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FUNGI ASSOCIATED WITH ROOT AND CROWN ROT OF WHEAT IN THE KERMAN PROVINCE OF IRAN

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ABSTRACT

In the growing season of 2016-17, the fungal agents associated with crown and root rot of wheat in the Kerman province of Iran were identified. For this purpose, different fields were randomly selected for sampling and percentages of disease were estimated. Infected parts of root and crown were surface sterilized and cultured on Potato Dextrose Agar medium. A total of 260 isolates were obtained and identified on the basis of macroscopic and microscopic characters and valid keys. Of the total isolates, 212 belonged to *Fusarium*, 28 to *Bipolaris* and 20 isolates belonged to *Drechslera* species. Fungal species included *F. oxysporum* (96 isolates), *F. nivale* (20 isolates), *F. poae* (18 isolates), *F. anthophilum* (9 isolates), *F. subglutinans* (22 isolates), *F. solani* (32 isolates), *F. culmorum* (11 isolate), *F. proliferatum* (2 isolate), *F. chlamyosporum* (2 isolates), *B. kusanoi* (11 isolates), *B. australiensis* (17 isolates) and *D. tetrarrhene* (20 isolates). The pathogenicity test was conducted using greenhouse root dip technique. The pathogenicity confirmed five *Fusarium* species, two *Bipolaris* and one *Drechslera* species associated with wheat root and crown rot in Kerman province of Iran. It is concluded that wheat root and crown rot is fairly distributed in the Kerman province and showed virulence of varying degrees demanding strict control measures to minimize losses.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important crop in the world, particularly in Iran. Wheat crown and root rot is one of the most common diseases in wheat growing regions around the world which has led to a decline in annual yields (Mohammadipour and Ilkhechi, 2004). The term common crown and root rot is often used to refer to a group of complex disease that is characterized by blighting, stunting and death of seedling, stunting of mature plants, necrotic lesions on

seminal and crown roots, sub-crown internodes, crown and stem tissue (Sallans, 1965). Common root and crown rot is caused by several soil borne pathogens. Species of *Bipolaris* and *Drechslera* have roles for inducing foot and root decays. *Bipolaris sorokiniana* is distributed worldwide in all of the major cereal growing regions. Within a region, it is rare to find a field that does not harbor the pathogen at some level of inoculum (Mathre et al., 2003). Two species of *Bipolaris* were identified as *B. sorokiniana* and *B. australiensis*. The first

one had highest prevalence and the latter had lowest prevalence. *B. australiensis* was isolated from wheat root in Damghan and this was the first report of this pathogen on wheat root from Iran (Ommaty et al., 2000). In the other study, three species including *B. australiensis*, *B. kusanoi* and *Drechslera tetrahene* were identified from wheat fields of Kerman province (Hashemi et al., 2011).

Several species of *Fusarium* are common causative agents of brown rot of cereals, especially in temperate and semi-tropical areas (Hajieghrari, 2009). *Fusarium* crown rot has been reported from all wheat cultivating areas including Australia, North America, Africa and China. Identification and pathogenicity of *Fusarium* spp. isolated from wheat fields in Queensland and Northern New South Wales appeared to be increasingly important globally in part, at least, because resistant wheat varieties are commercially unavailable (Beccari et al., 2018).

Another study in Turkey showed the existence of a wide range of *Fusarium* species associated with crown rot of wheat. *F. culmorum*, *F. pseudograminearum* and *F. graminearum* caused severe crown rot disease on durum wheat. *F. avenaceum* and *F. hostae* were weakly to moderately virulent (Gebremariam et al., 2018). Several species of *Fusarium* (*F. culmorum*, *F. nivale*, *F. solani*, *F. oxysporum*, *F. solani*, *F. oxysporum*, *F. subglutinans*, *F. reticulatum*, *F. anthophilum*, *F. chlamidosporum*, *F. proliferatum* and *F. poae*) were detected from wheat fields of Kerman province of Iran (Dehghan et al., 2011). Similarly, two species of *Curvularia* (*C. ramose* and *C. specie-do*) were included in the common root rot and crown rot complex (Sallans, 1965).

