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Research Article

Identification of bean root rot casual and associated fungal agents in Khomein county, Markazi province, Iran

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Abstract: Beans are major feed crops belonging to the family Leguminosae, representing one of the most consumed legumes in Iran. Root rot diseases account for high yield losses in bean crops annually, driving the need to identify causative pathogenic agents. During the cropping season of 2019, samples were collected from rhizosphere and infected plant tissues in Khomein county. Of the total 80 purified isolates, 19 fungal isolates were selected for morphological and molecular identification studies. *Fusarium equiseti, Fusarium acuminatum,* and *Macrophomina phaseolina* were isolated from the infected crown and root tissues. Their pathogenicity on bean cultivars (Yaghout, Dorsa, and Koosha) is reported for the first time from Markazi province. However, the pathogenicity of fifteen saprophytic and pathogenic fungi species that are isolated from the rhizosphere needs to be confirmed in further studies. Disease symptoms were found to be more severe on pinto beans than red and white beans. In all cases, molecular studies using ITS and *tef* genomic region confirmed the morphological results. Briefly, these fungal species are introduced as bean root rot causal agents in Khoemin county, which needs to be considered in upcoming breeding programs.

Keywords: Bean, *Fusarium* spp., molecular identification, root and crown rot

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Introduction

Regarding food security, legumes are the most important agricultural products after cereals, which account for 71% of global consumption in the human food basket (Hayat *et al.,* 2014; Castro-Rosas *et al.,* 2016). According to the World Food Organization, Iran is ranked the 19th country globally in bean production with 220740 tons (FAO, 2018). In the cropping season of 2017-18, Fars, Lorestan, Zanjan, Markazi, and Khuzestan provinces had the highest area under cultivation, respectively (Ahmadi *et al.,* 2019). Plant pathogens are among the principal factors responsible for different levels of yield loss in bean crops and affect revenue and food security worldwide (Burke and Hall, 1991; Cerda *et al.,* 2017; Mardani-Mehrabad *et al.,* 2019). Fungal diseases such as Rhizoctonia root and crown rot (*Rhizoctonia solani* Kuhn), Fusarium wilt *Fusarium oxysporum* (Mart.) Sacc., Fusarium root rot *Fusarium solani* (Mart.) Sacc., Ascochyta blotch *Ascochyta rabiei* (Kovatsch) Arx., Alternaria blight *Alternaria alternata* (Fr.) Keissl. and Charcoal rot *Macrophomina phaseolina* (Tassi) Goid. have been reported as some of the severe diseases of legumes in Iran (Ahari Mostafavi *et al.,* 2012).

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Bean root rot is one of the most important and common diseases in most bean-growing regions of the world. The disease was first observed in 1916 in New York, USA (Burke and Hall, 1991). Significant disease damage occurs under adverse growing conditions and environmental tensions; however, the crop loss depends on weather conditions, soil, and specific cultural practices in each region (Miller *et al.,* 1995; Harveson *et al.,* 2005). The disease also causes substantial yield loss every year in Iran (Moini *et al.*, 1998). In heavily infected areas, it is responsible for the loss in crop yield of 51 up to 85% (Ahari Mostafavi, 2009). Bean root rot diseases are caused by soil-borne fungal pathogens such as *R. solani*, *M. phaseolina*, *Pythium* spp., and *Fusarium solani* f. sp. *phaseoli*. *R. solani* has been reported as one of the primary root rot causal agents and results in significant crop loss in Iran, Turkey (Erper *et al.,* 2007), Kenya (Mwangombe *et al.,* 2007), USA (Harveson *et al.,* 2005), Australia (O'brien *et al.,* 1991) and Canada (Hall and Phillips, 1992). The disease is prevalent in bean cultivation areas in Markazi, Charmahal Bakhtyari, and Zanjan provinces (Heydarian and Ershad, 2002; Naseri, 2008). The infection rate of *R. solani* in Zanjan province has been reported as 50 % by Moini *et al.* (1998).

In addition, bean root rot caused by *F. solani* f. sp. *phaseoli* has been reported in most bean-growing regions of the world (Naseri and Marefat, 2011).). Fusarium species are important soil-borne fungi both as a pathogen (Sahampoor *et al.,* 2020) and elicitor (Torkamani *et al.*, 2014) and has been reported as a causal agent of bean root rot in Iran (Kaiser *et al.,*1968; Nelson *et al.,* 1983; Saremi, 1998). The importance of this fungus is due to various diseases on a variety of crops (Jarvis and Schoemer, 1978; Burgess *et al.,* 1994; Rezaee *et al.,* 2018). The diversity of species and their adaptation to different climatic conditions have caused considerable damages in other countries, which their distribution varies according to the type of climate (Saremi, 2003). Moini *et al.* (1998) reported the genus *Fusarium* as the most important agent of bean root rot. Several species of *Fusarium* have been reported as causal or associated agents of bean root rot in different parts of the world, including; *F. acuminatum*, *F. anthophilum* (Braun) Wollenweber, *F. avenaceum* (Fries) Sacc., *F. culmorum* (Smith) Sacc., *F. equiseti*, *F. oxysporum*, *F. proliferatum* (Mats) Nirenberg, *F. redolens* Wollenweber, *F. crookwellense*, *F. verticillioides* (Sacc.) Nirenberg (Montiel-González *et al.,* 2005; Asan, 2011).

