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Identification of *Pythium* species and their pathogenicity on cool season turfgrass in Tehran province

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ABSTRACT: Turfgrasses have been widely used in many residential areas and sport fields for many years. *Pythium* species can cause severe losses on cereals and other crops as well as ornamental plants such as turfgrass. To study the role of *Pythium* species in causing seed and root rot and damping-off, plant and soil samples were collected from different regions of Tehran province. Isolates were identified on the basis of morphological characters and cardinal temperature. Pathogenicity of the recovered species was determined on common cool season turfgrasses (Loliumperenne, Poaparatensis and Festucaarundinaceae). Of the 48 recovered *Pythium* isolates, three species were identified include 66.7% *P. aphanidermatum*, 18.7% *P. catenulatum and* 14.6% *P. okanoganense*. Comparative pathogenicity of *Pythium* species (total diseases) on turfgrasses showed that P.aphanidermatum was the most aggressive species and *P. catenulatum* and *P. okanoganens* were in second and third levels respectively.

Keywords: Festuca- Lolium-Poa-total diseases

INTRODUCTION

Nowadays, turf grassis used for production of green space in homes, business buildings, airports, highways and sport fields. Turf grasses used in this way has many benefits, for example, they reduce air and noise pollution, control soil erosion and eliminate dust (Kafi and Kaviani 2002). Moreover, the relaxing effects of turfgrass on human cannot be denied (Beard and Green 1994). Turfgrasses are monocots plant that belonged to Poaceae family. Based on their temperature range for growth, they can be divided into two groups: cool-season and warm-season grass.. Regarding to the weather condition in our investigation area (Iran, Tehran), the most common cool season turfgrass used are Poa, Festuca and Lolium. According to turfgrass important role in green space and sport field and its demonstrated usefulness, their maintenance is necessary and need to be concentrated. Due to high cost of turfgrass establishment and maintenance, and environmental problem of improper pesticide application, importance of turf diseases identification and their management is obvious.

Worldwide many pathogens are reported to affect turfgrasses including *Bipolaris, Fusarium, Magnaporthe, Pythiumand Rhizoctonia* species (Abad and Lucas 1990; Nelson and Craft 1991; Dong *et al.* 2007; Farr and Rossman 2013). The high level of irrigation commonly used for turfgrasses and poor root aeration (Hensley *et al.* 1999; Kafi and Kaviani 2002), contribute to the humid condition which are highly suitable for *Pythium* infection resulting in *Pythium* species being the major pathogen of concern compared with other pathogenic agents. Under these condition *Pythium* can cause widespread damage and significant losses in turfgrass (Saladini *et al.* 1983; Khodashenas Rudsare and Atharipour 2010). *Pythium* species are found in many regions and cause a wide range of damage and symptoms including blight, damping off, crown and root rots on plants (vander Plaats-Niterink 1981; Zamaninoor *et al.* 2004; Uzuhashi and Kakishima 2008). Various surveys have revealed that *Pythium* species are aggressive pathogens of turfgrass in different regions of the world (Abad and Lucas 1990; Nelson and Craft 1991; Allen *et al.* 2004). On bentgrasses, *Pythium* species such as *P. aphanidermatum*, *P. graminicola*, *P. ultimum* and *P. aristosporum* were reported to be highly aggressive whilst others including *P. irregulare*, *P. vanterpoolii* and *P. violae* are moderately and *P. catenulatum*, *P. torulosum* and *P. multisporum* are modestly aggressive (Abad et al. 1994). *Pythium graminicola* was reported asthe main pathogen causing root rot diseases on ryegrass (Nelson and Craft 1991). Additionally, Nelson and Craft (1991) isolated and reported *P. intermedium* and *P. dissotocum* from bluegrass and *P. aphanidermatum*, *P. carolinianum* and *P. volutum* from fescue. On wheat, *P. aristosporum* were the mainpathogenic species and *P. ultimum* and *P. irregular* were the next most important (Chamswarng and Cook 1985).

Pythium species have been reported from turfgrasses in Iran including *P. oligandrum, P. aphanidermatum, P. vexans, P. ultimum* varsporangiiferum, *P. deliens, P. vanterpooli* and *P. torlosum* (Mirabolfathy and Ershad 2002; Barzegar and Banihashemi 2011), but their morphology was not fully described and their pathogenicity has not been verified. This study was undertaken to identify the *Pythium* species associated with cool season turfgrasses used in Tehran green spaces and sport fields and to determine their pathogenicity on turfgrasses with the aim to determine which species were the most aggressive on the three commonly used turfgrass species, Lolium, Poa and Festuca.

