as well.

**Table 2.** *Fusarium* spp. recovered from plants of Lamiaceae and Asteraceae in Kerman, Iran.

Species	Frequency (%)	Hosts
<i>F. solani</i>	24.8	<i>Achillea wilhelmsii</i> (10) <sup>a</sup> , <i>Teucrium polium</i> (3), <i>Ziziphora tenuior</i> (8), <i>Thymus carmanicus</i> (4), <i>Ziziphora cliniopodiodes</i> (5)
<i>F. oxysporum</i>	4.1	<i>Achillea wilhelmsii</i> (5)
<i>F. nygamai</i>	9.1	<i>Achillea wilhelmsii</i> (11)
<i>F. redolens</i>	3.3	<i>Mentha piperita</i> (4)
<i>F. tricinctum</i> species complex	22.3	<i>Teucrium polium</i> (6), <i>Ziziphora tenuior</i> (5), <i>Mentha piperita</i> (6), <i>Thymus carmanicus</i> (4), <i>Achillea wilhelmsii</i> soil (6)
<i>F. brachygibbosum</i>	7.4	<i>Mentha piperita</i> (9)
<i>F. equiseti</i>	5	<i>Mentha piperita</i> (6)
<i>F. proliferatum</i>	19.0	<i>Teucrium polium</i> (5), <i>Ziziphora tenuior</i> (4), <i>Mentha piperita</i> (7), <i>Ziziphora cliniopodiodes</i> (7)
<i>F. merismoides</i>	2.5	<i>Mentha piperita</i> (3)
<i>F. compactum</i>	2.5	<i>Mentha piperita</i> (3)

<sup>a</sup>. Numbers in parentheses are the numbers of isolates recovered from each plant species.



**Fig. 2.** *Fusarium nygamai* (a-e): a. Colony on PDA incubated for five days at 24 °C; b. Macroconidia; c-d. False heads and microconidia in chain; e. Chlamydospores; *Fusarium brachygibosum* (f-h): f. Colony incubated for five days at 24°C grown on PDA; g. Chlamydospore. h. Macroconidia; *Fusarium redolens* (i-l): i. Colony incubated for seven days at 24°C grown on PDA; j. Macroconidia; k. Microconidia. l. False heads; Scale bars = 10 µm

## DISCUSSION

The current study was conducted to develop a better understanding of the soilborne fungal communities associated with selected medicinal plant species of Lamiaceae and Asteraceae causing diseases or potentially involved in host adaptation and interaction to their ecosystem. During the surveys, a range of fungal species were found to be associated with medicinal plants of the two families, including members of genus *Fusarium*, which the members of this genus are among the most important plant pathogens, worldwide. Morphological and molecular phylogenetic analyses were used to identify *Fusarium* species. This is the first comprehensive study of *Fusarium* spp. associated with medicinal plants of Lamiaceae and Asteraceae in Iran.

In this study, pathogenicity assays showed that *F. nygamai* was pathogenic on *A. wilhelmsii* causing foliar yellowing and necrosis. The diseased samples were collected in Kerman, koohpayed region (N30°26'9.90", E57°12'34.64"). There are no formal reports of *F. nygamai* infecting *A. wilhelmsii*. To the best of our knowledge, this is the first report of *Fusarium* wilt on *A. wilhelmsii* caused by *F. nygamai* in Iran and probably, the first worldwide report on this host species.

Molecular phylogenetic analysis using partial sequencing of *TEF1- $\alpha$*  gene indicated clustering of pathogenic *Fusarium* isolates obtained from *A. wilhelmsii* with reference isolates of *F. nygamai*. *Fusarium* isolates obtained from symptomatic *A. wilhelmsii* tissues belonged to *F. nygamai*, *F. oxysporum* and *F. solani*. Sequencing of the *TEF1- $\alpha$*  gene showed that most of the isolates from *A. wilhelmsii* were *F. nygamai* while in few cases, we had *F. oxysporum* and *F. solani* isolates which proved to be nonpathogenic on *A. wilhelmsii*.