Moreover, *F. solani* has been reported from rotten bean roots in Zanjan (Khodagholi *et al.,* 2013). Safarloo and Hemmati (2014) reported *Fusarium* species involved in bean root rot in Zanjan province as *F. solani*, *F. acuminatum*, *F. equiseti*, *F. oxysporum*, *F. crookwellense,* and *F. sambucinum*.

Eventually, widespread incidence of some root disease fungi in important bean growing areas of Iran indicates an urgent need for studying the influential factors affecting the occurrence and spread of these diseases. Therefore, disease damage reduction in bean crops serves as one of the research priorities both in Iran and worldwide. Since Khomein is a bean production center in Markazi province, it was considered representative of the region in this research. Hence, this study aimed to identify the associated and causal fungal agents of bean root rot disease in Khomein county.

Materials and Methods

Sampling and isolation of fungal isolates

Samples were collected from the rhizosphere and infected plants (roots and crowns) in Khomein county, Markazi province, Iran, during the growing season of 2019 to identify causal and associated agents of bean root rot. The samples were randomly chosen from infected plants with visible wilting, yellowing, root, and crown rot and transferred to the laboratory. After surface disinfestation, fragmentation with disease symptoms was cultured on potato-dextrose-agar (PDA) medium by 1% sodium hypochlorite (active ingredient). Cultures were maintained at 25 ± 2 °C. Fungal colonies grown on agar were sub-cultured and purified on PDA and water-agar (WA) media (Ghaneie *et al.*, 2012). For fungal isolation from the rhizosphere, serial dilutions

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were prepared from the soil samples and cultured on PDA and Rose Bengal agar media. The obtained colonies were transferred to PDA medium and purified by the hyphal tip and single spore methods on WA medium.

Morphological and molecular identification

Morphological identification of the isolates was made based on hyphae, conidiophores, and conidia characteristics using identification keys (Barnett and Hunter, 1998; Leslie and Summerel, 2006). For molecular identification, fungal isolates were first cultured in potato-dextrose broth (PDB) medium and placed on a shaker at 135 rpm for 5 to 7 days at room temperature. The fungal mass was then collected using sterile filter paper under vacuum conditions. Total genomic DNA was extracted using Safaie *et al.* (2005) method and used as a template for PCR programs. Finally, the quality and quantity of the obtained genomic DNA were examined using electrophoresis on 0.8% agarose gel and nanodrop (IMPLEN N-60), respectively.

ITS1-5.8s-ITS2 region was amplified using ITS1 and ITS4 primers (White *et al.,* 1990; Haratian *et al.*, 2013).). For accurate identification of *Fusarium* species, the *tef-1α* gene (Translation Elongation Factor 1-α) was amplified using specific primers TEF-4f and TEF-4r (O'Donnell*,* 2000). Primer sequences are given in Table 1.

Table 1 Name and sequence of the primers used in this study.

Primer Name	Sequence (5' → 3')	Annealing Temperature (°C)	Reference
ITS1	TCCGTAGGTGAACCTGCGG	56	White *et al.*, 1990
ITS4	TCCTCCGCTTATTGATATGC	56	White *et al.,* 1990
TEF-4r	GGARGTACCAGTSATCATG	58	O'donnell *et al.,* 2000
TEF-4f	ATGGGTAAGGARGACAAGAC	58	O'donnell *et al.,* 2000

The PCR programs were performed with a final volume of 20 µl, including; 10 µl of the reaction mixture, 1 µl of each forward and reverse primer, 1 µl of template DNA (50 ng/ µl) and the residual volume of deionized water. PCR process for the ITS region amplification includes; an initial denaturation for 5 min at 94 °C, followed by 35 cycles including 1 min at 94 °C, 30 seconds at 57 °C, 1 min at 72 °C, and final extension at 72 ºC for 10 min. Besides, the amplification program for *tef1α* gene consisted of an initial denaturation for 5 min at 95 °C, then 30 cycles including 1 min at 95 °C, 75 seconds at 57 °C, and 1 min at 72 °C and final extension at 72 ºC for 10 min.

The PCR products were examined by electrophoresis on 1% and 1.2% agarose gel and then sequenced by Biomagic Gene Company (www. Bmgtechno. com). The obtained sequences were compared with the deposited sequences at the NCBI database. Similar sequences were aligned with the obtained sequences in this study using MEGA X software, and the phylogenetic tree was drawn based on the Neighbor-joining method.