MATERIAL AND METHODS

Isolation and identification. Turfgrass root and crown and soil samples were collected from various locations of green spaces and sport fields in Tehran province of Iran. The most commonly cultivated cool season turfgrass Poaparatensis L., *Lolium perenne* L. and Festucaarundinaceae Schreb were sampled. *Pythium* spp. were isolated from roots and crowns of mentioned turfgrasses exhibiting characteristic disease symptoms and also from soil. For soil samples, *Pythium* isolates were recovered using a bating method described by Ershad (1977). In this method, 300 g of each soil sample was placed in a container and saturated with water to one cm above the soil surface. A few discs (two mm diameter) cutfrom freshlime, orange and eucalyptus leaves were surface sterilized using alcohol and floated on the surface of the water and incubated for 24 hours at room temperature. After 24 hours the leaves were removed, washed with distilled water, dried and transferred to cornmeal agar (17 g L^{-1}; Difco CMA) amended with pimaricin 0.01 g, rifampin 0.01g, ampicillin 0.25g, PCNB 0.1g (CMA-PARP). CMA-PARP media. To prepare CAM-PARP, Difco CAM was used as basic medium. After autoclaving 17 g/L of this powderat 121°C for 20 min and cooling, pimaricin 0.01g, rifamp in 0.01g, ampicillin 0.25g, PCNB 0.1g added to this medium. After incubation for 36-48 hours in dark and 25°C, plates were evaluated for *Pythium* appearance. For isolations from the plant samples, the root and crown samples were washed, small sections (approx. 0.5 cm) from the edge of necrotic or rotten tissues on the main and secondary roots or the crown were surface sterilized with sodium hypochlorite, rinsed with sterile distilled water, dried and were placed on CMA-PARP plates. After incubation for 36-48 hours at 25°C in the dark, the plates were examined for growth of colonies characteristic of *Pythium* spp. From the leaf or root/crown tissues. The resulting *Pythium* spp. colonies were purified by hyphal tip subcultured onto 2% water agar (contained 20 g agar at 1L distilled water; WA).and purified by hyphal tip. In this way, after 24-48 hr emerging hyphal tips were moved to new 2%WAandsubsequent pure colonies were transferred to CMA, PCA and HSA to enable for morphological survey. To prepare PCA, 20 g of potatoes and 20 g of carrots were chopped and boiled for 10 min in 1 L of distilled water, filtered with mesh cloth and distilled water added to the filtrate to a final volume of 1 L. Then 20 g agar was added and autoclaved. To prepare HSA, 20 g of fresh hemp seeds were boiled in 1 L of distilled water for 30 min, then filtered and distilled water added to a final volume of 1 L. Then 20 g agar was added and autoclaved. In order toinduce sporangia and subsequent zoospores production, pond water and hemp seed and grass leaf methods were utilized. In this way boiled pieces of grass leaf and hempseed were placed on fresh *Pythium* colonies. After 24- 48 hours the colonized hemp seed and grass leaf were transferred to a Petri dish containing one part of sterile water to one part of pond water and incubated at distance of 20 cm from fluorescent lamps. Sporangium were produced on the floating hemp seed and leaf pieces after several hours to several days with zoospore release also observed using this method. For stimulating the production of sexual organs, PCA, HSA and CMA amended with0.1- 0.5 ppm beta-sitosterol was used (Ershad 1977). In addition, colony growth pattern of the different isolates were observed on both CMA and PCA were. Identification of *Pythium* isolated species were done using the Plaats-Niternik key (vander Plaats-Niterink 1981) with respect to observation of asexual and sexual organs, their characteristics, size, cardinal temperature for growth, daily growth rate and colony morphology. A minimum of 30 measurements was taken of each structure.

Pathogenicity test. From the three *Pythium* spp. Morphotype group observed, a representative number of isolates from each group (five isolates from the first group, two isolates each from the second and third group) were selected to determine their pathogenicity on the three turfgrasses Poaparatensis, Festucaarundinaceae and Loliumperenne. The pathogenicity of each isolate was assessed in a greenhouse experiment whereby the three turfgrasses were grown insterilized soilartificially infested with vermiculite inoculums of each *Pythium* spp. Isolate. To produce inoculum for each isolate, 100 g vermiculite and 60 ml hempseed extract were placed in a 250 ml flask and autoclaved at 120°C for 20 min. Five agar plugs from a 3- day-old culture of each *Pythium* isolate grown on CMA were transferred to the flask and incubated at 25°C for one month. Each pot (6 diameter) was filled with sterile silty-sand soil to a depth of three cm. Then a mixture of soil and *Pythium* inoculum at a ratio of 1 to 5 was added to the pot. The seeds of each turfgrass were hydrated by soaking in water and then surface sterilized with Sodium hypochlorite and rinsed in sterile water. For each replicate, 40 seeds were sown at a depth of one cm in the soil in each pot. The pots were placed on benches in a greenhouse and were irrigated every day. An untreated control was set up consisting of sterile soil and uncolonised vermiculite/hemp seed extract mix as described for the *Pythium* inoculated treatments.