Results of the present study revealed that *F. redolens* and *F. brachygibbosum* were pathogenic on *M. piperita*. *F. brachygibbosum* (isolates O16 and O19) caused whole plant decline while plants inoculated with *F. redolens* isolates (isolates O31 and O46) showed stem base yellowing. No reports on the pathogenicity of these two species on *M. piperita* have been published. *Mentha piperita* in Kerman is cultivated with no rotations which can cause accumulation of soilborne fungal pathogens. Among the *Fusarium* species obtained from *M. piperita* plants, *F. solani* has been previously reported to be pathogenic on *M. piperita* causing stolon decay (Green & Skotland 1993, Kalra et al. 2005). However, *F. solani* isolates in this study were not pathogenic on *M. piperita* plants as well as isolates of *F. proliferatum* and *F. equiseti*. As regards other *Mentha* spp., *F. oxysporum* had been reported as the causal agent of *Mentha arvensis* (Japanese mint) wilt in India (Sattar & Husain 1980). *Fusarium* spp. have been reported to occur on other Lamiaceae species worldwide as well (Borges et al. 2018, Nasr Esfahani & Monazzah 2011, Ortu et al. 2018). Interestingly, antifungal effects of some

Lamiaceae and Asteraceae species on *Fusarium* spp. had been reported in a number of recent studies (Askun et al. 2008, Rongai et al. 2015).

*Fusarium solani* and *F. proliferatum* isolates from symptomatic *Ziziphora clinopodioides*, were not pathogenic on host plants. No other fungal species being able to cause serious plant diseases were isolated as well. Therefore, the causal agent of the observed foliar yellowing and necrosis symptoms remained unknown.

Recently the commercial medicinal plant species production in Iran is getting more and more attention. This interest in monoculture of these plant species favors the conditions for occurrence of plant pathogens (de Melo et al. 2017). New reports of soil borne diseases provide important new knowledge to further research on monitoring yield and quality losses due to fungal diseases as well as developing management strategies.

Members of the *F. avenaceum*/*F. acuminatum*/*Fusarium tricinctum* species complex, here referred to as *F. tricinctum* species complex, were difficult to be assigned taxonomically using morphological traits particularly *F. acuminatum* and *F. avenaceum*, in consistence with previous studies (Harrow et al. 2010, Turner et al. 1998, Yli-Mattila et al. 2002). *TEF1- $\alpha$*  gene as suggested by several researchers to be informative in species differentiation within the *Fusarium* genus (O'Donnell et al. 1998, Geiser et al. 2004), was used in this study to separate these closely related taxa. However, we suggest using additional sequence data from other genes for better classification of this clade.

In this study, species belonging to *F. tricinctum* and *F. solani* species complexes were isolated from most hosts tissues and rhizosphere. In the pathogenicity tests in *A. wilhelmsii*, no disease symptoms could be attributed to any of the isolates of *F. oxysporum*, *F. tricinctum* and *F. solani* species complexes. Moreover, isolates of *F. tricinctum* species complex were not pathogenic on *M. piperita*. These isolations of nonpathogenic *Fusarium* species suggest a symbiotic (*ie.* endophytic or mutualistic) association with their plant hosts. Several plants rely on their symbiotic fungi for the stress adaption in their habitat. Members of the genus *Fusarium* are able to serve as saprophytes or endophytes in asymptomatic tissues of several plant species in a nonpathogenic lifestyle (Sieber 2002, Skovgaard 2002, Zakaria & Ning 2013, Imazaki & Kadota 2015). Since medicinal plants of Lamiaceae and Asteraceae in Kerman are found in diverse habitats that many of them are rocky areas and scree and variations in rainfall and periods of drought are inherent to this area (Rajaei & Mohamadi 2012, Tabari et al. 2012), yet these plants have successfully been managed to grow, it is suggested that these *Fusarium* species may play roles in their hosts health by contributing in adaptation to environmental stresses (Redman et al. 2001). On the other hand, *Fusarium* spp. colonizing these plants have immediate access to plant nutrients made available as soon as hosts'



senescence. However, these associations with host plants are likely transitory (Bacon & Yates 2006) and it should be noted that these *Fusarium* spp. are able to switch symbiotic lifestyles and pathogenic isolates may arise especially in commercial monoculture plantations.