Pathogenicity test of the fungal isolates

Before planting the seeds, soil samples were prepared from the experimental farm of the Faculty of Agriculture, Shahrood University of Technology, and then sieved to achieve homogeneous soil and added to the perlite + cocopeat mixture to obtain a soil/perlite/cocopeat ratio of 1: 1: 1, and poured in small pots.

Three cultivars of bean seed, red beans (Yaghout cv.), white beans (Dorsa cv.) and pinto beans (Koosha cv.) were received from Markazi Agricultural and Natural Resources Research and Education Center- Khomein Research Station. The seeds were disinfected with 1% sodium hypochlorite for 15 min and then washed and placed in a dark and suitable environment for 4-5 days to germinate. Three germinated seeds were planted in each pot and placed in a growth chamber with a photoperiod

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of 8: 16 (L: D) h. Three replications were considered for each fungal isolate and control. The pathogenicity test was repeated twice.

Petri dishes containing a one-week-old fungal colony were washed with sterile distilled water to prepare spore suspension of *Fusarium* spp. The number of spores was adjusted to 2×10^6 spores/ml using a hemocytometer, and seedlings at the two-leaf stage were inoculated by 10 ml of suspension, which was added to the rhizosphere of each plant (Karimian *et al.,* 2010; Keshavarz *et al.,* 2019). Tap water was used as a control. The inoculation was performed twice at 10 days intervals. Then, disease symptoms in pots kept at 25 ± 2 °C were evaluated after 30 to 40 days by removing the seedlings from the soil. Possible disease symptoms such as yellowing, leaf chlorosis, necrosis, wilting, early leaf drop, narrow red streaks on the hypocotyl, root browning, and hollow stalk were examined (Schwartz *et al*., 2005). In addition, the disinfected roots of inoculated plants were cultured on PDA medium to recover the fungal isolate.

The inoculation with *M. phaseolina* was performed using the method of Alagheh Bandzadeh (2007) so that the colonized toothpicks by the pathogen were used as inoculum. Consequently, infected toothpicks were inserted into the crown at a depth of 2 cm in the soil, and un-inoculated toothpicks were used as control. The inoculation was carried out on 5-7-leaf seedlings. After that, the plants were kept in a growth chamber for 30 d at 27 ± 2 °C. Finally, disease symptoms were examined in different treatments and compared with the control.

Results

In the present study, sampling was conducted at ten fields in Khomein county, and 44 samples of soil and infected plant tissues were collected. Subsequently, 80 fungal isolates were purified, and a total of 19 isolates were selected for further molecular and morphological identification studies. The list of studied isolates is given in Table 2. As shown in the table, three species, including *F. equiseti*, *F. acuminatum,* and *M. phaseolina* were isolated from infected plants' crown and root tissues. Other species were isolated from the plants' rhizosphere as common saprophytic and pathogenic fungi. However, functions as pathogen or saprophyte require further investigation on the host plants. Due to the importance of *Fusarium* species as causative agents of bean root and crown rot, morphological description and molecular study of the identified species in this research are given below.

Table 2 List of the identified fungal species in the present study.

No.	Code	Origin	Fungal Species	Accession Number
1	SM1	Soil	*Metarhizium anisopliae*	MV145182
2	SM2	Soil	*Alternaria* sp.	MV145183
3	SM3	Soil	*Aspergillus europaeus*	MV145184
4	SM4	Soil	*Alternaria* sp.	MV145185
5	SM5	Soil	*Acrostalagmus luteoalbus*	MV145186
6	SM6	Soil	*Penicillium* sp.	MV145187
7	SM7	Soil	*Penicillium* sp.	MV145188
8	SM8	Soil	*Fusarium solani*	MV145189
9	SM9	Soil	*Chaetomium globosum*	MV145190
10	SM10	Soil	*Chaetomium truncatulum*	MV145191
11	SM11	Soil	*Acrostalagmus luteoalbus*	MV145192
12	SM12	Soil	*Chaetomium iranianum*	MV145193
13	SM13	Soil	*Aspergillus* sp.	MV145194
14	SM14	Soil	*Acremonium sclerotigenum*	MV145195
15	SM15	Soil	*Fusarium solani*	-
16	RB1	Crown and root	*F. equiseti*	MW551800
17	RB2	Crown and root	*F. equiseti*	MW551801
18	WB1	Crown and root	*F. acuminatum*	MW551802
19	WB2	Crown and root	*Macrophomina phaseolina*	-

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Identified *Fusarium* species

F. acuminatum Ellis and Everhart

The fungal colony grew relatively slowly on PDA medium and produced white mycelia. Macroconidia were formed in pale orange sporodochium. Red (sometimes brown) pigments were formed on the agar medium. Microconidia were absent, but macroconidia had four to six septa. Although *F. acuminatum* is commonly known as a saprophytic and secondary agent invading necrotic tissues, some isolates can cause severe root rot in some legume species. The morphological characteristics of WB1 isolate in this study were consistent with the descriptions provided by Leslie and Summerel (2006), and the molecular analysis using the *tef1* region also confirmed the morphological findings. The results of the morphological and molecular studies are shown in Figs. 1c, 2d, and 3, respectively.