Assessment of emerging seedlings were carried out after 7 to 10 days, and the percentage of dead seedlings and root rots were conducted after 4 weeks. The pots were destructively harvested, the plants were removed carefully and the roots washed under water. Factors included the number of emerging seeds, number of healthy and dead seedling and the amount of root rot were assessed to compare aggressiveness of isolates. The aggressiveness of the isolates were rated based on a scale of 0 to 100% (Chamswarng and Cook 1985; Nelson & Craft 1991; Abad *et al.* 1994). Based on the above factors and according to the numbers of death, solates were evaluated to be nonpathogenic, 0; slightly aggressive, 1-20%; moderately aggressive, 21-60% and highly aggressive, 61-100%. Samples of dead seedlings, rot lesions on the roots and seeds which failed to germinate were surfaced sterilised, plated on CMA and incubated at 25°C. The colonies which grew from the diseased tissue were morphologically compared with that of the inoculating *Pythium* spp. Isolate to confirm causal agent For more comprehensive assessment of Pythium pathogenicity, statistical test was also used in this study. Statistical test, apart from comparison of the pathogenicity of the different Pythium isolates on the different genus of turfgrasses, provides the possibility of study their interactions. Therefore, treatments were arranged in a two factor randomized complete block design with four replications. First factor included three genus of turfgrasses and second factor consisted of 9 isolates (5 isolates of *P. aphanidermatum*, 2 isolates of *P. catenulatum*, 2 isolates of *P. okanoganense*) and one control. Analysis of variance were conducted using the MSTATC software with means compared using Duncan's least significant difference test.

RESULTS

Identification and description of Pythium species. Of the 48 Pythium isolates recovered, three species were identified including 32 isoltes (66.7%) *P. aphanidermatum* (Edson) Fitzp, 9 isoltes (18.7%) P. catenulatum Matthews and 7 isoltes (14.6%) *P. okanoganense* Lipps.

P. aphanidermatum. The colony morphology of the P. *aphanidermatum* isolates on PCA and CMA was without a special pattern and produced abundant aerial mycelium. Main hyphae width was 7.5-9.4 µm. The shape of the sporangia was swollen hyphal branches or filamentous in various dimensions which formed easily after a short time on both colonized grass blades and hemp seed incubated in pond water and agar media and produced zoospores in pond water at room temperature. A large number of oogonia and antheridia were produced simultaneously after some days on PCA, CMA and HSA. Oogonia were globose, with smooth wall and were produced terminally with 22-25µmdiameter. Antheridia were sac-shaped and were mostly formed intercalary. Antheridia were paragenous, and in most isolates were diclinous and for each oogonium one antheridium was formed. Oospores diameter was18.5-22.5µm and were aplerotic with smooth walls and 1-2 µm thick. Daily growth rate on PCA at 25°C was over 31-35 mm. Cardinal temperatures were minimum 10°C, optimum 35°C and maximum 40°C. The morphology of the reproductive and vegetative structures is illustrated in Figure 1.

***P. catenulatum*.** The *P. catenulatum* isolates on PCA produced few aerial mycelium and were without a special colony pattern. On CMA the colonies prod used a radiate pattern partly chrysanthemumpattern. Main hyphae width was 4-5 µm. Sporangia were produced on both colonized grass blades and hemp seed incubated in pond water and on agar media at room temperature were irregularly swollen branched. Hyphal swelling were produced in chains varying from 5 to 20 µm in diameter that germinated by producing some germ tubes. For some isolates, oogonia were produced on PCA and CMA after 25 days and on HSA after 20 days. For the remaining other isolates, no oogonia were produced. Amendment of the media with beta-sitosterol did not significantly affect the rate of production of sexual organs. Oogonia were globose with smooth walls and were produced terminally with 19-26 µm diameter. Antheridia were crook-necked and stalked. They were paragenous, monoclinous and diclinous and formed intercalary. For each oogonium more than one antheridium was produced, each having apical contact with the oogonium. Oospores had smooth walls which wall thickness was 1-1.4 µm, some being plerotic and other saplerotic. Daily growth rate on PCA at 25°C was 10-13 mm. Cardinal temperatures were minimum 5°C, optimum 30°C and maximum 40°C. The morphology of reproductive and vegetative structures is illustrated in Fig. 1.

P. okanoganense. The colony morphology of the *P. okanoganense* isolates on CMA was with a radiate pattern, while on PCA the pattern was partly chrysanthemum. Main hyphae width was 2.4-2.4 µm. Sporangia were globose and subglobose in shape and formed terminally after some days on colonized grass blades and hemp seed incubated in pond water and on agar media. Sporangia germinated by producing some germ tubes being from 2 to 20 µm in diameter. Oogonia were globose with 18-22.7 µm diameter with smooth wall and produced terminally forming after a week on PCA, CMA and HSA. Wall thickness was 1.7-2.3µm.Antheridia were stalked and usually formed simultaneously with the oogonia. In some isolates (more than one antheridia formed per oogonium but in one isolate mostly only one antheridium formed per oogonium. Antheridia were paragenous, stalked, mostly diclinous and had apical contact with oogonia. Oospores were mainly aplerotic with smooth walls with only a few being plerotic (Fig. 1). Daily growth rate of the isolates on PCA at 25°C was 4-6 mm.Cardinal temperatures were minimum 10°C, optimum 30°C and maximum 35°C.