In the obtained phylogenetic tree, nonpathogenic isolates grouped in the same clades with pathogenic isolates. This may suggest different scenarios: first that some of these nonpathogenic isolates have lost their ability to cause disease on their hosts and/or are plant endophytes, second that nonpathogenic isolates are ancestors of pathogenic ones which are the outcome of a recent evolutionary event. This is correspondent with the results of Gordon & Okamoto (1992) who found that *F. oxysporum* f. sp. *melonis* had identical VCGs and mtDNA haplotypes with nonpathogenic *F. oxysporum* strains. However, there are reports that pathogenic and nonpathogenic strains of *F. oxysporum* have different genetic fingerprints (*i.e.* different RFLP and RAPD profiles) (Woo et al. 1996). The lack of correlation between pathotypes and phylogeny being observed in this study might be due to sexual reproduction or the presence of transposable elements which remains to be investigated in further studies.

Species belonging to *F. tricinctum* and *F. solani* species complexes, *F. equiseti* and *F. proliferatum* were recovered almost from all hosts rhizosphere which indicated that these species are adopted in rhizosphere of medicinal plants of Lamiaceae and Asteraceae species. Rhizosphere microbial diversity is affected by the genetics of the host plant. Differences in rhizosphere fungal communities of different plant species may be as a result of difference in plant root exudates (Bakker et al. 2013). Further research is necessary to investigate correlation between the observed *Fusarium* species in the rhizosphere of Lamiaceae and Asteraceae and root exudates of these plants.

It is proved that temperature can effect on distribution of root inhabiting *Fusarium* spp. Some *Fusarium* species have been reported to occur in certain climatic zones such as *F. longipes* and *F. sambusinum* (Burgess et al. 1988). However, most of the species isolated in this study were widespread and could occur in different climatic zones. Therefore, the correlation between these *Fusarium* species and climatic zones in Kerman medicinal plants sampling sites was not considered to be analyzed.

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## گونه های *Fusarium* همراه با گیاهان دارویی تیره های Asteraceae و Lamiaceae

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**چکیده:** این پژوهش باهدف شناسایی گونه های *Fusarium* همراه با چندین گونه منتخب گیاه دارویی از خانواده های Lamiaceae و Asteraceae در استان کرمان انجام شد. گیاهان با علائم مشابه با آلودگی به *Fusarium* و نیز گیاهان بدون علائم از عرصه های رویش گیاهان دارویی و نواحی کشت این گیاهان جمع آوری گردید. گونه های جدا شده بر اساس ویژگی های ریخت شناختی همراه با توالی یابی بخشی از ژن *TEF1-a* شناسایی شدند. این گونه ها شامل *F. oxysporum*، *F. solani* و *F. nygamai* از گیاه *Achillea wilhelmsii* (Asteraceae)، *F. proliferatum*، *F. solani* و جدایه هایی از کمپلکس گونه ای *F. tricinctum* از گیاه *Teucrium polium* (Lamiaceae)، *F. proliferatum*، *F. solani* و جدایه هایی از کمپلکس گونه ای *F. tricinctum* از گیاه *Ziziphora tenuior* (Lamiaceae)، *F. solani* و جدایه ای از کمپلکس گونه ای *F. tricinctum* از گیاه *Thymus carmanicus* (Lamiaceae)، *F. redolens*، *F. proliferatum*، *F. brachygibbosum*، *F. tricinctum*، *F. compactum*، *F. equiseti* و *F. solani* از گیاه *Mentha piperita* (Lamiaceae) و *F. proliferatum* و *F. solani* از گیاه *Ziziphora cliniopodioides* (Lamiaceae) هستند. در این پژوهش، بیماری زایی *F. nygamai* روی گیاه *Achillea wilhelmsii* و *F. redolens* و *F. brachygibbosum* روی گیاه *Mentha piperita* برای اولین بار گزارش می شود.

**واژه های کلیدی:** پوسیدگی ریشه، میکروارگانیزم های ریزوسفر، ریخت شناسی، *TEF1-a*