Take-all of wheat is caused by the fungus, *Gaeumannomyces graminis* var. *tritici*. It is the deadliest root disease of wheat worldwide. It also affects triticale, barley and rye but to a lesser extent (Freeman and Ward, 2004) and causes stunting and nutrient deficiency symptoms in the tops and progresses upward into the bases of the stems. Here, it disrupts the flow of water to the tops and causes premature death of the plant (Cook, 2003).

Rhizoctonia solani Kuhn anastomosis group eight (AG8) caused "bare patch", colonized the roots of wheat producing symptoms of stunted shoot growth, whitehead, reduced blackened roots and premature grain ripening. It is more severe in sandy soils where rainfall is low (Gill et al., 2000). The present research is

focused on the identification and pathogenicity of fungal pathogens associated with wheat root and crown rot compared to previous study in 2009 in the Kerman province of Iran.

MATERIALS AND METHODS

Sampling: The sampling was done during the growing season of 2016-17 from wheat farms in the Kerman province of Iran. A total of 260 samples of durum and bread wheat from four regions were collected. Plants in ripe maturity stages with dubious symptoms such as necrosis, weakness, yellowing, and white heading were collected randomly (about 2- 3 km apart), placed in plastic bags and taken to the laboratory.

Isolation of wheat root and crown associated fungi:

The wheat root and crown collected samples were washed under tap water. The infested roots, lower crown internodes and crown pieces (3-5 mm) were surface disinfected by immersing in 2% sodium hypochlorite solution for 2-3 minutes. They were washed twice in sterile distilled water and dried on sterile filter paper in a laminar airflow cabinet (Heydarian and Ershad, 2000). Specimens were cultured on Potato Dextrose Agar (PDA) in 9 cm Petri dishes and incubated at 25 °C. Emerging fungi were subcultured on PDA and purified by hyphal tip and single spore culture.

Identification of common root and crown rot fungi:

Fusarium cultures were transferred to Carnation Leaf Agar (CLA). The cultures were identified on the basis of general colony morphology, mycelial morphology, reproductive structure and taxonomic description. *Fusarium* spp. were characterized by macroscopic features such as colony morphology and color and microscopic morphological characteristics of microconidia, macroconidia, chlamydospores and conidiophores of the *Fusarium* diagnostic keys (Booth, 1971; Leslie and Summerell, 2008; Nelson et al., 1983; Nirenberg, 1990). *Bipolaris* spp. isolates were characterized by a profuse production of *Bipolaris*-type dark conidia identified with a binocular microscope and the species were identified according to their description (Ellis, 1971; Sivanesan, 1987). The germination of conidia produced on PDA was also examined, using dispersed conidia in a drop of sterilized water on the surface of PDA plates (Hajieghrari, 2009). To identify and differentiate *Bipolaris* and *Drechslera* species, germination of conidia was studied. Conidia were dispersed in a drop of sterilized water on the surface

of PDA; plates were then placed under alternative light for 4-5 days. After stipulated period, the germination of conidia was examined with light microscope.

Pathogenicity test: For pathogenicity test, two wheat species were used in the tropical zone such as durum wheat (*Triticum durum*) var. Shabrang and bread wheat (*Triticum aestivum*) var. Chamran. In temperate zone, bread wheat var. Mihan was used. These varieties predominate in the tropical and temperate zones. Root dip method was used to determine the pathogenicity of all the fungi. Wheat seeds were disinfected with 1% sodium hypochlorite for 5 min followed by rinsing with distilled water 3 times and then sown in the sterilized sand. After germination, plants were removed from the sand and roots were washed with distilled water. Subsequently, the conidia of fungi were scraped off from the surface of Potato Dextrose Agar (PDA, Merck.39 g/l) and counted, using hemocytometer. They were adjusted to 10^6 conidia/ml.