Pathogenicity test of *Pythium* species. The average percentage of seed rot, root rot and seedling damping off recorded for the different isolates of the three *Pythium* species for the three turfgrasses are shown in Table 1.

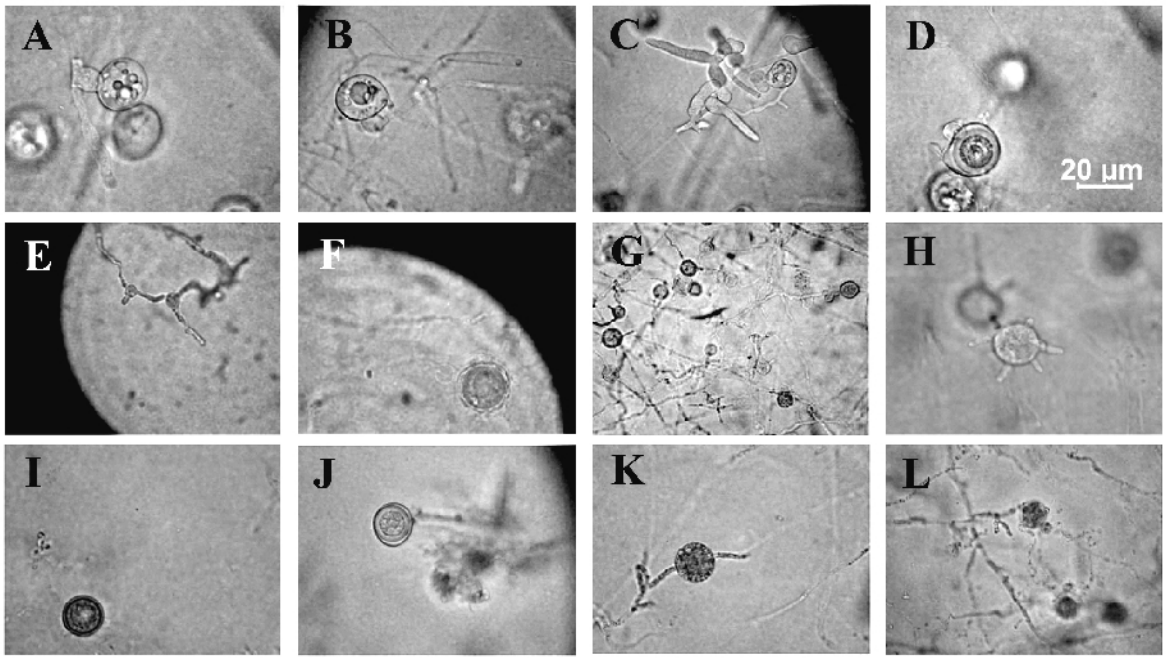

Fig. 1. Reproductive and vegetative structures of *Pythium* species in turfgrasses. A, intercalary antheridium and smooth terminally oogonium of *P. aphanidermatum* - B, stalked and hypogynusantheridumof *P. aphanidermatum*- C, toruloid sporangium of *P. aphanidermatum* - D, aplerotic oospore of *P. aphanidermatum* - E, sporangium of *P. catenulatum*– F, oogonium, antheridium and oosporeof *P. catenulatum*– G, hyphal swelling of *P. catenulatum* – H, germination of hyphal swelling of *P. catenulatum*- I,aplerotic oospore of *P. okanoganense*- J, plerotic oospore of *P. okanoganense* – K, golobus sporangium of *P. okanoganense* – L, antheridium and oogonium of *P. okanoganense*.

Table 1: Percentage of seed rot, root rot and damping off in *Loliumperenne*, *Poaparatensis* and *Festucaarundinaceae* grown in sterile soil inoculated with different isolates of *three Pythium* species.

