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ORIGINAL ARTICLE

Molecular characterization and distribution of *Fusarium* isolates from uncultivated soils and chickpea plants in Iran with special reference to *Fusarium redolens*

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Abstract

In this study, eighty isolates of *Fusarium* were obtained from the uncultivated soils and roots of chickpea plants showing typical black root rot symptoms from different areas of Kermanshah province, west Iran during 2015 to 2017. Based on colony morphology, growth pattern, and micromorphological characteristics, the most prevalent *Fusarium* species recovered from uncultivated soil were *F. redolens*, 38 isolates (64%), followed by *F. oxysporum*, 12 isolates (20%), *F. solani*, seven isolates (11%) and *Neocosmospora vasinfecta*, three isolates (5%). All isolates recovered from chickpea plants with black root rot symptom were identified as *F. redolens* according to macro-micromorphological and molecular characteristics. After grouping, 27 isolates were selected for molecular confirmation by phylogeny of DNA sequence data for the internal transcribed spacer (ITS) rDNA and translation EF1α intergenic regions. The results of the pathogenicity test under greenhouse condition revealed that all isolates of *F. redolens* obtained from chickpea plants with black root rot symptoms in this study and *N. vasinfecta* from uncultivated soil are pathogenic to chickpea cultivar Bivanij. Two weeks after inoculation with *F. redolens* and *N. vasinfecta*, symptoms developed as black cankers that extended upward and downward of roots of all emerged seedlings. Re-isolation from all inoculated plants after observation of symptoms were performed, and isolates were compared to original cultures thus fulfilling Koch's postulates. According to the results of canonical correspondence analysis, soil texture, altitude, $CaCO_3$, EC, carbon, organic matter and pH in descending order were recognized as the most important environmental variables for the distribution of *Fusarium* species in soil. *Fusarium redolens* reflected a soil with very low sand, carbon, organic matter and intermediate pH, EC and $CaCO_3$. Results of this study suggest that previously reported *F. oxysporum* in western Iran on chickpea might have been mistaken. This is the first report of pathogenicity of *F. redolens* and *N. vasinfecta* on chickpea from Iran.

Keywords Translation elongation factor 1-alpha · Phylogenetic analysis · Black root rot · Canonical correspondence analyses · *Fusarium redolens* · Iran

Introduction

Fusarium is one of the most important genera of soil-inhabiting fungi, commonly associated with plants as pathogens, saprobes, and endophytes (Summerell et al. 2011). The fungi have been isolated from various soil types of many parts of the world. This genus belongs to the division Ascomycota, sub-division Pezizomycotina, class Sordariomycetes, order Hypocreales, and family Nectriaceae (Kirk et al. 2008). *Fusarium* species play differing roles in the soil ecosystem. Some species of this genus have important roles through their saprobic ability and subsequent effects on decomposition and mineralization of organic residues in soil, releasing plant nutrients into the ecosystem for other soil organisms and nutrient cycling (Stoner 1981; Paul and Clark 1989; Ruiter et al. 1994). A number of *Fusarium* species are plant pathogens that cause various types of diseases on plants such as root rot, fruit and seed decay, bulb rot, stem rot, vascular wilt, canker, dieback, gall, and foliar disease (Dean et al. 2012; Chehri et al. 2017; Trabelsi et al. 2017; Sharma and Marques 2018). Non-pathogenic *Fusarium* spp. have been shown to grow endophytically in the endorhiza (Dababat and Sikora 2007). These non-pathogenic *Fusarium* have been used effectively for the control of soilborne plant pathogens (Steinberg et al.

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2007; Zhang et al. 2015; Šišić et al. 2017; Shadmani et al. 2018).

Kermanshah province is a mountainous area and situated at an elevation of 1200 m. The mean annual precipitation is 400–500 mm and the mean annual temperature is about 13 °C. There are seven different climatic zones in the province (Karam et al. 2014). With the exception of cold and highland climates, chickpea (Bivanij cultivar) is planted in the rest of the region. Bivanij cultivar is mostly common and popular than other chickpea cultivars (cultivars Azad, Hashem and ILC482) due to its shorter phenological stages time as complete ripening and a highest biomass and grain yield. In recent years, chickpea cultivation has increased and great damage is caused to the crop every year as a result of *Fusarium* disease (Jalali and Chand 1992). Chickpea cultivation has also increased in newly cultivated soils of foothill areas. Considering the importance of this crop, it is important to investigate the presence of *Fusarium* species infecting chickpea in these soils for proper management strategy.

The distribution of *Fusarium* species in soil is influenced by climatic factors and differs by adaptation to specific sets of climatic and environmental conditions in different areas (Saremi and Burgess 2000). Factors such as temperature, vegetation, rainfall, and soil organic matter have a major influence on distribution of some *Fusarium* species (Burgess and Summerell 1992). In Iran, a number of studies have been done on the distribution and presence of *Fusarium* species in cultivated soils compared to uncultivated soils (Hasanzade et al. 2008; Haji-Allahverdipoor et al. 2011; Zokaee et al. 2012; Nourollahi et al. 2017). In other parts of the world, due to the widespread distribution of *Fusarium* in different geographical areas, many scientists and researchers around the world have identified *Fusarium* species in cultivated and uncultivated soils (Jeschke et al. 1990; Stahl et al. 1999; Latiffah et al. 2007; Tafinta et al. 2018), but little has been published on the effect of environmental variables on *Fusarium* distribution especially in uncultivated soils. The aims of this study were (1) to isolate *Fusarium* species from uncultivated soils and from chickpea plants with disease symptoms, (2) to identify the isolates through morphological and molecular characterization, (3), to study the effect of environmental parameters on species distribution in uncultivated soils, and (4) to determine their pathogenicity on seedlings in a greenhouse to fulfill Koch's postulates.

Materials and methods

Soil sampling

During 2015–2017, a total of fifteen samples of uncultivated soils (rangelands and foothill) were collected from different areas of Kermanshah province (west Iran) including Bistoon, Harsin, Sarab-e Niloofar, Paveh, Sahneh, Kangavar, Eslamabad-e Ghrab, Ravansar, Javanrud, Dalahu, Sarpol-e Zhab, Sumar, Qasr-e Shirin, Gilan-e Gharb and Sonqor during 2015 to 2017 (Fig. 1). The sampling sites covered all localities of the study area. Twenty soil subsamples from each area (50 × 50 m) to a depth of 20 cm after the litter removal were randomly collected and pooled to get one composite sample (approximately 2 kg). These soil samples were placed in sterile labeled plastic bags and transferred to the laboratory and stored at 4 °C until processing. The geographic location information (longitude, latitude, altitude) were recorded using GPSMAP device model 76CSx.

Isolation from soil

The standard soil dilution plate method (Nash and Snyder 1965) was used to isolate species of *Fusarium* from uncultivated soil. For this purpose, each soil sample was first passed through two mm, 40 and 60 mesh sieves. Ten grams of each subsample (four subsamples from each composite sample) were ground using mortar and pestle and suspended in 90 ml of 0.1% water-agar containing 100 ppm NPX (nonyl phenyl polyethyleneglycol ether containing a concentration of 10.5 mol of ethylene oxide) and shaken for 30 min. Three replicates (0.5 ml) of the serially diluted suspension (10^{-2}, 10^{-3}) were plated on sterile Petri dishes containing peptone-PCNB-agar (Raper and Fennell 1965) amended with 250 ppm ampicillin to prevent bacterial growth. Petri dishes were incubated at 25 °C for 3 to 5 days under a photoperiod of 12 h light/12 h dark and under fluorescent illumination. All *Fusarium*-like colonies were transferred to water-agar medium and purified using single spore method.

