

IDENTIFICATION OF SOIL *PYTHIUM* SPECIES IN FARS PROVINCE OF IRAN*

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Abstract – During 2000-2001, soil *Pythium* species were studied in Fars province. Soil samples were collected from various parts of the province (Abadeh, Bajgah, Bayza, Borazjan, Darab, Estahban, Fasa, Khafr, Sepidan, Shiraz, Moharloo, and Zarghan). Isolates were recovered from soil by baiting. Species were identified based on morphological characters of sexual and asexual organs, growth rate at different temperatures, and colony morphology on various media. From 270 isolates of *Pythium* recovered, 12 species and two groups were identified as: *P. aphanidermatum**, *P. aquatile*, *P. deliense*, *P. diclinum*, *P. echinulatum*, *P. inflatum*, *P. okanoganense*, *P. oliganderum*, *P. orthogonon*, *P. ostracodes*, *P. rostratum*, *P. vexans*, *Pythium* Group “G”*, and *Pythium* Group “HS”* (species with asterisk were predominant).

P. aquatile, *P. diclinum*, *P. echinulatum*, *P. inflatum*, *P. okanoganense*, *P. oliganderum*, *P. orthogonon*, *P. ostracodes*, *P. rostratum*, and *P. vexans* are new for Fars, and *P. aquatile*, *P. echinulatum*, *P. inflatum*, *P. okanoganense*, *P. orthogonon*, *P. ostracodes*, and *P. rostratum* are new for Iran flora.

A key is also presented for identification of Fars *Pythium* species.

Keywords – *Pythium* species, Fars, soil mycoflora, taxonomy

1. INTRODUCTION

Pythium Pringshem species are often pathogenic to many plants and to animals such as fish or Crustacea. They can cause severe losses in cereals and other crops, as well as ornamental plants. From an ecological view, the species of this genus occur worldwide: from temperate to tropical, and even cold regions. They occur most abundantly in cultivated soils near the root region in superficial soil layers, less commonly in noncultivated or acid soils [1-5].

Species of *Pythium* can live saprophytically or parasitically. Their parasitic role often depends on external factors. When conditions are favorable for the pathogen, but less for the host. *Pythium* species can become very pathogenic and cause the rotting of fruit, roots or stems, pre- or post-emergence damping-off of seeds and seedlings [3, 5, 6].

The pathogenic capacity is largely determined by the available enzymes. Pectolytic, as well as cellulolytic enzymes have been demonstrated in several species. From a number of *Pythium* species, phytotoxins have been isolated which can have a role in their pathogenicity [3, 5]. Because of the variation between the species of this genus in the cultivated soils, and their various pathogenicity potential; the inclusion of these characteristics in the identification of *Pythium* isolates seems to be necessary.

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From 13 species of *Pythium* reported from Iran, only certain species including *P. aphanidermatum*, *P. coloratum*, *P. deliense*, *P. debaryanum*, *P. intermedium*, *P. oligandrum*, *P. paroecandum*, *P. ultimum*, and *P. vexans* have morphologically been described [1, 5-20]. The abstract of the present work has appeared earlier [21].

2. MATERIALS AND METHODS

Soil samples were collected from various parts of Fars province in Iran (Table 1). One hundred grams of each sample were placed in a plastic container and flooded with tap water to 1 cm above the soil surface. Isolates were recovered from soil by baiting with 5-mm citrus leaf disks at 20°C for 24 hours [22], and plating on PARP media (extract of 60 g ground maize, pimaricin 0.01g, ampicillin 0.25g, rifampin 0.01g, PCNB 0.1g, agar 15g, distilled water 1l) [23]. Colonies recovered after 36 hours were plated on water agar (agar 15g, distilled water 1l) and purified with the hyphal tip method. Colony morphology was studied on CMA (extract of 60 g ground maize, agar 15g, distilled water 1l) [24], and PCA (extract of 20 g carrots and 20 g potatoes, agar 15g, distilled water 1l) [24] at 25°C. Growth rate was measured on PCA at 5, 15, 20, 25, 30, 35, and 37°C. Morphological characters of asexual organs were studied on PCA and V8A (V8 vegetable juice 200 ml, calcium carbonate 4 g, agar 15 g, distilled water 800 ml) [24]. To induce the formation of sporangia, 5 mm boiled pieces of grass leaf (*Poa annua*) were placed on PCA at 25°C, and after 24 hrs were transferred on to a Petri dish in a shallow layer of sterile water or pond water under fluorescence illumination. Sexual organs were studied on HAS (extract of 20 g ground hemp seeds, agar 15g, distilled water 1l) containing 30 mg β -sitosterol [25]. All isolates were identified by authentic keys [1, 3, 5, 26].