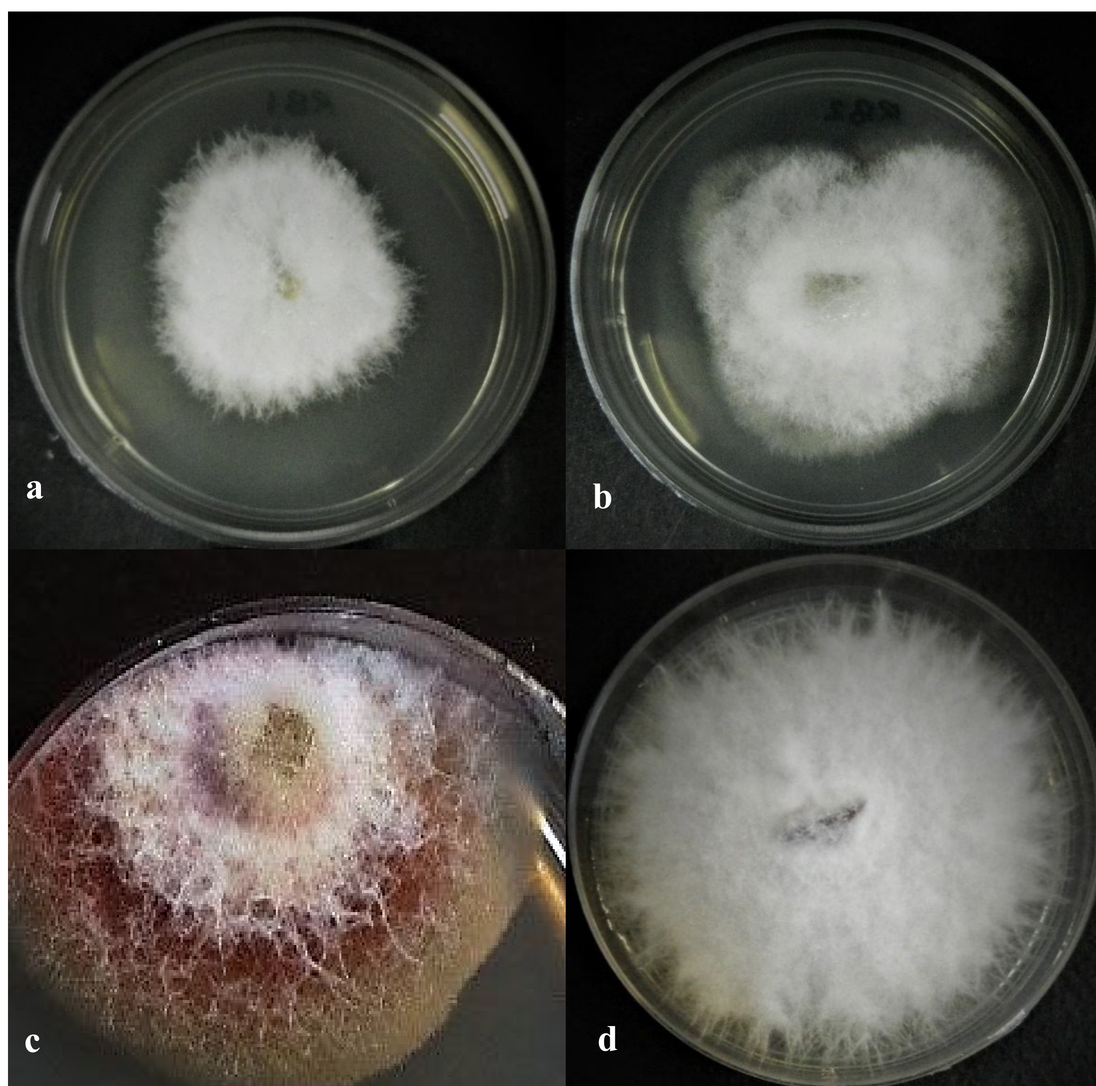

Figure 1 Colony of *Fusarium* species on PDA medium 5 days after incubation. a*) Fusarium equiseti* isolate RB1, b) *Fusarium equiseti* isolate RB2, c) *Fusarium acuminatum* isolate WB1, d) *Fusarium solani* isolate SM15.