Isolation from chickpea plants

During 2016–2018, twenty isolates of *Fusarium* were obtained from roots of chickpea plants showing typical black root rot symptoms (Fig. 2a, b) from 20 different fields which they distributed in a similar pattern to the uncultivated soils sampled in Kermanshah province, west Iran (Fig. 1). Root samples were collected and carried separately to the laboratory for isolation. In order to isolate the pathogen, after washing roots to remove soil and debris with running tap water for 30 min, small root pieces, approximately 5 mm in size were taken from the interface of healthy and diseased tissues. Then, the pieces were surface disinfected with 1.5% solution sodium hypochlorite (2% available chlorine v/v) for 30 s, rinsed three times in sterile distilled water to remove surface sterilization agents, and plated on potato dextrose agar amended with chloramphenicol (25 μg/ml) (Jamali and Nasimi 2014). Plates were incubated at 25 °C for 3 to 5 days to allow the fungi to grow. All *Fusarium*-like colonies were transferred to water-agar medium and purified using single spore method.

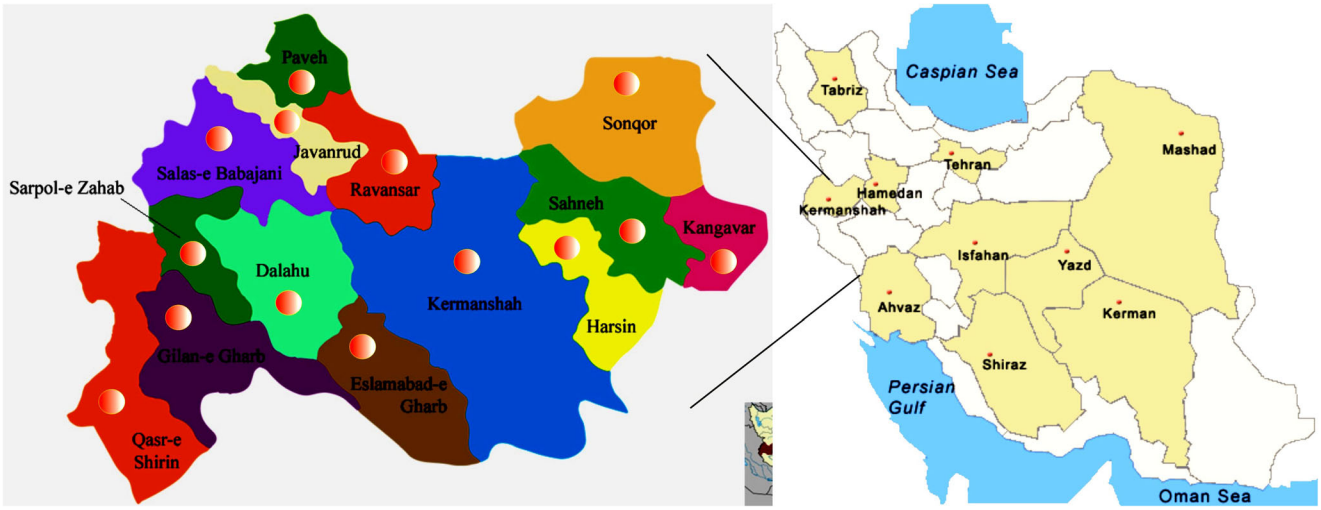

Fig. 1 Map of study site location in the Kermanshah province, western Iranian state of Iran. Color circles refer to the locations in this study from which *Fusarium* isolates were obtained from chickpea fields and uncultivated soils. [S1 = Paveh; S2 = Javanrud; S3 = *Ravansar*; S4 = *Qasr-e Shirin*; S5 = *Dalahu*; S6 = *Sarab-e Niloofar*; S7 = Sahneh; S8 = *Harsin*; S9 = *Eslamabad-e Gharb*; S10 = *Bistoon*; S11 = *Sarpol-e Zahab*; S12 = *Kangavar*; S13 = *Gilan-e Gharb*; S14 = *Sonqor*; S15 = Sumar]

For fungal identification, during the incubation period, plates were observed daily for the appearance of fungal colonies.

Pathogenicity tests

Inoculation tests were performed with 20 purified isolates of *F. redolens* isolates obtained from chickpea plants and three isolates of *Neocosmospora*. For this, 200 g of wheat grain and 120 ml of distilled water were autoclaved for 1 h at 121 °C three times in 24 h interval in 1 L Erlenmeyer flasks. The wheat was inoculated with 5–6 blocks of 7-day-old potato dextrose agar plate containing the fungus and incubated at 25 °C for 2 weeks in the dark (Westerlund et al. 1974). Inocula were mixed thoroughly with a sterilized vermiculite: perlite: soil (2:1:1 v:v:v) mix at a rate of 1:12 (wt/wt) to reach an inoculum density of approximately 10^5 CFU/g of soil for each of the isolates (Jimenez-Fernández et al. 2011). Noninfested wheat grain with the autoclaved soil mixture at the same rate as above served as the control. Chickpea seeds of the cultivar Bivanij were disinfected with sodium hypochlorite 10% w/v, and then washed three times with sterile distilled water, germinated in transparent plastic germination boxes (Gerbox) (11 × 11 × 3.5 cm), selected for uniformity (length of radicle = 1 to 2 cm), and sown into pots with a 0.5 kg capacity. Four seeds were used in each pot and inoculated plants placed in a greenhouse at 24 °C for 35–42 days in a completely randomized design with four replications. Starting 5 days after inoculation, the seedlings were examined for symptomatic leaves and dead plants at 3 days intervals until the end of the experiment, 6 weeks after inoculation. Disease severity on each plant was rated using the following scale: 0 = no symptoms; 1 = 1 to 33% of leaves with symptoms; 2 = 34 to 66% of leaves with symptoms; 3 = 67 to 100% of leaves with symptoms; and 4 = plant dead. The disease severity index was calculated for each isolate according to the parameters in the disease scale (Landa et al. 2006; Navas-Cortés et al. 2007) DSI = Σ (A × n)/Σ (B) × 100. In this formula; A: disease scale, n: number of plants in specific scale, and B: total number of plants.

Morphological identification

Morphological and micromorphological characteristics of isolates in the pure culture were studied using a light microscope (Olympus model BH2). *Fusarium* isolates were grown on carnation leaf agar (CLA), Synthetischer Nährstoffarmer agar (SNA) and potato dextrose agar in Petri plates at 25 °C with 12 h light/12 h darkness for 10 days. Species of the *Fusarium* isolates were identified based on their colony texture, growth pattern, growth rate, pigmentation on potato dextrose agar, and micromorphology of phialides, microconidia, macroconidia, and chlamydospores on SNA (Leslie and Summerell 2006). Images were captured with a camera (Canon Powershot model SX10). Measurements of the observed fungal structures were made using the BioloMICS Measure software (Robert et al. 2011). The identification was carried out according to the authentic mycology taxonomic keys (Nelson et al. 1983; Leslie and Summerell 2006).

Genomic DNA isolation

For molecular studies, isolates were grown on potato dextrose agar for 5 days at 25 °C in the dark. Approximately 100 mg of fungal mycelium was scraped from the pure cultures and mechanically disrupted by grinding the mycelium with a mortar and pestle to a fine powder under liquid nitrogen (Gardes

Fig. 2 Chickpea field affected with *Fusarium redolens* (**a**), Black root rot caused by *Fusarium redolens* on chickpea in fields (**b**), Pathogenicity tests of *Fusarium redolens* from chickpea plants with black root rot symptoms under greenhouse conditions: chickpea plants non-inoculated with *Fusarium redolens* (**c**), inoculated plants with *Fusarium redolens* (**d**), black root rot symptom on inoculated plants with *Fusarium redolens* (**e**, **f**), and control (**g**) in greenhouse trial

and Bruns 1993). Fungal genomic DNA was extracted using a genomic DNA purification Kit (50 t-PR881613-EX6011, Sinagen co., Iran) according to the manufacturer's instructions. The nuclear ribosomal DNA internal transcribed spacer (ITS) of the fungal isolates were amplified using the forward primer, ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and the reverse primer, ITS4 (5′-TCCTCC GCT TAT TGA TAT G-3′) (White et al. 1990). All PCR reactions were conducted in 25 µl containing 2.5 µl of a diluted genomic DNA (1:10 or 1:100 dilutions of the original extract), 20 pmol of each primer, 1.25 nmol of each deoxynucleotide, 0.5 U *Taq* DNA polymerase (CinnaGen, Iran), 1.5 mM of MgCl2, 2.5 µl of 10 × PCR buffer (CinnaGen, Iran), and 14.5 µl H2O. The PCR reactions were performed on a Biometra model T-Personal thermocycler. Thermocycling parameters for ITS were as follows: an initial denaturation step at 95 °C for 2 min, then 35 cycles at 95 °C for 60 s, 55 °C for 80 s, and 72 °C for 90, with a final extension step of 72 °C for 10 min before cooling or removing the tubes. A portion of the translation elongation factor 1-alpha (TEF-1α) gene was amplified using primers EF1F (5-ATGGGTAAGGAGGACAAGAC-3) and EF2R (5-GGAAGTACCAGTGATCATGTT-3) (Geiser et al. 2004) under these parameters: initial denaturation step at 95 °C for 5 min, then 35 cycles at 95 °C for 60 s, 57 °C for