Table 1. Characteristics of the soil samples collected in 2000 from Fars province used for isolating *Pythium* species

Location	ID	Date of sampling	Site of sampling
Darab	A	Apr.13	Chick pea field
Fasa	B	Apr.13	Wheat field
Darab	C	Apr.13	Potato field
Shiraz	D	Apr.9	Blackberry crown
Bajgah	E	Apr.21	Wheat field
Bajgah	F	Apr.21	Rose plot
Bajgah	G	Apr.21	Alfalfa field
Bajgah	H	Apr.21	Wheat field
Bajgah	I	Apr.21	Apple orchard
Bajgah	J	Apr.21	Liquorice crown
Bajgah	K	Apr.21	Wheat pot
Bajgah	L	Apr.21	Pine crown
Shiraz	M	Apr.22	Sweet orange crown
Borazjan	N	Apr.26	Tomato field
Borazjan	O	Apr.26	Tobacco field
Estahban	P	May.24	Wheat field
Sepidan	Q	Jun.4	Onion field
Sepidan	R	Jun.4	Wheat field
Sepidan	S	Jun.4	Barley field
Bayza	T	Jun.4	Wheat field
Khafri	U	Jun.8	Tangerine orchard
Khafri	V	Jun.8	Almond orchard
Khafri	W	Jun.8	Walnut orchard
Moharloo	X	Jun.8	Potato field
Shiraz	Y	Jun.21	Evonymus nursery
Zarghan	Z	Nov.29	Pine crown
Abadeh	AA	Dec.22	Grapevine orchard

3. RESULTS

Based on morphological characters of sexual and asexual organs, colony morphology, and growth rates at different temperatures (Table 2.), the isolates were identified as 12 species and two heterothallic groups (Table 3.). A key is also constructed for the identification of I species from Fars province (Table 4).

Table 2. Growth rate of isolates of *Pythium* species from Fars province

<i>Pythium</i> species	Isolate	Temperature (°C)						
		5*	15	20	25	30	35	37
<i>P. aphanidermatum</i>	C30	0	17	23	30	37	40	40
<i>P. aquatile</i>	Q177	2	10	13	14	5	0	0
<i>P. deliense</i>	A5	0	10	11	21	35	38	35
<i>P. diclinum</i>	N143	0	19	21	30	35	37	35
<i>P. echinulatum</i>	A8	0	6	7	8	4	N	0
<i>P. inflatum</i>	K117	0	5	6	7	10	7	7
<i>P. okanoganense</i>	Q173	0	11	18	23	28	24	18
<i>P. oliganderum</i>	W231	0	14	20	24	30	27	25
<i>P. orthogonon</i>	X243	0	5	5	8	8	12	5
<i>P. ostracodes</i>	B11	N	5	6	7	8	9	8
<i>P. rostratum</i>	J104	0	5	5	8	8	7	7
<i>P. vexans</i>	AA270	0	6	10	12	13	1	0

N=Negligible (less than 1 mm/24h)

* (mm/24h)