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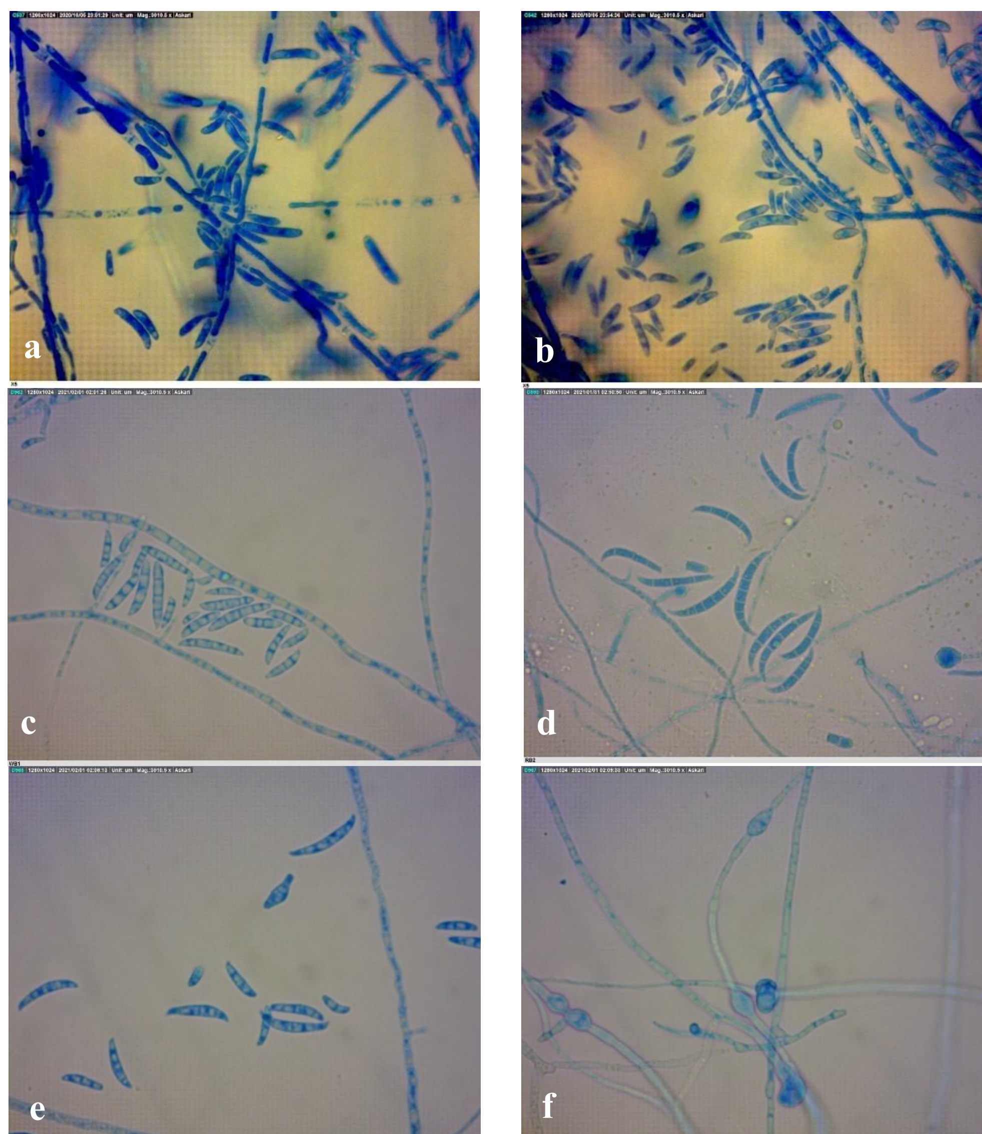

Figure 2 Macroconidia of *Fusarium* species using 40X magnification, a and b) *Fusarium solani* isolate SM15, c) *Fusarium acuminatum* isolate WB1, d) *Fusarium equiseti* isolate RB2, e and f) *Fusarium equiseti* isolate RB1.

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F. equiseti (Corda) Saccardo

Macroconidia were abundant and had a thick wall with a strong posterior dorsal curve and 5 to 7 septa. Chlamydospores were formed rapidly and turned to pale brown, golden, or brown color by aging. The mycelium formed in the PDA medium was initially white and turned brown with age. The morphological characteristics of RB1 and RB2 isolates in this study were consistent with the descriptions provided by Nelson *et al.* (1983), and molecular analysis using the *tef 1* region also confirmed the morphological findings. The results of the morphological study are shown in Fig. 1 (a and b) and Fig. 2 (d, e, and f), and the results of the molecular study are shown in Fig. 3.

F. *solani* (Martius) Appel and Wollenweber emend.

The fungal colony had white to creamy mycelium on PDA medium. Macroconidia were slightly curved with 3 to 7 septa and rounded ends. Macroconida were abundant and creamy in color. Oval and reniform microconidia were

also observed. These characteristics distinguish this species from a similar species, *F. oxysporum*. Chlamydospores usually form quickly within 2-4 weeks. The morphological characteristics of the isolate SM15 in this study were consistent with the descriptions provided in the identification keys (Summerbell, 2003; Larone, 2011), and molecular analysis using the *tef 1* region also confirmed the morphological findings. The results of the morphological and molecular studies are shown in Figs. 1d, 2a, b, and 3, respectively.

Pathogenicity test of the fungal isolates

The disease symptoms appeared 30 days after inoculation on the upper- and underground parts of the plants of all cultivars with the tested pathogens. Most of the symptoms were in the form of crown wounds and brown discoloration of secondary roots. The symptoms observed on seedlings treated with *F. equiseti*, *F. acuminatum,* and *M. phaseolina* isolates are shown in Fig. 4.