50 s, and 72 °C for 90, with a final extension step of 72 °C for 7 min. The amplification products were visualized under UV light after electrophoresis on 1% TBE-agarose gel stained with ethidium bromide and run in 1× TBE buffer. The controls, with no DNA, were included in every set of amplification to check the DNA contamination in reagents and reaction buffers.

Sequencing of the amplified ITS and TEF-1α regions

The amplification products were purified using the GeneJET PCR purification Kit (Fermentas, UK) to remove excess primers and nucleotides. Sequencing reaction was performed on purified PCR products in forward orientation using the same primers as in PCR. The sequence was determined with an Applied Biosystems (ABI prism 377) DNA sequencer according to the manufacturer's instruction (Macrogene, South Korea). All DNA sequences were deposited in the National Center for Biotechnology Information GenBank (NCBI, http://www.ncbi.nlm.nih.gov/Entrez) (Bethesda, MD, USA) and are listed in Table 1.

Phylogenetic analysis

After sequencing, the nucleotide sequences were edited using BioEdit Sequence Alignment Editor version 7.2.5 software (Hall 1999), and sequence similarity searching in the GenBank sequence database was performed using BLAST service in NCBI (http://blast.ncbi.nlm.nih.gov). The multiple alignment program ClustalW (http://www.clustal. org/download) (Thompson et al. 1994) was implemented using MEGA5 software (http://megasoftware.net/) to align the ITS and TEF-1α sequences generated in this study (Table 1) with sequences available in GenBank (Table 2), mainly those from previously published studies. Alignments were manually optimized with BioEdit version 7.2.5. Poorly aligned positions and gaps of the sequences were excluded from the final alignment of each dataset using Gblocks software version 0.91b (Castresana 2000) under the less stringent parameters selected. The evolutionary history was inferred using the Neighbor-Joining (Saitou and Nei 1987) and Maximum Likelihood (Kimura 1980) methods based on the p-distance and Kimura 2-parameter model respectively. The analysis involved 72 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 334 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 software (http://megasoftware.net/). Evaluation of the reliability of tree topologies were performed by calculating 1000 bootstrap re-samplings (Felsenstein 1985).

Soil physicochemical parameters

The collected soil from uncultivated soils and chickpea fields (4 subsamples per pooled soil sample) was analyzed for physicochemical parameters included organic carbon, pH, electrical conductivity (EC), texture, and $CaCO_3$ content. Analysis of soil particle size distribution was performed by a Bouyoucos hydrometer method (Gee and Bauder 1986). The soil pH in the saturation extract was measured according to Thomas (1996) and electrical conductivity (EC) measured using an electrical conductivity meter in a soil and water suspension (Consort C833 pH/EC Meter). Total carbonates ($CaCO_3$) were measured with a Scheibler calcimeter (Loeppert and Suarez 1996). Organic carbon was determined by the Walkley and Black (1934). These characteristics are listed in Table 3. Soil parameters were tested for significant differences between the sites using Tukey's *HSD* test (Tukey 1953) in the statistical analysis system (SAS) software for Windows version 8.2 (SAS, Institute Inc., Cary, NC, USA).

Effect of pH on *Fusarium redolens* radial growth

To evaluate the influence of pH on *F. redolens* mycelial growth (19 isolates that were molecularly identified), a 5 mm diameter mycelial block was cut from the margin of 3-day-old *F. redolens* colonies and placed on potato dextrose agar plates with pH adjusted to 5.8, 7.3, 8.4, 9.19, 9.72, 10.44 with 0.1 N HCl and NaOH before autoclaving, and incubated at 25 °C. The colony diameters of the *F. redolens* colonies were measured in 2 directions for each isolate replicated on 4 separate potato dextrose agar plates at each pH level 1 day after inoculation. The means were analyzed by analysis of variance (ANOVA) at 5% significant level with SAS software for Windows version 8.2 (SAS, Institute Inc., Cary, NC, USA).

Statistical analysis

Normality and homogeneity of the variances of the disease severity data were assessed using the Shapiro–Wilk and Levene tests, respectively. The Levene statistics for testing the homogeneity of variances indicated unequal variances. Therefore, Welch's F test was used and post hoc comparisons conducted with the Games-Howell procedure (SPSS software version 18.0).

To examine the relationships between environmental variables and *Fusarium* species, at first detrended correspondence analysis (DCA) was used to determine the length of the gradient (length > 2 SD: indicates unimodal variation and a length < 2 SD indicates a linear variation). Gradient analysis showed a length of 7 for DCA, therefore

Table 1 Origins of Iranian isolates studied and their GenBank sequence accession numbers for ITS and TEF sequences

Isolate no.	Species	Province-Locality	Substrate	GenBank- TEF	GenBank- ITS
KBSR2–1	*Fusarium redolens*	Kermanshah-Paveh	Uncultivated soil	MK869741	MG820750
KSSR1–1	*Fusarium redolens*	Kermanshah-Kerend-e Gharb	Uncultivated soil	MK869742	MG820751
KKSR2–2	*Fusarium redolens*	Kermanshah-Niloofar Sarab	Uncultivated soil	MK869743	MG820753
KPSR4	*Fusarium redolens*	Kermanshah-Paveh	Uncultivated soil	MK869744	MG820754
KPSR1	*Fusarium redolens*	Kermanshah-Sahneh	Uncultivated soil	MK869745	MG820755
KCSR2–3	*Fusarium redolens*	Kermanshah-Kerend-e Gharb	Uncultivated soil	MK869746	MG820756
KSSR5	*Fusarium redolens*	Kermanshah-Bistoon	Uncultivated soil	MK869747	MG811580
KDSR3–1	*Fusarium redolens*	Kermanshah-Kangavar	Uncultivated soil	MK869748	MH819237
KDSR4–3	*Fusarium redolens*	Kermanshah-Sahneh	Uncultivated soil	MK869749	MG811581
KGSSO3–4	*Fusarium oxysporum*	Kermanshah-Javanrud	Uncultivated soil	MK869750	MG820759
KJSO1–3	*Fusarium oxysporum*	Kermanshah-Gilan-e Gharb	Uncultivated soil	MK869751	MG820760
KJSO1–4	*Fusarium oxysporum*	Kermanshah-Javanrud	Uncultivated soil	MK869752	MG820761
KGSO3–1	*Fusarium oxysporum*	Kermanshah-Javanrud	Uncultivated soil	MK869753	MG811578
KGSS2	*Fusarium solani*	Kermanshah-Sarpol-e Zahab	Uncultivated soil	MK869754	MG820757
KSASS3–1	*Fusarium solani*	Kermanshah-Gilan-e Gharb	Uncultivated soil	MK869755	MG820758
KJSS2–1	*Fusarium solani*	Kermanshah-Javanrud	Uncultivated soil	MK869756	MG811577
KSSN1–1	*Neocosmospora vasinfectum*	Kermanshah-Sumar	Uncultivated soil	MH976665	MG811579
FuRe-1	*Fusarium redolens*	Kermanshah-Paveh	chickpea	MN865692	–
FuRe-2	*Fusarium redolens*	Kermanshah-Qasr-e Shirin	chickpea	MN865693	–
FuRe-3	*Fusarium redolens*	Kermanshah	chickpea	MN865694	–
FuRe-4	*Fusarium redolens*	Kermanshah- Kuzran	chickpea	MN865695	–
FuRe-5	*Fusarium redolens*	Kermanshah-Sarpol-e Zahab	chickpea	MN865696	–
FuRe-6	*Fusarium redolens*	Kermanshah-Gilan-e Gharb	chickpea	MN865697	–
FuRe-7	*Fusarium redolens*	Kermanshah-Harsin	chickpea	MN865698	–
FuRe-8	*Fusarium redolens*	Kermanshah-Ravansar	chickpea	MN865699	–
FuRe-9	*Fusarium redolens*	Kermanshah- Kuzran	chickpea	MN865700	–
FuRe-10	*Fusarium redolens*	Kermanshah-Sonqor	chickpea	MN865701	–

the canonical correspondence analysis (CCA) (unimodal method) was used (Gauch and Wentworth 1976). Analyses were performed in CANOCO for Windows v4.5 (Ter Braak 2003).