Table 3. Taxa of the genus *Pythium* isolated in Fars province

<i>Pythium</i> taxa	Code of isolates *
<i>P. aphanidermatum</i>	C30-C39, L121-L129, T207-T209, Y250-Y259
<i>P. aquatile</i>	Q177-Q179
<i>P. deliense</i>	A1-A5
<i>P. diclinum</i>	F60-F69, J100-J105, N140-N149
<i>P. echinulatum</i>	A6-A10
<i>P. inflatum</i>	K110-K119
<i>P. okanoganense</i>	W231-W239
<i>P. oliganderum</i>	Q170-Q176, X241-X249, Z260-Z269
<i>P. orthogonon</i>	E50-E59
<i>P. ostracodes</i>	B11-B20, M130-M139
<i>P. rostratum</i>	J106-J109
<i>P. vexans</i>	AA270-AA274
<i>Pythium</i> Group "G"	G70-G79, H80-H89, T204-T206, U210-U211, U216-U220
<i>Pythium</i> Group "HS"	D40-D49, I90-I99, O150-O159, P160-P169, R180-R189, S190-S199, T200-T203, U212-U215, V221-V229

* See Table 1. (Letters refer to location followed by isolate number)

Table 4. Key to *Pythium* species isolated from Fars province of Iran

1	Oogonia produced in single culture	2
	Oogonia not produced in single culture	13
2(1)	Oogonial wall ornamented with obtuse or blunt projection	3
	Oogonial wall smooth	4
3(2)	Sporangia consisting of irregular complexes of (sub)globose and filamentous elements	<i>P. oliganderum</i>
	Sporangia single, (sub)globose or elongated, terminal, occasionally intercalary	<i>P. echinulatum</i>
4(2)	Sporangia filamentous, inflated or not inflated	5
	Sporangia (sub)globose	9
5(4)	Sporangia not inflated	6
	Sporangia inflated	7
6(5)	Antheridia monoclinal	<i>P. aquatile</i>
	Antheridia diclinous	<i>P. diclinum</i>
7(5)	Antheridia mostly intercalary	8
	Antheridia terminal, diclinous	<i>P. inflatum</i>
8(7)	Oogonial stuck mostly curved towards the antheridia	<i>P. deliense</i>
	Oogonial stuck straight	<i>P. aphanidermatum</i>
9(4)	Sporangia proliferating	<i>P. ostracodes</i>
	Sporangia not proliferating	10
10(9)	Oospores plerotic or nearly so	11
	Oospores aplerotic	12
11(10)	Oogonia intercalary	<i>P. rostratum</i>
	Oogonia mostly terminal; antheridia often crook-necked	<i>P. orthogonon</i>
12(10)	Antheridia bell shaped, monoclinal	<i>P. vexans</i>
	Antheridia is not bell shaped	<i>P. okanoganense</i>
13(1)	Sporangia not formed	Pythium Group "HS"
	Sporangia (sub)globose	Pythium Group "G"

Description of *Pythium* species:

1. *P. aphanidermatum* (Edson) Fitzp.: isolates produced colonies with cottony aerial mycelium on CMA, and no aerial mycelium on PCA and HAS, without a special pattern on all of the three media; main hyphae 6-9 μm wide; sporangia consisting of a terminal complex of swollen hyphal branches of varying length; oogonia terminal, globose, smooth, 24-29 μm diameter; antheridia mostly intercalary sometimes terminal, broadly sac-shaped 9-12 μm wide; oospores aplerotic, 18-25 μm diameter, wall 2-3 μm thick (Fig. 1, a-c). Daily growth rate on PCA 30 mm at 25°C.

2. *P. aquatile* Hohnk: isolates produced submerged colonies on CMA, and indistinct to radiate colonies on PCA and HAS; main hyphae 5 μm wide; sporangia filamentous without inflation, 5-6 μm wide; vesicles globose, about 30 μm diameter; zoospore production ability at 20°C high, encysted zoospores 8-10 μm diameter; oogonia terminal, globose, smooth, 25 μm diameter; antheridia 1-2 per oogonium, clubbed, curved, paragynous, monoclinal, 7 μm wide; oospores aplerotic, 8-28.5 μm diameter, wall 7 μm thick (Fig.1, d-f). Daily growth rate on PCA 14 mm at 25°C.