ol at	*Pythium*	seed rot			root rot			damping off		
	spp.	*Festuca*	*Poa*	*Lolium*	*Festuca*	*Poa*	*Lolium*	*Festuca*	*Poa*	*Lolium*
1	*P. aph*†	47.0±6 AB	79.8± 3 A	44.0 ± 6 ABC	9.8±2 BCD	14.0± 3 BC	8.3 ± 3 B	4.8 ± 1 CD	5.8 ± 1 BC	5.0±1.4 ABCD
2	*P. aph*	53.0±8 AB	76.8 ± 4 AB	49.0 ± 9 AB	12.0±3 AB	14.5 ± 4 ABC	9.3 ± 3 AB	6.0± 1.4 BC	8.3 ± 1.3 AB	4.3 ± 1.7 BCD
3	*P. aph*	57.8 ±3 A	80.0 ± 4 A	52.8 ± 6 A	9.3 ±3 BCD	12.0± 3 BCD	13.3 ±3 A	9.8 ± 1.9 A	7.8 ± 1.5 AB	6.3 ± 1.3 AB
4	*P. aph*	52.8±17 AB	71.0 ± 3 AB	48.5 ± 10 AB	14.8 ±5 A	18.8 ± 3 A	7.8 ± 4 B	6.8 ± 2.2 BC	9.8 ± 1.7 A	5.3 ± 1.7 ABC
5	*P. aph*	57.5±9 A	72.5 ± 3 AB	52.0± 6 A	12.5 ±4 AB	16.5 ± 3 AB	9.5 ± 4 AB	6.0± 1.4 BC	9 ± 0.8 A	3.8± 0.5 BCDE
1	*P. oka*‡	37.0±8 B	48.0 ± 9 D	22.0 ± 6 DE	6.8 ±3 CD	9.0± 4 D	3.0± 2 C	4.3 ± 1.7 CD	3.5 ± 0.6 CD	2.8 ± 1 CDE
2	*P. oka*	38.5±6 B	54.3 ± 7 CD	22.5 ± 5 DE	6.5 ±2 CD	10.0± 2 CD	3.0± 3 C	4.3 ± 2.1 CD	3.8 ± 1.3 CD	2.5 ± 0.6 DE
1	*P. cat*ǂ	47.8±13 AB	65.3 ± 5 ABC	34.8 ±13 BCD	11.3 ±5 ABC	13.8 ± 3 BCD	7.0± 4 BC	8.5 ± 1.3 AB	7.5 ± 0.6 AB	7.3 ± 1 A
2	*P. cat*	46.8±12 AB	63.0± 7 BCD	31.5 ± 7 CD	11.3 ±3 ABC	12.5 ± 2 BCD	6.3 ± 3 BC	10.3 ± 1.7 A	8.8 ± 1 A	6.3 ± 1.7 AB
C*		11.8±2 C	12.8 ± 2 E	9.3 ± 2 E	6 ±1 D	4.0± 1 E	2.5 ± 1 C	2.5 ± 0.6 D	2.3 ± 0.5 D	1.5 ± 1 E

P.aph†: *Pythium aphanidermatum, P. oka*‡: *Pythium okanoganense , P. cat*ǂ : *Pythium catenulatum*

C*= control, Values are the means from four replications, Means in each column followed by the same letter are not significantly different ($P \leq 0.01$) according to Duncan's least significant difference test.