Results

Sampling and morphological identification

A total of 60 *Fusarium* isolates were obtained from uncultivated soil in different areas of Kermanshah province (west Iran). Based on their colony texture, growth pattern, and micromorphological characteristics, four different species were identified, namely *F. redolens*, *F. oxysporum*, *F. solani* and *Neocosmospora vasinfecta*. The most prevalent *Fusarium* species recovered were *F. redolens*, 38 isolates (64%), followed by *F. oxysporum*, 12 isolates (20%), *F. solani*, seven isolates (11%) and *N. vasinfecta*, three isolates (5%).

Isolation from chickpea plants

The disease was present on chickpea plants in all of the chickpea growing regions. In this study, many samples with black root rot symptoms were collected from each field and cultured in media, but because the *Fusarium* isolates obtained from each plant were the same in terms of morphological characteristics, so only one plant was randomly selected from each field and each isolate was from a different field. A total of 20 fungal isolates were obtained from 20 fields in Kermanshah province, west Iran. All the 20 isolates were identified as *F. redolens* on the basis of colony texture, growth pattern, growth rate, pigmentation on potato dextrose agar and micromorphological characteristics. Morphological characteristics were consistent with descriptions of *F. redolens* in Leslie and Summerell (2006). Fungal colonies were white to pink color with abundant aerial mycelium. Colony diameter on potato dextrose agar varied from 25 mm to 30 mm after 3 days of incubation at 25 °C in the dark. Sporodochia produced on CLA were cream to orange. Abundant spherical

Table 2 GenBank accession numbers of translation elongation factor 1-alpha (TEF 1α) gene regions of *Fusarium* species used for phylogenetic studies and origin of these species

Species	Isolate no.	Country	Substrate	GenBank Accession No.	Authors
Fusarium redolens	Fre076	Finland	onion	KT239482	Haapalainen et al. 2016
Fusarium redolens	strain O8	Germany	sugar beet	HQ702568	Christ et al. 2011
Fusarium redolens	34.92	Spain	chickpea	HQ731063	Jimenez-Fernández et al. 2011
Fusarium redolens	31B3	Iran	oak trees	MF563935	Alidadi et al. 2018
Fusarium redolens	strain O52	Germany	sugar beet	HQ702585	Christ et al. 2011
Fusarium redolens	9914I	Spain	chickpea	HQ731060	Jimenez-Fernández et al. 2011
Fusarium redolens	NRRL 52657	USA	soil	GU250584	Balmas et al. 2010
Fusarium redolens	strain O37	Germany	sugar beet	HQ702579	Christ et al. 2011
Fusarium redolens	NRRL 46665	USA	soil	GU250580	Balmas et al. 2010
Fusarium redolens	ER 1276	Italy	Lentil	EU281660	Riccioni et al. 2008
Fusarium redolens	181-2a	Spain	chickpea	HQ731067	Jimenez-Fernández et al. 2011
Fusarium hostae	NRRL29642	USA	–	AF324322	Baayen et al. 2001
Fusarium hostae	NRRL 29889	USA	–	HM057340	Huang et al. 2011
Fusarium nisikadoi	NRRL25179	USA	–	AF324329	Baayen et al. 2001
Fusarium nisikadoi	NRRL25183	USA	–	AF324330	Baayen et al. 2001
Fusarium proliferatum	9233	Malaysia	*Hylocereus polyrhizus*	JX869031	Masratul Hawa et al. 2013
Fusarium proliferatum	9224	Malaysia	*Hylocereus polyrhizus*	JX869030	Masratul Hawa et al. 2013
Fusarium proliferatum	MUCL 31970	USA	wheat	AF336913	Kwon and Anderson 2001
Fusarium subglutinans	KACC 47736	Korea	*Cymbidium orchids*	KM213994	Han et al. 2015
Fusarium subglutinans	NRRL22016	USA	–	AF160289	O'Donnell et al. 2015
Fusarium subglutinans	MUCL52468	Belgium	maize	HM067691	Scauflaire et al. 2011
Fusarium verticillioides	663ES	Mexico	maiz	KR905560	Velarde Félix et al. 2018
Fusarium verticillioides	610CS	Mexico	maiz	KR905566	Velarde Félix et al. 2018
Fusarium verticillioides	676ES	Mexico	maiz	KR905562	Velarde Félix et al. 2018
Fusarium oxysporum	NRRL 46589	USA	Human pathogen	FJ985438	O'Donnell et al. 2009
Fusarium oxysporum	SH-LQ01	China	*Dendrobium candidum*	JQ809657	Xiao et al. 2012
Fusarium oxysporum	FRC 0–2519	USA	Human pathogen	JN235330	Short et al. 2011
Fusarium oxysporum	NRRL 36251	USA	human pathogen	FJ985337	O'Donnell et al. 2009
Fusarium oxysporum	Fo156	France	vanilla	KM065851	Koyyappurath et al. 2016
Fusarium solani	ER026	Austria	soil	KU738440	Haas et al. 2016
Fusarium solani	QZ15051822	China	eggplant	KX981060	Li et al. 2017
Fusarium solani	Fso016	Finland	onion	KT239480	Wang et al. 2018
Fusarium solani	CPO 3.04	Mexico	pepper	KR935888	Rivera-Jiménez et al. 2018
Fusarium solani	1L3H129a	Mexico	*Agave tequilana*	MH179126	López-Bautista et al. 2020
Fusarium solani	GuangX17	China	Bitter grand	KY785024	Wen and Guo, submitted for publication
Fusarium solani	SPL16099	South Korea	sweet potato	KY796232	Yang et al. 2018
Fusarium solani	KU Fs4	Germany	pisi	KY556500	Šišić et al. 2017

chlamydospores, 8–12 μm in size, were formed either singly or grouped in chains on CLA medium after 4 weeks. Macroconidia with 3 to 5 septa and 3–5.4 × 35–44 μm size were also present. Microconidia were abundant, oval or cylindrical shape and 3–5.1 × 6–16 μm in size.