The washed roots were placed in conidial suspension of 10^6 conidia for 24 hours. For control pots, the roots were placed in distilled water until 24 hours (Heydari and Ershad, 2000). Treated seedlings were planted in the sterilized sand and pots were randomly placed in the greenhouse. There were five replicates for each isolate. After four weeks, seedlings were evaluated. The root and crown inoculated plants were scored after decay and color change using 0 to 5 scale. The scale depends on the browning percentage on the basal stem i.e. 1 = 1-9%, 2 = 10-29%, 3 = 30-69%, 4 = 70-89% and 5 = 90-99%. The

lines were ranked from resistant to highly susceptible as follow; R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible, HS = Highly Susceptible (Sudhir, 2015).

RESULTS

Of the total 260 recovered isolates, three genera viz. *Fusarium*, *Bipolaris* and *Drechslera* were identified with frequencies of 212, 28 and 20 respectively. Out of total 212 *Fusarium* isolates, 9 *Fusarium* species were recognized. These species included *F. oxysporum*, *F. nivale*, *F. poae*, *F. anthophilum*, *F. subglutinans*, *F. solani*, *F. culmorum*, *F. proliferatum* and *F. chlamyosporum*. Two *Bipolaris* species consisting of *B. kusanoi* and *B. australiensis* and one *Drechslera* species, *D. tetrarrhena*, were identified.

The infection of the disease ranged from 2.1% to 14.5% in the 52 areas of study. *Fusarium* spp. were found predominant that caused root and crown rot. The species were isolated from wheat crown and root tissue from different districts in Kerman province. Two species i.e. *F. oxysporum* and *F. solani* were the most commonly isolated from wheat crown and root isolates. These species of *Fusarium* may happen individually or in conjunction with other recognized species of *Fusarium* and in some areas with other specified fungi. With the exception of four species of *Fusarium*, most of the isolated fungi were pathogens. Based on greenhouse pathogenicity trials, *Fusarium* species i.e. *F. oxysporum*, *F. nivale*, *F. culmorum*, *F. poae* and *Bipolaris kusanoi*, *B. australiensis* and *D. tetrarrhena* were virulent at varying degrees (Table 1, 2, 3).

Table1: Fungi associated with root and crown rot of durum wheat var. Shabrang from tropical zone.

Fungal Species	Pathogenicity test	Severity Index	Number of positive isolates in			
			Vakilabad	Shahmaran	Soghan	Dolatabad
<i>F. oxysporum</i>	+	4	8	8	7	8
<i>F. nivale</i>	+	4	2	2	0	2
<i>F. poae</i>	+	4	3	2	2	2
<i>F. anthophilum</i>	-	0	2	0	0	2
<i>F. subglutinans</i>	-	0	2	2	2	1
<i>F. solani</i>	+	4	2	2	2	2
<i>F. culmorum</i>	+	5	1	0	0	12
<i>F. proliferatum</i>	-	0	0	0	0	0
<i>B. kusanoi</i>	+	4	1	1	0	1
<i>B. australiensis</i>	+	4	2	2	2	2
<i>D. tetrarrhena</i>	+	4	2	2	2	2

The scale depending on the browning percentage on the basal stem 1 = 1-9%, 2 = 10-29%, 3 = 30-69%, 4 = 70-89% and 5 = 90-99%.

Table 2: Fungi associated with root and crown rot of bread wheat var. Chamran from tropical zone.

Fungal Species	Pathogenicity test	Severity Index	Number of positive isolates in			
			Vakilabad	Shahmaran	Soghan	Dolatabad
<i>F. oxysporum</i>	+	4	11	11	8	8
<i>F. nivale</i>	+	5	2	5	2	5
<i>F. poae</i>	+	5	3	2	2	2
<i>F. anthophilum</i>	-	0	2	0	1	2
<i>F. subglutinans</i>	-	0	2	2	2	1
<i>F. solani</i>	+	5	2	4	2	4
<i>F. culmorum</i>	+	5	2	0	0	2
<i>F. proliferatum</i>	-	0	2	0	0	0
<i>B. kusanoi</i>	+	5	1	1	0	1
<i>B. australiensis</i>	+	5	3	2	2	2
<i>D. tetrarrhene</i>	+	5	2	2	2	2

The scale depending on the browning percentage on the basal stem 1 = 1-9%, 2 = 10-29%, 3 = 30-69%, 4 = 70-89% and 5 = 90-99%.