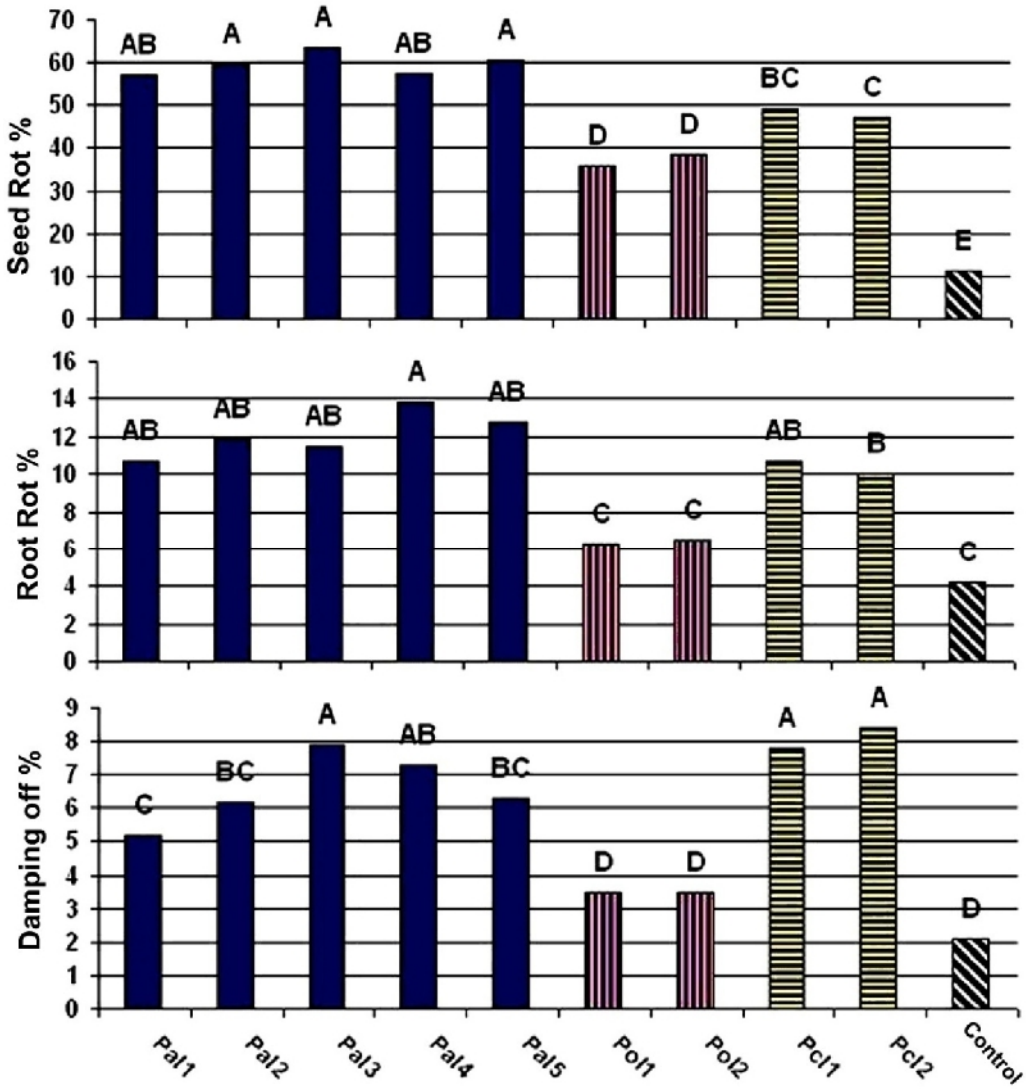

Fig. 2. Comparison between seed rot, root rot and damping off caused by inoculation with different isolates of three *Pythium* species across three turfgrass genera, PaI1-PaI5: isolates 1- 5 of *P. aphanidermatum*, PoI1-PoI2: isolates 1 and 2 of *P. okanoganense*, PcI1-PcI2: isolates 1 and 2 of *P. catenulatum*. Means followed by the same letters are not significantly different according to Duncan's least significant difference test at $p \leq 0.01$.

As can be seen from the data given, there are significant difference between various isolates of *Pythium* in seed rot, root rot and damping off in three turfgrass genus. But due to the many interactions in this multi factorial design, the main effects of factors were covered. So with extra sum of squares, the pathogenicity of *Pythium* different isolates across all three turfgrass genera (Fig. 2) and for each individual turfgrass genus (Fig. 3) were determined.

From the data given in Fig. 2, data variance analysis and means comparison test about seed rot in three studied genus of *Pythium* showed significant differences with the control which indicate the pathogenicity potential of these species on the average of three demonstrated turf genera that *P. aphanidermatum* was the most aggressive species and *P. catenulatum* and *P. okanoganense* were in second and third levels respectively. According to Duncan's least significant difference test, all isolates within *P. aphanidermatum* showed significant differences with the control ($p \leq 0.01$) and isolates 2, 3 and 5 located in different group with isolates 1 and 4.

Also percentage of seed rot ranged based on the scale from 0 to 100% which isolates 2, 3 and 5 were highly aggressive (61- 100%) and isolates 1 and 4 were moderately aggressive (21- 60%). These results corresponded with the Duncan's outcomes. *P. catenulatum* and *P. okanoganense* were moderately aggressive (21- 60%) and there was no significant difference between their isolates. The lack of difference between these isolates corresponded with the results of the Duncan test.

All isolates of *P. aphanidermatum* and *P. catenulatum* significantly increased the percentage of root rots with the untreated control ($p \leq 0.01$) while both *P. okanoganense* isolates not being significantly different from the control In the other words, *P. aphanidermatum* was more aggressive pathogen than *P. catenulatum* whereas *P. okanoganense* was nonpathogenic. Isolates within *P. aphanidermatum* (according to Duncan's test, $p \leq 0.01$) showed significant difference with each other which isolate 4 by mean 13.75% located in distinct group with isolates 2, 3 and 5.There was no significant difference ($p \leq 0.01$) between the percentage root rots caused by the two isolates of *P. catenulatum* (Fig. 2). On the scale of 0-100%, both species (*P. aphanidermatum* and *P. catenulatum*) caused low level of disease (1-20%). Also all isolates of *P. aphanidermatum* with low pathogenicity rating (1-20%) were slightly aggressive and there was any difference between them that this result did not match with the Duncan test outcome. About isolates within *P. catenulatum*, all of these caused ≤20% seed rot and showed any difference with each other which corresponded with the results of the Duncan test.