Molecular identification

In order to confirm the morphological identification, after grouping the isolates based on morphological and cultural characteristics, the molecular techniques were used for 17 isolates from uncultivated soils and 10 isolates from chickpea plants. The TEF-1α partial gene was successfully amplified using EF1/EF2 primers from these 27 isolates, and yielded a single band ranging in size from about 600 to 700 base pair. Results of the sequencing and blast search for similar TEF-1α sequences in the Genbank DNA database using Blast program (http://blast.ncbi.nlm.nih.gov/blast.cgi) showed that all ten isolates recovered from chickpea plants showed 100% identity with valid sequences of *F. redolens* deposited in GenBank. From 17 isolates obtained from uncultivated soil, nine isolates showed 100% similarity with *F. redolens* isolates deposited at GenBank, four isolates showed 100% homology with *F. oxysporum* at GenBank, one isolate showed 100% homology with *N. vasinfecta* at GenBank and three isolates showed 100% homology with those previously identified as *F. solani* at GenBank. Analysis of the ITS sequence of 17 isolates from uncultivated soil revealed that nine isolates had 98–99% identity with the reference sequences of *F. redolens*, four isolates had 98% identity with *F. oxysporum* at GenBank

Table 3 Physico-chemical parameters of the uncultivated soil samples collected from different parts of Kermansha province

Location	Altitude	Longitude	Latitude	pH	EC (µs/m)	C (%)	OM (%)	Sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	Soil texture
Paveh (S1)	1554	46.2100	35.0300	8.70 a	536 i	0.273 i	0.4706 i	22 fg	43.8f	34.2 b	10.0 k	Loamy
Javanrud (S2)	1590	46.5172	34.7961	8.45 ab	250 o	0.663 h	1.1430 h	32 d	41.8 g	26.2 f	13.6 gh	Loamy
Ravansar (S3)	1660	46.6560	34.7081	8.40 abc	591 h	1.443 d	2.4877 d	20 g	47.8e	32.2 c	16.7 e	Sandy-Clay-Loamy
Qasr-e Shirin (S4)	373	45.5917	34.4999	6.85 d	269 n	1.053 e	1.8153 e	58 a	29.8j	12.2 k	12.5 hij	Sandy-Loamy
Dalahu (S5)	1547	46.2605	34.2668	8.40 abc	405 m	2.145 b	3.6979 b	16 h	47.8e	36.2 a	11.5 j	Sandy-Clay-Loamy
Sarab-e Niloofar (S6)	1305	46.8574	34.4048	7.40 d	737 d	1.521 c	2.6222 c	22 fg	55.8b	22.2 g	12.3 ij	Silty-Loamy
Sahneh (S7)	1368	47.6872	34.4844	9.00 a	596 g	0.819 g	1.4119 g	16 h	55.8b	28.2 e	23.1 bc	Sandy-Clay-Loamy
Harsin (S8)	1508	47.5806	34.2719	8.35 abc	487 l	0.117 k	0.2017 k	22 fg	57.8a	20.2 h	24.5 a	Silty-Loamy
Eslamabad-e Gharb (S9)	1414	46.5277	34.1113	7.60 cd	907 a	1.443 d	2.4877 d	38 c	43.8f	18.2 i	13.2 hi	Loamy
Bistoon (S10)	1294	47.4364	34.3853	8.35 abc	762 c	2.145 b	3.6979 b	16 h	57.8a	26.2 f	15.1 f	Silty-Loamy
Sarpol-e Zahab (S11)	540	45.9897	34.4262	7.65 bcd	691 e	4.953 a	8.5389 a	26 e	51.8d	22.2 g	14.6 fg	Silty-Loamy
Kangavar (S12)	1450	47.9600	34.5000	7.20 d	527 j	0.858 f	1.4791 f	24 ef	53.8c	22.2 g	22.0 c	Silty-Loamy
Gilan-e Gharb (S13)	946	45.7494	34.1218	7.35 d	814 b	0.858 f	1.4791 f	22 fg	47.8e	30.2 d	18.0 d	Loamy
Sonqor (S14)	1601	47.5900	34.7700	8.45 ab	503 k	0.195 j	0.3361 j	40 c	39.8 h	20.2 h	17.6 de	Loamy
Sumar (S15)	682	45.9590	33.9657	8.55 a	643 f	1.053 e	1.8153 e	52 b	33.8i	14.2 j	23.5 ab	Loamy

Data are presented as means from three replicate samples; letters show significant differences between sites according to Tukey's HSD test ($p < 0.05$). S1 to S15 refers to site numbers corresponding to Fig. 4

and three isolates 100% identity with *F. solani* at GenBank. The sequenced isolates with accession numbers are listed in Table 1.

Phylogenetic analysis

Phylogenetic reconstruction of the combined ITS1 + 5.8S + ITS2 regions of the genomic ribosomal RNA tandem gene repeat which was applied for preliminary identification of species, resulting in poor resolution of species discrimination between the investigated *Fusarium* taxa. The results indicated that ITS sequences did not have significant nucleotide variations (data not shown). The TEF-1α gene had a higher resolution than ITS for discrimination between different *Fusarium* species (Fig. 3). Both methods used for phylogenetic analysis showed the same topology, in spite of slight differences in bootstraps value among equivalent branches. In neighbor-joining method, the optimal tree was with the sum of branch length = 0.636 (data not shown). In maximum likelihood method, the tree with the highest log likelihood (−1357.57) is shown (Fig. 3). In this method the Kimura 2-parameter model was determined as the best fit nucleotide substitution model of evolution for each dataset based on the Bayesian information criterion (BIC) by MEGA 5. Phylogenetic analysis of isolates based on TEF-1α gene put them into four monophyletic lineages. Two main clades supported by high bootstrap values were distinguished. Clade I was divided into five subclades. Subclade I comprised isolates from uncultivated soil (9 isolates) and chickpea plants (10 isolates), which together with authentic isolates of *F. redolens* constitute a monophyletic group with high bootstrap value (97% NJ, 87% ML). The reference isolates were from Spain, Italy, Germany, USA and Finland (Riccioni et al. 2008; Balmas et al. 2010; Jimenez-Fernández et al. 2011; Christ et al. 2011; Haapalainen et al. 2016). Subclade II comprised two isolates described as *F. hostae*. *Fusarium hostae* with high bootstrap value (99% NJ, 88% ML) is a sister taxon of *F. redolens*. Subclade III comprised two isolates described as *F. nisikadoi*. Subclade IV comprised *Gibberella fujikuroi* complex, three described as *F. verticillioides*, three as *F. subglutinans* and three as *F. proliferatum*. Subclade V comprised four isolates from uncultivated soil in this study and *F. oxysporum* from other authors within *F. oxysporum* complex (O'Donnell et al. 2009; Short et al. 2011; Koyyappurath et al. 2016). The distances among TEF-1α gene of *Fusarium* species were calculated using Mega 5 software both between and within the five subclades. The sequence divergence between the five subclades was generally much higher than the distances within the subclades. The two highest within-clade distances characterized subclades 4 and 5, respectively, which included *Gibberella fujikuroi* complex and *F. oxysporum* complex isolates. The lowest sequence divergence was between subclade *F. redolens* (subclade I) and *F. hostae*