Table 3: Fungi associated with root and crown rot of bread wheat var. Mihan from temperate zone.

Fungal Species	Pathogenicity test	Severity Index	Number of positive isolates in				
			Baft	Bardsir	Negar	Mahan	Shahrbabak
<i>F. oxysporum</i>	+	3	6	6	6	4	2
<i>F. solani</i>	+	5	4	2	2	2	2
<i>F. culmorum</i>	+	5	2	2	0	0	0
<i>F. subglutinans</i>	-	0	2	2	2	2	0
<i>F. chlamidosporum</i>	-	0	0	12	0	0	0
<i>B. kusanoi</i>	+	5	1	2	1	1	0
<i>D. tetrarrhene</i>	-	0	2	2	0	0	0

The scale depending on the browning percentage on the basal stem 1 = 1-9%, 2 = 10-29%, 3 = 30-69%, 4 = 70-89% and 5 = 90-99%.

DISCUSSION

Results from the study of wheat farms in Kerman province showed that root and crown rot is a major disease in these farms resulting in yield losses by causing damping off, decreasing number of tillers, head size and seed yields. Wheat crown and root rot problem was caused by multiple fungi. Among these fungi, the species of *Fusarium* were found to be the most prevalent of all the associated fungi and are in line with the findings of Gonzalez and Trevathan (2000). The most common infection in wheat fields was due to *Fusarium* genus which comprises about 76% of isolated fungi whereas results of other studies in these districts in 2011 showed that 99% of isolates were *Fusarium* (Dehghan et al., 2011). The prevalence and frequency differentiation of the genus *Fusarium* and certain species in a region is also primarily affected by crop rotation and environmental circumstances, especially temperature

and humidity requirements. The results showed that the frequency of isolates in the tropical zone between varieties was similar. *F. nivale*, *F. poae* and *F. proliferatum* exist in the tropical zone and *F. chlamidosporum* exists only in the temperate zone. Three species *F. oxysporum*, *F. solani* and *F. subglutinans* were the most prevalent in each case. Forty eight of the isolates in the present study belonged to two species of *Bipolaris* and one that to *Drechslera*.

In the present study, *B. kusanoi*, *B. australiensis* and *D. tetrarrhene* were isolated from tropical zone and *B. kusanoi* and *D. tetrarrhene* were isolated from temperate zone. The present findings are similar to the reports of different regions of world and Iran (Fedel-Moen and Harris, 1987; Hekimhan et al., 2004; Kazemi, 2001; Moradzadeh et al., 1997; Van, 2004; Zare and Ershad, 1997).

The population of *Bipolaris*, *Drechslera* and *Fusarium* species was boosted in this research compared to the

previous research conducted by Dehghan et al. (2011). The reason of this increase is change in irrigation method i.e. from flood irrigation to sprinkler and tap drip irrigation in recently years. These new methods decreased irrigation interval (4-6 days) compared to traditional irrigation (10- 15 days), therefore, soil remained wet for a long time. The prevalence of *Bipolaris* and *Drechslera* were reported in South Asia at high temperature and humidity (Kumar et al., 2002). The present studies also show that *Fusarium* infection increases at high humidity and corroborated the findings of Gherbawy et al. (2006).

It is concluded that wheat root and crown rot is fairly distributed in the Kerman province of Iran. The fungi associated with this disease were *F. oxysporum*, *F. nivale*, *F. culmorum*, *F. poea*, *Bipolaris kusanoi*, *B. australiensis* and *Drechslera tetrarrhenes*. The fungi showed virulence of varying degrees and demand strict control measures to minimize losses.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

MR and GRB designed the study, executed the experiment, collected and analyzed the data, wrote and edited the manuscript. Both the authors approved the final manuscript.

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