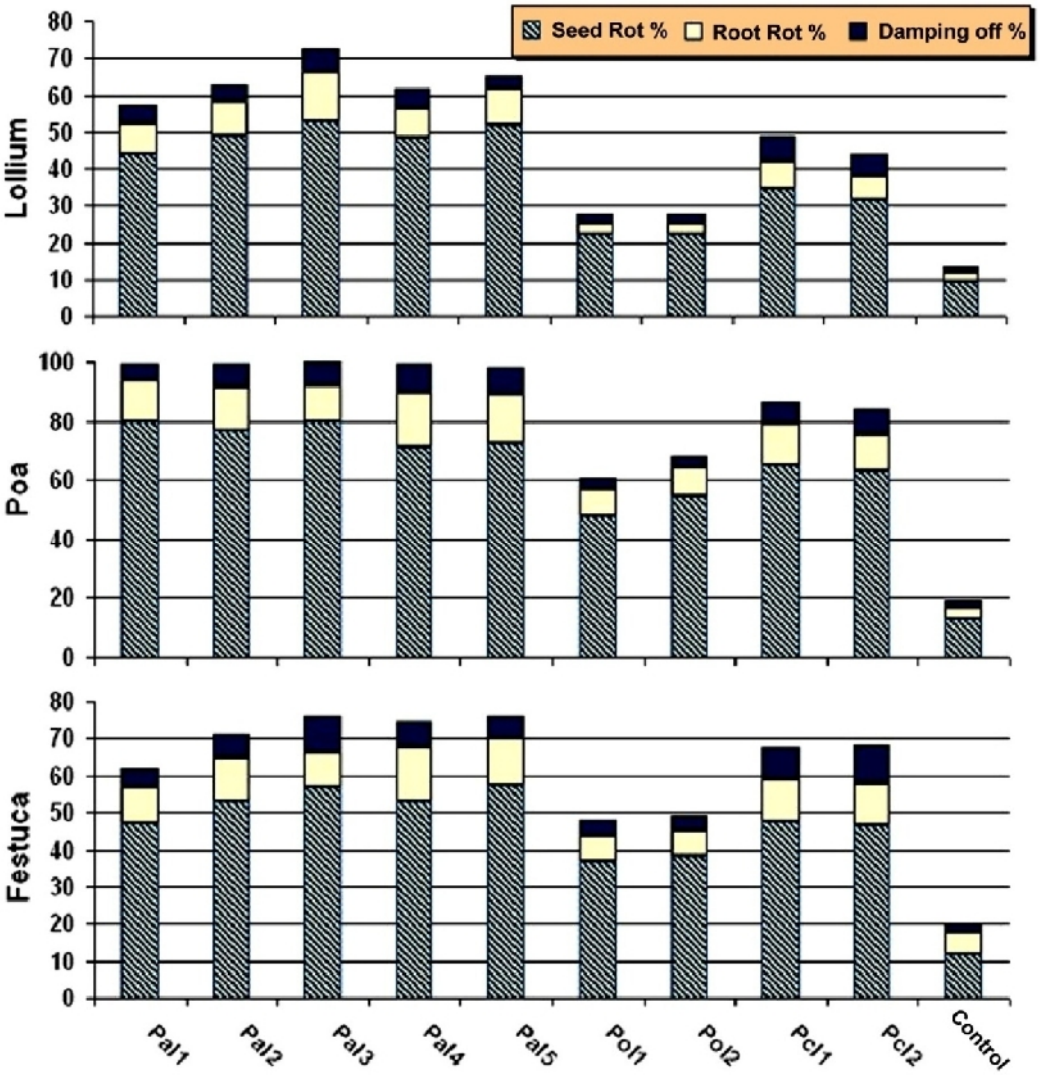

Fig. 3. Total disease as a sum of seed rot, root rot and damping off in three turfgrasses, *Loliumperenne*, *Poaparatensis* and *Festucaarundinaceae* grown in soil inoculated with different isolates of three *Pythium* species, *P. aphanidermatum* isolates Pal1-Pal5, *P. okanoganense* isolates PoI1-PoI2 and *P. catenulatum* isolates PcI1-PcI2.

With respect to damping off, given species according to the Duncan test divided into four groups. Group 1 included isolates 1 and 2 of *P. catenulatum* and isolate 3 of *P. aphanidermatum*, had the most aggressive species. The second most aggressive species contained three isolates of *P. aphanidermatum* (isolates 2, 4 and 5) located in group 2. Isolate 1 of *P. aphanidermatum* situated in third group and damping off caused by both isolates of *P. okanoganense* were not significantly different to that recorded in the untreated control and were nonpathogenic. Isolates within *P. catenulatum* did not show difference (according to Duncan's test) while there was difference between isolates of *P. aphanidermatum* and they located in three distinct groups (Fig. 2). On the scale of 0-100%, all isolates of both species (*P. aphanidermatum* and *P. catenulatum*) caused ≤ 20% damping off so were slightly aggressive. Also isolates within *P. catenulatum* showed any difference with each other which corresponded with the results of the Duncan test. There was any difference between isolates of *P. aphanidermatum* which did not match with the Duncan test result.

Comparative pathogenicity of *Pythium* species (total of seed rot, root rot and damping-off) on each genus of turfgrasses (Poaparatensis, Festucaarundinaceae and Loliumperenne) showed that all three *Pythium* species caused more plant losses on Poaparatensis where as Festucaarundinaceae was less susceptible and Loliumperenne had the most resistance against *Pythium* species (Fig. 3). In addition, comparative pathogenicity assessment of demonstrated *Pythium* indicated that *P. aphanidermatum* was the most aggressive species and *P. catenulatum* and *P. okanoganense* were in the second and third levels respectively (Fig. 3).