As the last step of the pathogenicity test, the fungal agents were reisolated from the wounds of the infected plants. Morphological studies showed that these isolates were quite similar to the ones initially used for inoculation.

This study showed the most apparent disease symptoms in the plants treated with the pathogens *F. acuminatum* and *F. equiseti* in Dorsa, Koosha, and Yaghout cultivars. No disease symptoms were observed in roots, crowns, and aerial parts of the control plants. Symptoms varied depending on the studied species. In inoculated plants with *M. phaseolina*, hollow stalk and leaf drop were frequently observed before maturity. In infected plants by *F. equiseti* and *F. acuminatum*, symptoms such as leaf chlorosis and necrosis, wilting, narrow red streaks on hypocotyl, root, and especially secondary root browning were observed. Besides, vascular tissue browning was usually kept in the cross-section of the stem. The root rot symptom severity varied from reddish-brown to dark brown.

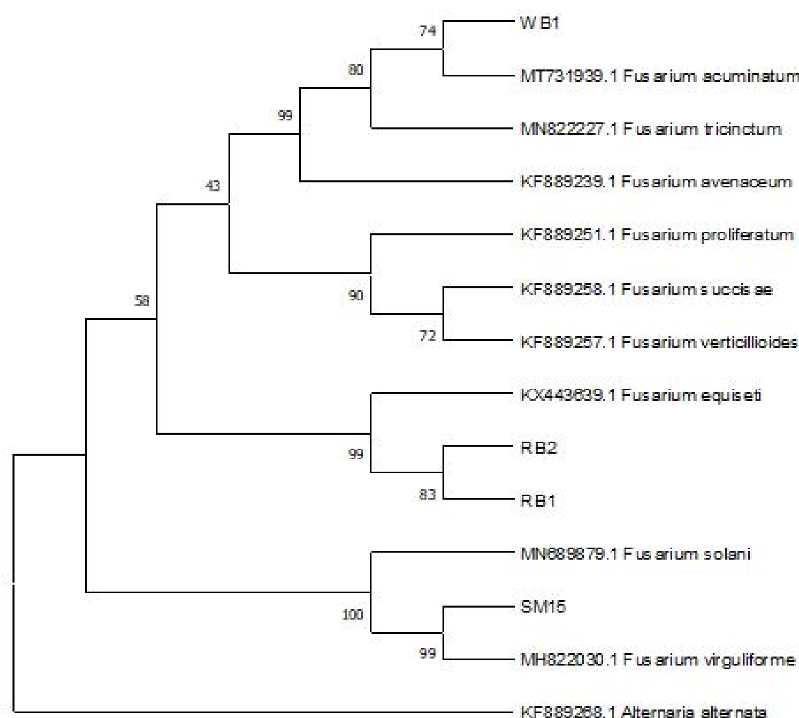

Figure 3 Phylogenetic position of the studied *Fusarium* species. The most parsimonious tree obtained by *tef-1 α* gene sequence using Neighbor Joining method, MEGA X software. The bootstrap support from 1000 replications is indicated on the branches. *Alternaria alternata* is used as outgroup.