3. *P. deliense* Meurs: isolates produced aerial mycelium and colonies with indistinct patterns on CMA, HAS and PCA; main hyphae 8 μm wide; sporangia inflated, filamentous, terminal, 5-10 μm wide; oogonia globose, smooth, terminal or intercalary, 18-23 μm diameter; oogonial stalk bending towards the antheridium; antheridia mostly 1 per oogonium, with a straight stalk or sessile, terminal or intercalary, paragynous, diclinous, 7 μm wide; oospores aplerotic, 18-20 μm diameter, wall 2 μm thick (Fig. 1, g-I). Daily growth rate on PCA 21 mm at 25°C.

4. *P. diclinum* Tokunaga: isolates produced loose aerial mycelium and colonies with indistinct pattern on CMA, HAS and PCA; main hyphae 8 μm wide; sporangia filamentous without inflation, terminal, 3-8 μm wide; oogonia (sub)globose, smooth, mostly terminal sometimes intercalary, 25-27 μm diameter; antheridia mostly 1 per oogonium, terminal or intercalary, paragynous, diclinous, 9 μm wide; oospores aplerotic, 20-22 μm diameter, wall 2 μm thick (Fig. 1, j-k). Daily growth rate on PCA 21 mm at 25°C.

5. *P. echinulatum* Matthews: isolates produced submerged colonies with a rosette pattern on CMA, and colonies with an indistinct pattern on HAS and PCA; main hyphae 7 μm wide; sporangia globose to cylindrical, terminal sometimes intercalary, 18 μm wide; oogonia (sub)globose, smooth, 8-20 μm diameter, provided with acute, conical projections 3 μm long; antheridia 1-2 per oogonium, hypogynous sometimes paragynous, monoclinal; oospores aplerotic or plerotic, 18 μm diameter, wall 1 μm thick (Fig. 1, l-n). Daily growth rate on PCA 8 mm at 25°C.

6. *P. inflatum* Matthews: isolates produced colonies with indistinct to radiate patterns on PCA, and colonies with vague radiate patterns on HAS and CMA; main hyphae 3 μm wide, with irregular hyphal swellings; sporangia inflated, filamentous, containing irregular or globose outgrowths, variable wide; oogonia globose, smooth, mostly terminal sometimes intercalary, 15-17 μm diameter; antheridia 1-3 per oogonium, paragynous, monoclinal or diclinous; oospores aplerotic, 15 μm diameter, wall 2 μm thick (Fig. 1, o-p). Daily growth rate on PCA 7 mm at 25°C.