DISCUSSION

Pythium aphanidermatum was first described as *Rheosporangium aphanidermatum* in 1915 (Edson 1915) and recognized as *Pythium* in 1923 (Fitzpatrick 1923). There are many reports about this species causing root, stalk and fruit rots, damping off and blight on various plants included turfgrass throughout the world (Saladini *et. al* 1983; Smiley *et. al* 1992; Agrios 1997). Nearly all of the characteristics of *P. aphanidermatum* isolates in this study were consistent with descriptions of vander Plaats-Niterink (van der Plaats-Niterink 1981).The most frequently recovered species in this survey was *P. aphanidermatum* (66.7%) whereas Abad *et al.* (1994) reported *P. torulosum* as the most frequent species and Nelson and Craft (1991) reported *P. graminicola* as the most frequently isolated species. In these studies *P. aphanidermatum* frequency was 1.3% and 17.4% respectively. In Iran this species was reported from turfgrass by Ershad in 1977 (Ershad 1977) but in his survey the frequency of isolation and pathogenicity of the recovered *P. aphanidermatum* isolates and pathogenicity was not investigated. *Pythium aphanidermatum* was reported as being a highly aggressive pathogen (total pathogenicity) in past studies (Abad *et al.* 1994) which agreed with our results. Moreover our results also compared the susceptibility of the cool-season turf to Pythium species infection indicating that Poaparatensis was most susceptible compared with Festucaarundinaceae and Lolium perenne respectively.

P. catenulatum was recovered from plant debris for the first time in 1931 (Matthews 1931)
and has reported as a damping off and root rot pathogen from a range of plant species including sugar beet, rice, tomato, potato throughout the world. This species was reported to only be slightly to non pathogenic on bentgrass (Abad *et al.*1994) while in our study it was shown to be moderately to slightly aggressive on the three cool- season turfgrasses tested. To confirm its importance as a pathogen of turfgrasses more assessments under different temperature and humidity conditions are needed. In our study the minimum temperature for growth of the *P. catenulatum* isolates was 5°C lower than that described by van der Plaats-Niterink. However the other characteristic of this species matched with vander Plaats-Niterink description. Two of the four isolates of *P. catenulatum* tested in our study produced oospores in single culture and were therefore homothallic, while two isolates did not produce oospores and indicating they are heterothallic. This agrees with the findings of van der Plaats-Niterink (1981) and Abad *et al.* (1994) where some isolates of *P. catenulatum* were homothallic and others being heterothallic. In addition, Hendrix and Papa (1974) reported that of the 24 isolates of *P. catenulatum* from turf soil that eight isolates were homothallic and the others were heterothallic. In our study, *P. catenulatum* was the second most frequent species isolated corresponding with that reported by Abad *et al.* (1994). This species was not recovered from turfgrass in the survey by Nelson and Craft (1991). *Pythium catenulatum* has not been reported from turfgrass of Iran until now. *P. okanoganense* was first described as a wheat pathogen in Washington (Lipps 1980) and subsequently has been isolated from wheat and barley in Japan (Ichitani *et al.* 1986). In the current study *P. okanoganense* was less frequently isolated compared with the other two species and was shown to only be moderately aggressive to slightly on the three cool- season turfgrasses. *Pythium okanoganense* has previously been isolated from soil in Iran, but was shown to be nonpathogenic on sugarbeet (Zamaninoor *et al.* 2004) but has not been reported from turfgrasses in Iran and other countries yet. Uzuhashi *et al.* described *Pythium okanoganense* as Globisporangium (2010) but it has not recognized by “mycobank.org” yet. Information about its distribution, ecology, phylogeny and host range is limited and needs further studies. In our study characteristic of this species corresponded to van der Plaats-Niterink (1981) except for the minimum temperature and the number of antheridia. This means that the number of antheridium and minimum temperature (two degrees) in our study were upper than van der Plaats-Niterink description.

Since the maximum activity and virulence of *Pythium* species is at alkaline pH, high humidity and poor soil drainage conditions, the avoidance of excessive irrigation (especially during establishment), improvement of soil drainage and increasing with soil aeration, maintenance of the soil pH in the range from slightly acidic to neutral, and avoiding high fertilization can be effective in preventing the occurrence of disease (Smiley *et al.* 1992; Clark and Kenna 2001). However complementary studies on the biology, ecology and epidemiology of these *Pythium* species is needed to provide information on the potential control strategies.

According to the disease levels caused by the different *Pythium* species isolates, Poaparatensis, was seen to be the most susceptible whilst Loliumperenne was the most resistant. Therefore in turfgrass areas susceptible to *Pythium* infection it would be preferable to choose to grow Loliumperenne. However, consideration of the botanical characteristics of each genus of turfgrass such as drought and wear tolerance, tillering rate and germination speed must also be taken and where a mixture of three grass species are recommended for planting increase in the amount of Loliumperenne and decrease in Poaparatensis is suggested.

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