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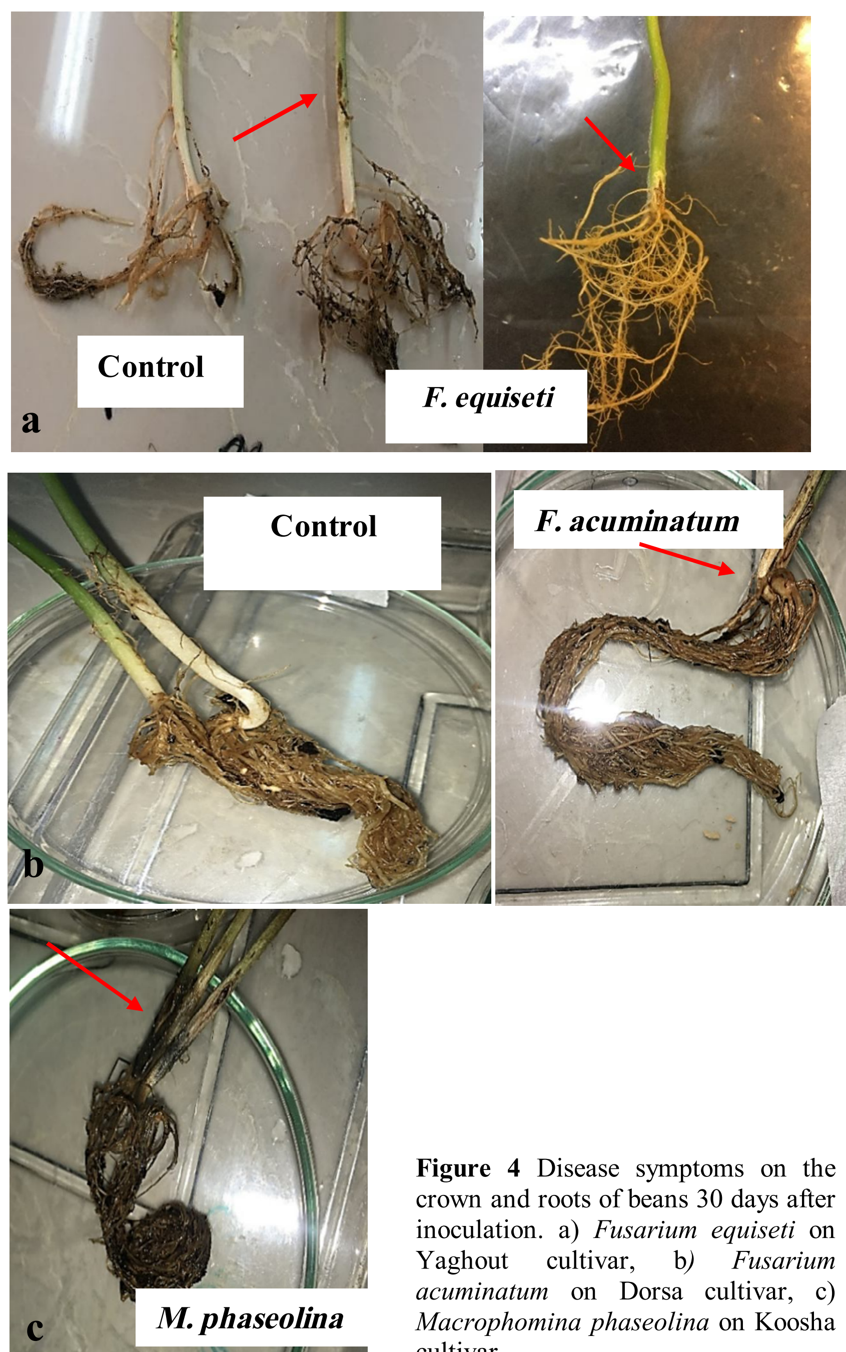

Figure 4 Disease symptoms on the crown and roots of beans 30 days after inoculation. a) *Fusarium equiseti* on Yaghout cultivar, b) *Fusarium acuminatum* on Dorsa cultivar, c) *Macrophomina phaseolina* on Koosha cultivar.

Discussion

In this study, several pathogenic and associated fungal agents were isolated from the tissue and rhizosphere of the infected bean plants. Among them, the purified fungal isolates from the infected plants were picked for further studies. According to the earlier studies, different *Fusarium* species such as *F. solani*, *F. oxysporum*, *F. crookwellense*, *F. equiseti*, *F. moniliform*, *F. acuminatum,* and *F. sambacinum* have been reported as root and crown rot causal agents in various hosts (Latani *et al.,* 2006; Khodagholi *et al.,* 2013; Safarloo and Hemmati, 2014).

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Azimi *et al*. (2005) isolated 84 isolates belonging to 6 species of *Fusarium* from the bean plants' crown, root, and rhizosphere. The species *F. solani*, *F. oxysporum,* and *F. moniliform* were the most abundant, and *F. equiseti*, *F. semitectum,* and *F. prolifratum* had the lowest frequency. In the present study, *F. acuminatum* and *F. equiseti* were identified as causative agents of bean crown and root rot from Khomein county, consistent with the results of other researchers mentioned above.

As shown in Fig. 3, both *F. solani* and *F. virguliform*e were grouped in one cluster along with SM15 isolate. The *F. solani* complex (FSSC) contains more than 60 phylogenetically distinct species (O'Donnell, 2000; Zhang *et al.,* 2006; O'Donnell *et al.,* 2008; Nalim *et al.,* 2011). The only fusarium species currently recognized as species complex is *F. solani.* Most group members have been identified as phytopathogens and divided into *formae speciales* based on their hosts (O'Donnell, 2000; O'Donnell *et al.,* 2008; Coleman, 2016). Soybean sudden death syndrome (SDS) is a root and crown rot disease of soybean *Glycine max* (L.) Merr is traditionally caused by *Fusarium solani* f. sp. *glycines* (Roy *et al.,* 1997). Four pathogens, including *F. virguliforme*, *F. tucumaniae*, *F. brasiliense,* and *F. cuneirostrum*, cause SDS and are members of FSSC (Aoki *et al.,* 2003, 2005). The species *F. virguliforme* has a wide host range and can cause root necrosis on different host species, including alfalfa, pinto and navy beans, white and red clover, pea, and Canadian milk vetch. However, they cause no symptoms on some other plants (Kolander *et al.,* 2012). The current study introduced the isolate SM15 as *F. solani* species according to morphological characteristics and host species.