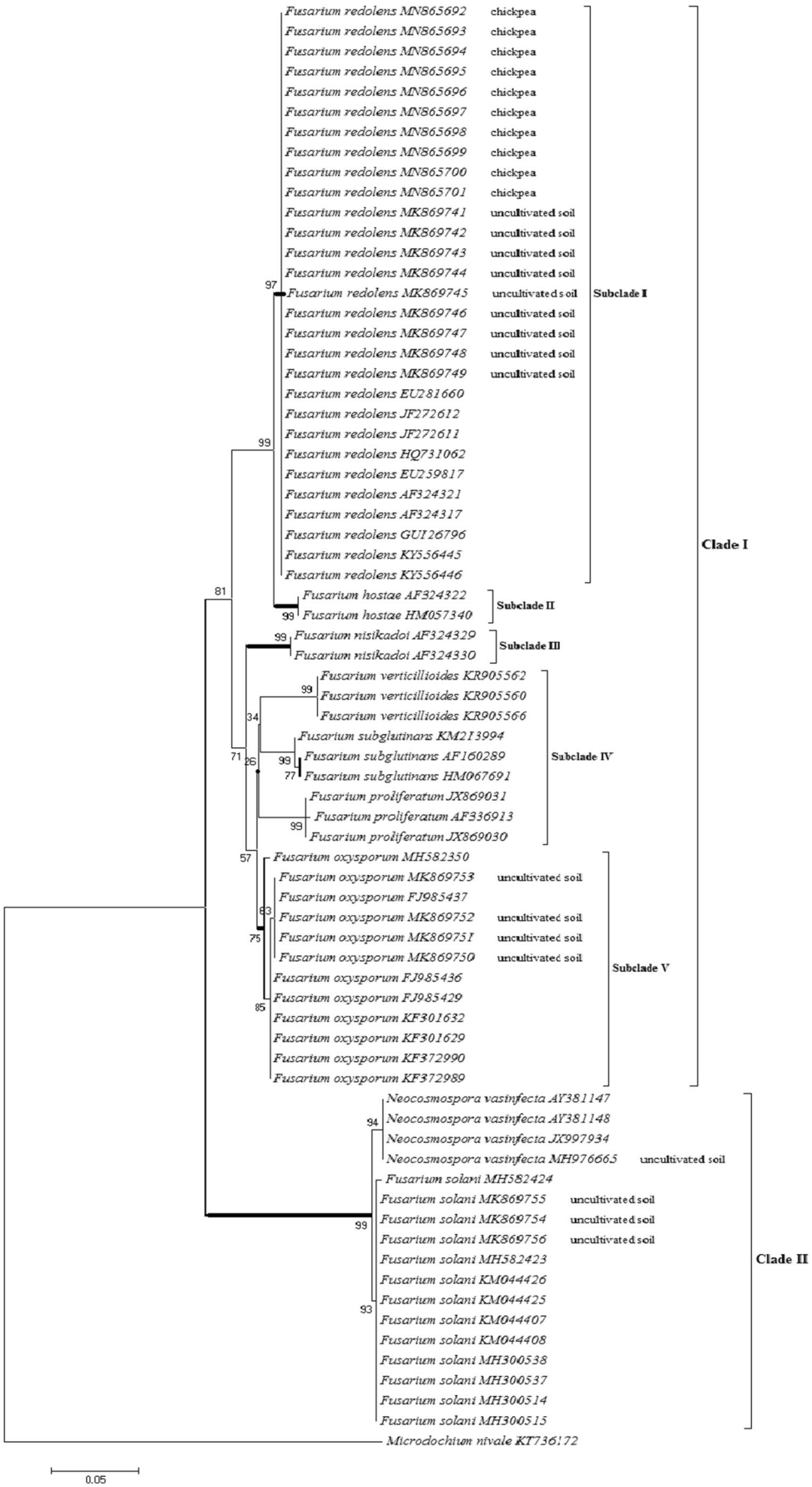

Fig. 3 Maximum likelihood phylogram generated in Mega from the alignment of 72 translation elongation factor 1-alpha (TEF 1α) gene regions sequences of *Fusarium* species using Kimura 2 parameter model with complete deletion gap handling and 1000-replication bootstrapping

(subclade II). In clade II, three of the *Fusarium* isolates in our study grouped with *F. solani* and one isolate grouped with *N. vasinfecta* isolates within the *F. solani* complex (99% NJ, 99% ML).

Soil analysis

The results of physico-chemical analyses on uncultivated soil samples from different parts of Kermanshah province are shown in Table 3 (physico-chemical data for chickpea fields not shown). Statistical analysis using Tukey's *HSD* test showed differences in soil structure parameters among sites. Soils collected from different parts of Kermanshah province (field and uncultivated soils) were mostly loamy and silty loamy, alkaline pH and low in organic matter. These soils are considered as non saline and basic soils.

Relationships between *Fusarium* species and environmental factors

The relative importance of the determined soil parameters and altitude to the distribution of *Fusarium* species is shown in canonical correspondence analysis (CCA) biplot (Fig. 4) where altitude, soil parameters and *Fusarium* species are arranged on the basis of their scores on two axes. The eigenvalue for the first axis was 0.98 and 0.78 for the second axis. The correlation between species and environmental parameters was highly significant (99.3% for the first axis and 89.4% for the second axis). Where electrical conductivity (EC), carbon, organic matter, silt, clay and altitude are negatively correlated with the first CCA axis, pH, sand and $CaCO_3$ correlated positively (Fig. 4). Also, the species on the second axis have a negative correlation with $CaCO_3$, pH, conductivity, silt and altitude and have a positive correlation with clay, sand, organic matter and soil carbon. The species at the margins of the axes are usually uncorrelated with environmental variables, and those at the center of the biplot can be highly correlated or uncorrelated to environmental variables. In canonical correspondence analysis arrow length indicates the importance of variables on species. According to the arrow lengths, soil texture (sand, silt, clay), altitude, $CaCO_3$, EC, carbon and organic matter and pH in descending order were recognized as the most important environmental variables on the distribution of *Fusarium* species in soil. *Fusarium redolens* was most numerous in soil with very low sand, while *F. oxysporum* and *F. solani* responded to soil with intermediate sand (Fig. 4). *Fusarium redolens* were most numerous in uncultivated soil with intermediate pH, EC and $CaCO_3$. The two species *F. solani* and *F. oxysporum* are much more abundant in soil with very low pH, EC and $CaCO_3$. *F. solani* and *F. oxysporum* were most common at higher carbon and organic matter; *F. redolens* at very low carbon and organic matter. For the altitude, *F. solani* and *F. oxysporum* were found at lower altitude than *F. redolens*. *Neocosmospora vasinfecta* is

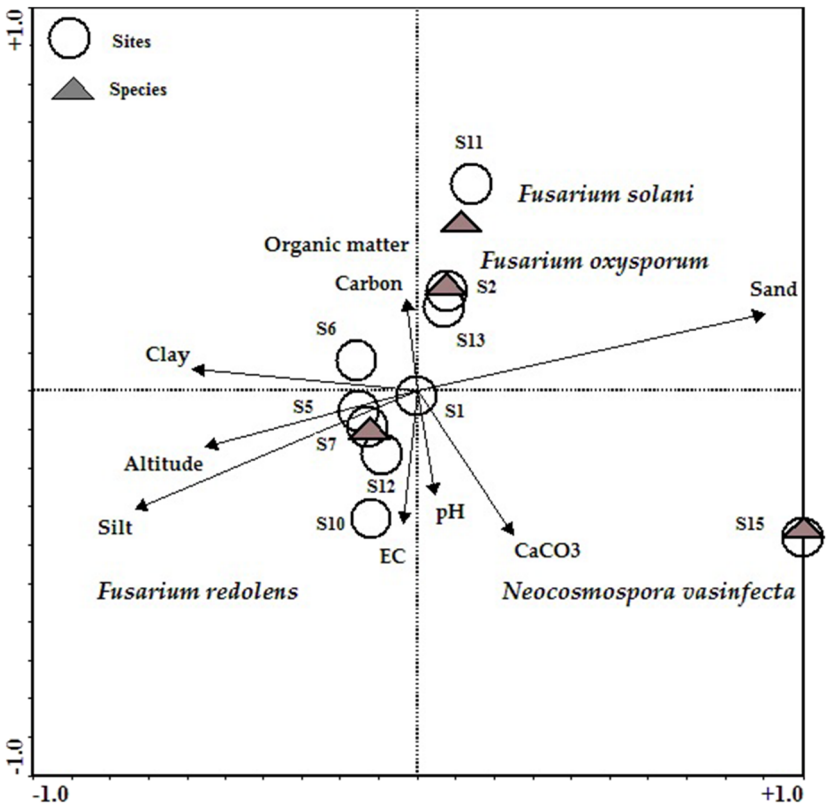

Fig. 4 Correspondence analysis (CA) of the *Fusarium* species communities found in different parts of Kermanshah province. The eigenvalues of the first and second axes in the two dimensional ordination diagrams are as: CA1 = 0.98 and CA2 = 0.78. [S1 = Paveh; S2 = Javanrud; S3 = *Ravansar*; S4 = *Qasr-e Shirin*; S5 = Dalahu; S6 = *Sarab-e Niloofar*; S7 = Sahneh; S8 = *Harsin*; S9 = *Eslamabad-e Gharb*; S10 = *Bistoon*; S11 = *Sarpol-e Zahab*; S12 = *Kangavar*; S13 = *Gilan-e Gharb*; S14 = *Sonqor*; S15 = Sumar]

located on the edge of the axis and was isolated only from the soil of Sumar region with its $CaCO_3$ content higher than the other locations.