M. phaseolina, the causative agent of root rot and charcoal rot, is one of the most important plant pathogens in hot and dry regions and has a widespread distribution and extensive host range (Su *et al.,* 2001). The disease severity varies depending on the host susceptibility, suitable climatic conditions including high temperature and low humidity, soil contamination, and irrigation system (Su *et al.,* 2001). Naseri (2007) reported *M. phaseolina* for the first time from the bean plants in Zanjan province and proved its pathogenicity on pinto, white and red beans. This pathogen is reported as a causal agent of root rot disease in bean plants from Khomein county in the present study. Also, Jafari Petroudi *et al.* (2014) considered charcoal rot disease caused by *M. phaseolina* as one of the limiting factors of crop cultivation in Iran, which represents the most important fungal disease of soybean, especially in the northern regions of Iran and even in the north of Khuzestan (Orojnia *et al.,* 2021).

Regarding the pathogenicity of *Fusarium* isolates on bean cultivars, Khodagholi *et al.* (2013) demonstrated the pathogenicity of *F. solani* on red beans (Naz cultivar). They evaluated the susceptibility of three-bean cultivars to the pathogen and reported that disease severity was higher on pinto beans than red and white beans. Naseri and Marefat (2011) also reported higher pinto and white beans contamination than red beans from Zanjan province. Therefore, red beans are recommended in areas with a long history of contamination rather than white and pinto beans. In the present study, the contamination in pinto beans was more severe than red and white beans based on disease symptoms and agreed with the results of the two studies mentioned above.

The host plants' symptoms caused by *F. solani* and *F. equiseti* have been reported as root and crown rot. Root rot usually appears as dark brown or completely black discoloration, mainly of secondary roots. Also, the rot occurring on the crown area is primarily dark reddish-brown, and sometimes stems turn black and thin, and leaves are small, dark, and completely wrinkled (Azimi *et al.,* 2005). In another study, root and crown necrosis and cracks in infected parts of the plant due to infection caused by *F. solani* and *F. equiseti* have been reported. Moreover, in the longitudinal section of roots and crowns, all species show varying degrees of internal tissue discoloration in infected areas. Root and crown rot caused by different species vary in color, from red to light brown to dark. Aerial

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symptoms included a range of symptoms such as chlorosis, slow growth, stunting, mosaic, and wilting. The type and severity of symptoms caused by different species are diverse (Safarloo and Hemmati, 2014). The symptoms observed in infected plants with *F. equiseti* and *F. acuminatum* pathogens in the present study were consistent with those of other researchers. They were similar to the symptoms mentioned above.

Finally, due to the importance of Khomein county as a bean seed production center in the country, susceptibility evaluation of typical local and commercial cultivars to *F. acuminatum, F. equiseti, F. solani* pathogens is suggested. Also, the resistance of bean cultivars to these pathogens should be included in breeding programs.

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شناسایی عوامل قارچی بیماری‌زا و همراه پوسیدگی ریشه لوبیا در شهرستان خمین (استان مرکزی)

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چکیده: لوبیا از محصولات مهم خانواده حبوبات و یکی از پرمصرف‌ترین و مهم‌ترین حبوبات در ایران می‌باشد. هر ساله بیماری پوسیدگی ریشه لوبیا سبب بروز خسارت می‌شود، از این‌رو شناسایی قارچ‌های بیماری‌زا ضروری می‌باشد. در طول فصل زراعی سال ۱۳۹۸، نمونه‌برداری از ریزوسفر و بافت گیاهی آلوده در مزارع شهرستان خمین انجام شد. در مجموع، ۱۹ جدایه از بین ۸۰ جدایه قارچی به‌دست آمده و خالص‌سازی شده برای مطالعات ریخت‌شناسی و مولکولی انتخاب شدند. سه گونه *Fusarium equiseti* و *F. acuminatum* و *Macrophomina phaseolina* از بافت‌های آلوده طوقه و ریشه گیاهان جداسازی و بیماری‌زایی آن‌ها از استان مرکزی گزارش می‌شود. بیماری‌زایی این گونه‌ها روی ارقام لوبیا چیتی، سفید و قرمز بررسی و تأیید شد. هم‌چنین، ۱۵ گونه قارچی از خاک اطراف ریشه گیاهان جداسازی شدند که شامل قارچ‌های ساپروفیت و بیمارگر می‌باشند و تأیید بیماری‌زایی آن‌ها نیاز به بررسی‌های بیش‌تر و آزمون بیماری‌زایی روی گیاه میزبان دارد. در این بررسی شدت علایم آلودگی در لوبیا چیتی بیش‌تر از لوبیا قرمز و سفید بود. در تمامی موارد نتایج بررسی‌های مولکولی با استفاده از ناحیه ITS و *tef*، تأییدکننده نتایج بررسی‌های ریخت‌شناسی بود. به‌طور خلاصه، این عوامل قارچی برای اولین بار به‌عنوان عوامل بیماری‌زای پوسیدگی ریشه گیاه لوبیا در شهرستان خمین معرفی می‌شوند، که نیاز است در برنامه‌های اصلاحی آینده مورد توجه قرار گیرد.

واژگان کلیدی: لوبیا، پوسیدگی ریشه و طوقه، *Fusarium* spp.، شناسایی مولکولی

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