Greenhouse pathogenicity tests

The results of the pathogenicity test under greenhouse condition revealed that all 20 isolates of *F. redolens* obtained from chickpea plants with black root rot symptoms in this study are pathogenic to the chickpea of the cultivar Bivanij (Fig. 2d). Two weeks after inoculation with *F. redolens*, symptoms developed as foliar yellowing and black cankers that extended upward and downward of roots of all emerged seedlings (Fig. 2e, f). Discoloration in the vascular tissues of inoculated chickpea plants were not observed. Disease severity index (DSI) ranged between 46 and 89 among the *F. redolens* isolates on chickpea cultivar Bivanij. *Fusarium redolens* isolate FuRe12 was most virulent with DSI 89 (Fig. 5). Three isolates of *N. vasinfecta* obtained from uncultivated soil could infect chickpea plants. Disease symptoms were observed as yellowing and black root rot. Except for three isolates of *Neocosmospora*, the *Fusarium* isolates from uncultivated soil samples were not tested for pathogenicity on chickpea. Re-isolation from all inoculated plants after observation of symptoms were performed, and isolates were compared to original cultures all fulfilling Koch's postulates.

Effect of pH on *Fusarium redolens* mycelial growth

The results of pH test on *F. redolens* growth showed the mycelial growth of this species was highest at pH 9.72. The mycelial growth was lowest at pH 5.8 (data not shown).

Discussion

In this study, a total of 60 *Fusarium* isolates were obtained from uncultivated soil in different areas of Kermanshah province (west of Iran) with *F. redolens* most prevalent. In most studies *F. oxysporum* and *F. solani* are considered cosmopolitan species and have been reported to be among the most frequently isolated fungi from different soils such as sandy soils (Mandeel 2006), grassland soils (Burgess and Summerell 1992), desert soils (El Gindy and Saad 1990), forest soils (Latiffah and Azaman 2011), cultivated soils (Saremi and Saremi 2013) and different climates such as tropical, temperate, arctic, arid and mediterranean regions (Sangalang et al. 1995; Mandeel 2006; Manshor et al. 2012; Stefańczyk et al. 2016).

In all of these studies, the identification was based on morphological characteristics. However, due to the overlapping in several characters among morphologically similar *Fusarium* spp., such as *F. oxysporum* and *F. redolens* some misidentifications may have been made when using these characteristics (Jimenez-Fernández et al. 2011; Jamali 2017). Recent molecular studies have shown that the causal agent of *Fusarium* yellows of some plants is *F. redolens* that was misidentified as *F. oxysporum*(Baayen et al. 1997; Baayen et al. 2000a, 2000b). Due to similar morphological characteristics, *F. redolens* has been considered to be conspecific with *F. oxysporum* or a variety of *F. oxysporum* (Baayen et al. 2001; Jimenez-Fernández et al. 2011) or even *F. solani* (Šišić et al. 2018). Our results showed that the abundance of *F. redolens* in uncultivated soils was higher than other species. Shadmani et al. (2018), showed that the most isolated *Fusarium* isolate from barley roots was *F. redolens* (Shadmani et al. 2018). In their study, the identification was performed by molecular characters.

In this study, the pathogenicity of all isolates of *F. redolens* obtained from symptomatic chickpea plants that showed

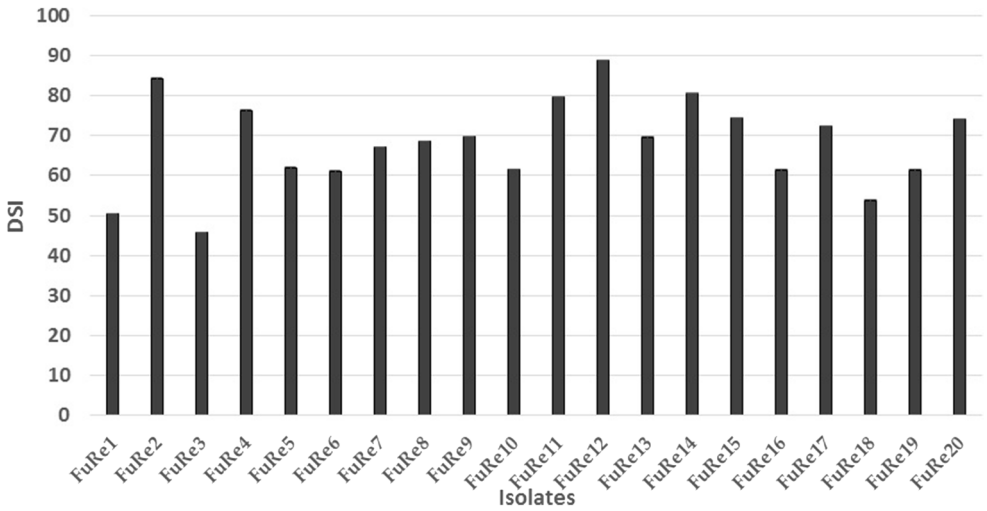

Fig. 5 Disease severity index of *Fusarium redolens* isolates on chickpea

yellowing (aerial parts) and black root rot were confirmed. In Iran, most studies are based on morphological characteristics, and *F. oxysporum* was reported as the most pathogenic agent of chickpea with black root rot and yellowing symptoms in most parts of the country (Afshari-Azad 1998; Mohammadi and Banihashemi 2005, 2006; Zamani et al. 2001, 2004; Hasanzade et al. 2008; Haji-Allahverdipoor et al. 2011; Zokaee et al. 2012; Nourollahi et al. 2017). In this study, all isolates obtained from chickpea plants were identified as *F. redolens* based on morphological and molecular characteristics.

Based on the morphological characters, *F. oxysporum* f. sp. *ciceri* has been reported as the most important causal agent of chickpea disease in many parts of the world, including India, Ethiopia, Egypt, Turkey, Spain, Syria, Pakistan, Peru, Australia, United States, Tunisia, Canada, and other countries (Chattopadhyay and Sen Gupta 1967; Echandi 1970; Westerlund et al. 1974; Trapero-Casas and Jimnez-Diaz 1985; Nene et al. 1996; Esmaeili Taheri et al. 2011). Then, in a number of these countries including Tunisia, Netherlands, Morocco, Pakistan, Canada, Spain and Lebanon, using molecular methods, *F. redolens* was identified as the causal agent of chickpea root rot (Baayen et al. 2000a, 2000b; Esmaeili Taheri et al. 2011; Jimenez-Fernández et al. 2011; Leisso et al. 2011; Bouhadida et al. 2017; Rafique et al. 2020).

Among various species of *Fusarium* being reported as the most important causal agent of chickpea disease in Iran, *F. solani* and *F. oxysporum* are the most common isolated species. To our knowledge, this is the first report of *F. redolens* causing root rot of chickpea in Iran. There was no previous report of this species causing diseases on chickpea and other crops in Iran. Different crop management procedures including sanitation, crop rotation, resistant chickpea cultivars and use of fungal or bacterial antagonists have been proposed to control the disease. Thus, accurate identification and clarifying the ecology and biology of this fungus are crucial for proper management strategy, especially if resistant cultivars are the most effective control measures. Differentiation between *F. oxysporum* and *F. redolens*, based on morphology, is difficult due to the presence of isolates with intermediate forms and it is possible that *F. redolens* has been found previously and misidentified as *F. oxysporum*. Use of molecular methods is needed in order to identify and separate *Fusarium* species correctly. We used the combined ITS1 + 5.8S + ITS2 regions of the genomic ribosomal RNA tandem gene repeat and partial TEF-1α gene for identification of species. Our results showed poor resolution of species discrimination between the investigated *Fusarium* taxa with the ITS regions, but the TEF-1α gene had a higher resolution than ITS for discrimination between different *Fusarium* species (Fig. 3). These results agree with the results of other authors (Zhao et al. 2011; Raja et al. 2011; Šišić et al. 2018; Alhawatema et al. 2019). Based on previous research, DNA-based studies showed that *F. redolens* is distinct from *F. oxysporum* (O'Donnell et al. 1998; Baayen et al. 2000a, 2000b; Baayen et al. 2001; Bogale et al. 2007). These studies revealed that they even lack a sister taxon relationship. Baayen et al. (2001) showed that the *F. nisikadoi-F. miscanthi* clade is more closely related to the *F. oxysporum* clade than it is to the *F. redolens-F. hostae* clade. In this study, the estimated transition/transversion bias (*R*) was 2.2, that could be suitable for phylogenetic analysis and clearly resolved species boundaries in the constructed phylogram (Fig. 3). The maximum Log likelihood for this computation was −1357.573. In our phylogenetic trees, *F. hostae* with high bootstrap value (99% NJ, 88% ML) was a sister taxon of *F. redolens*. Our results agree with results of Baayen et al. (2001) and other authors (Jimenez-Fernández et al. 2011). To clarify the ecology and biology of this fungus in Iran, more investigation is needed.

Little is known about the influence of environmental factors on the distribution of *F. redolens* under agricultural and natural soil conditions. Environmental factors and climate that contribute to the distribution of *Fusarium* species could predict the potential presence of different species of *Fusarium* in specific locations. The study of the effective factors on the distribution of *Fusarium* fungi and modeling species distribution using new and advanced software is useful and valuable, but has received far less attention. Saremi and Burgess (2000), showed that the distribution of *Fusarium* species is restricted due to adaptation to specific sets of soil environmental conditions. Their studies have demonstrated that some species of *Fusarium* are cosmopolitan while others are restricted to a particular climatic region. It has been concluded that the distribution of *Fusarium* species is closely related to a variety of climatic factors (Burgess et al. 1993; Saremi et al. 1997). It has been reported that non-pathogenic *F. oxysporum*, *F. solani* and *F. equiseti* typically were cosmopolitan and occur in most parts of the world, in contrast *F. acuminatum* and *F. sambucinum* that were restricted to the cool temperate areas (Abbas et al. 1987; Backhouse and Burgess 1995; Burgess et al. 1988; Backhouse et al. 2001). Summerell et al. (2010) have reported that Fusarium species distribution is influenced by environmental factors, such as temperature, soil texture, rainfall, drought tolerance, and local vegetation.

The three isolates of *Neocosmospora* obtained from uncultivated soil in Qasr-e Shirin, Kermanshah province, west Iran with an arid and hot climate, were identified as *N. vasinfecta* and their pathogenicity on chickpea cultivar Bivanij was confirmed. The locality was situated at an elevation of 682 m. In the CCA analysis *N. vasinfecta* is located on the edge of the axis. The species at the margins of the axes are usually uncorrelated with environmental variables (Ter Braak 1988). Qasr-e

Shirin is a hot area of Iran and therefore the correlation with temperature is likely. In most studies, disease severity is positively correlated with soil temperature, so planting this cultivar in this area with warm weather can be a threat for this crop. Cannon and Hawksworth (1982), showed that *N. vasinfecta* is found mostly in the soil of tropical or subtropical areas. This species has been reported as phytopathogenic fungus from chickpea in Pakistan (Ali et al. 2011), Hungary, Ethiopia and India (Nene et al. 1996), peanuts in South Africa, Australia, Vietnam and Taiwan (Fuhlbohm et al. 2007), *Arachis hypogaea* plant in Guinea (Lombard et al. 2015), and other plants (Cannon and Hawksworth 1982; Manikandan et al. 2007). This species has also been reported from clinical materials in Senegal and France (Ben Hamida et al. 1993; Kac et al. 1999; Gabriel et al. 2013), soil in India and South Africa (Lombard et al. 2015), and animal dung (Doveri 2011). This is the first report of pathogenicity of *N. vasinfecta* on chickpea from Iran. More studies are needed to clarify the ecology, biology and host range of this fungus in Iran.

The results of CCA analysis showed that the correlation between species and environmental parameters was highly significant (99.3% for the first axis and 89.4% for the second axis). In this study, all the soils sampled were mostly alkaline in the range of 7.2 to 9. Jones and Woltz (1981), showed that the most *Fusarium* wilt (*F. oxysporum*)- suppressive soils have a pH value greater than 7. Such soils are inhibitory to *F. oxysporum* species and increasing soil pH reduces *Fusarium* wilt (Borrero et al. 2004; Fang et al. 2012; Deltour et al. 2017). In contrast, *F. redolens* responded to a soil with intermediate pH, while *F. oxysporum* and *F. solani* responded to soil with very low pH (Fig. 4) and all isolates obtained from chickpea plants with black root rot symptom were identified as *F. redolens*. The results of acidity test on *F. redolens* growth showed the mycelial growth of this species was highest at pH 9.72. The mycelial growth was lowest at pH 5.8. The soil in most parts of Iran and Kermanshah province (in this study) is alkaline, and has a high pH of 7.4 and 8.2 (Qadir et al. 2008; Heidari et al. 2008) so it can be expected that root rot of chickpea is caused by *F. redolens*.

Soil pH modulates the bioavailability of macro- and micronutrients such as manganese, iron, copper and zinc (Collins and Buol 1970). To obtain micronutrients many organisms produce siderophores with stability constants differing in magnitude and pH dependence. Therefore, the relative ability of different species to obtain essential micronutrients differs with pH, due to the effect of pH on solubility of the metals and stability of the chelated forms (Boukhalfa and Crumbliss 2002; Dhungana and Crumbliss 2005). According to the arrow lengths in the CCA analysis, soil texture (sand, silt, clay), altitude, $CaCO_3$, EC, carbon and organic matter and pH in descending order were recognized as the most important environmental variables on the distribution of *Fusarium* species in soil. *Fusarium redolens* was most numerous in soil with low clay, while *F. oxysporum* and *F. solani* responded to soil with very low clay (Fig. 4). Deltour et al. (2017) identified a negative correlation between clay content and *Fusarium* wilt severity. Clay may influence suppression by pH buffering, availability of nutrients, and altering oxygen diffusion (Lavie and Stotzky 1986; Dominguez et al. 2001).

The abundance of *F. redolens* was significantly highest at very low carbon and organic matter. Organic matter and microbial biomass carbon contents in soil with loam and sandy loam texture are low (Vujanovic et al. 2006). Several studies have found a positive correlation between organic matter and suppression of *Fusarium* disease of melon, chrysanthemum, and flax (van Rijn et al. 2007; Saadi et al. 2010). Soil organic matter impacts the structure of soil, pH, pH buffering capacity, and nutrient availability (Brady and Weil 2000; Baum et al. 2015). Gehlker and Scholl reported that low soil pH, high soil organic matter, high clay content, or inadequate drainage favor *Fusarium* disease of asparagus (Gehlker and Scholl 1974). Our results showed that *F. redolens* was most abundant in uncultivated soil with intermediate EC and $CaCO_3$. Increased EC of the nutrient solution in hydroponic culture can also influence plant diseases positively or negatively. Nam et al. (2018) showed that the increase of EC of the nutrient solution had no significant effects on the *Fusarium* wilt of lettuce. Research on the effect of $CaCO_3$ on the survival of *Fusarium* is rather limited. Calcium carbonate ($CaCO_3$) could not only serve as a soil amendment to change soil pH but also increase soil Ca^{2+} content (He et al. 2014). Ca^{2+} has been reported to affect many soil-borne diseases (Benson et al. 2009).

Despite the overwhelming impact of *F. redolens*, there is limited information available on the pathogenicity of this species in relation to soil and climate and further research is required to investigate the effect of soil texture, pH, EC, $CaCO_3$, temperature, nutrients, and organic matter on disease severity of *F. redolens*. According to phylogenetic studies, the alkalinity of the soils, the frequency of *F. redolens* in the studied soils, optimal mycelial growth of *F. redolens* at pH 9.72, suppressiveness of alkaline soils against *Fusarium* wilt caused by *F. oxysporum*, and its pathogenicity on chickpea, it is possible that *F. redolens* also is the causal of agent of chickpea black root rot in other parts of Iran, and likely has been mistakenly called *F. oxysporum* based on morphological characteristics. Further studies in other regions of Iran should be performed to confirm whether *F. redolens* is the major causal agent of chickpea root rot.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42161-020-00698-w.

Compliance with ethical standards

Conflict of interest The authors state no conflict of interest.

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