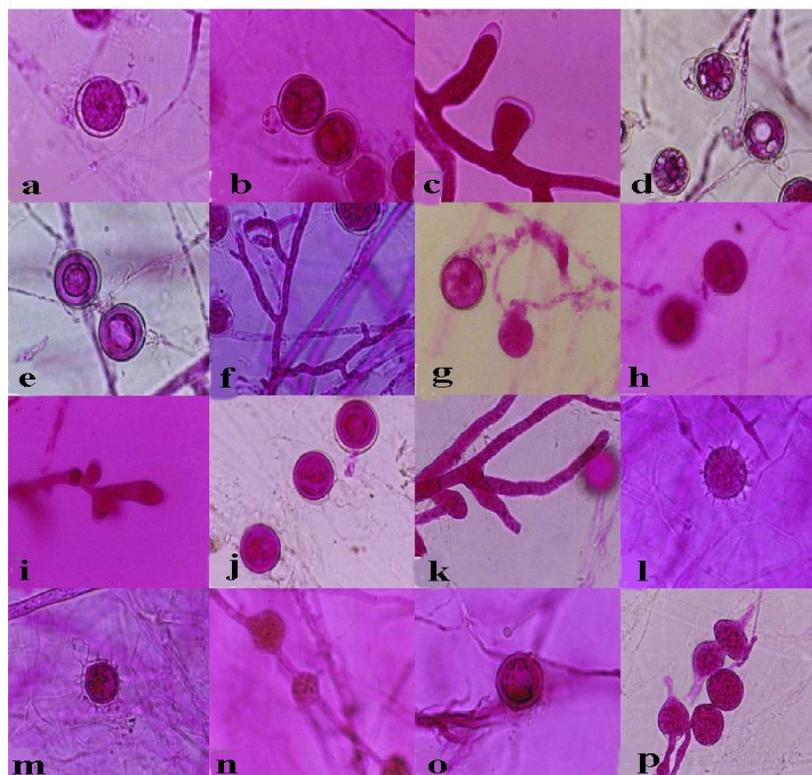


Fig. 1. Sexual and asexual organs of *Pythium* species isolated in Fars province of Iran. a-c: *P. aphanidermatum*, a: oogonium and antheridium, b: oospore, c: sporangium; d-f: *P. aquatile*, d: oogonium and antheridium, e: oospore, f: sporangium; g-i: *P. deliense*, g: oogonium and antheridium, h: oospore, i: sporangium; j-k: *P. diclinum*, j: oogonium and antheridium, k: sporangium; l-n: *P. echinulatum*, l: oogonium, m: oospore and antheridium, n: sporangium; o-p: *P. inflatum*, o: oospore, p: sporangium (1050X)

7. *P. okanoganense* Lipps: isolates produced submerged colonies with indistinct patterns on CMA, PCA and HAS; main hyphae 10 μm wide; sporangia globose to pyriform, terminal sometimes intercalary, infrequently proliferating, 30 μm wide; oogonia (sub)globose, smooth, terminal, 24 μm diameter; antheridia 1-2 per oogonium, paragynous, monoclinous sometimes diclinous, 5 μm wide; oospores aplerotic, 20-22 μm diameter, wall 2 μm thick (Fig. 2, a-b). Daily growth rate on PCA 23 mm at 25°C.

8. *P. oligandrum* Drechsler: isolates produced colonies with indistinct patterns on CMA and HAS, and colonies with indistinct to radiate patterns on PCA; main hyphae 3 μm wide; sporangia contiguous, forming irregular aggregates consisting of one or more subglobose elements with connecting filamentous parts, mostly intercalary, average 23 μm wide; oogonia globose, terminal sometimes intercalary, 27 μm diameter, provided with conical pointed protuberances, protuberances 4 μm long, 2 μm diameter at bases; antheridia 1-2 per oogonium, paragynous, monoclinous or diclinous, 7 μm wide; oospores aplerotic, 20-23 μm diameter, wall 1 μm thick (Fig. 2, c-d). Daily growth rate on PCA 24 mm at 25°C.

9. *P. orthogonon* Ahrens: isolates produced submerged colonies with indistinct patterns on CMA, and indistinct patterns on CMA, PCA and HAS; main hyphae 5 μm wide, sporangia globose to pyriform, 20-25 μm diameter; oogonia globose, smooth, terminal, 20 μm diameter; antheridia 1 per oogonium, paragynous, monoclinous, often crook-necked, 8 μm wide; oospores aplerotic, 17-20 μm diameter, wall 3 μm thick (Fig. 2, e-g). Daily growth rate on PCA 8 mm at 25°C.

10. *P. ostracodes* Drechsler: isolates produced aerial mycelium on CMA, HAS, and PCA, colonies with a chrysanthemum pattern on CMA, and submerged colonies on PCA and HAS; main hyphae 5 μm wide, sporangia lemoniform to subglobose, proliferating, sometimes sympodial, 25-30 X 35-40 μm ; oogonia globose, smooth, terminal or intercalary, 18-22 μm diameter; antheridia 1 per oogonium, paragynous, monoclinous or diclinous; oospores aplerotic, 16-20 μm diameter, wall 5 μm thick (Fig. 2, h-j). Daily growth rate on PCA 7 mm at 25°C.

11. *P. rostratum* Butler: isolates produced submerged colonies with an indistinct pattern on CMA, colonies with a chrysanthemum pattern on PCA, and colonies with indistinct patterns on HAS; main hyphae 8 μm wide, sporangia globose to ellipsoidal, terminal sometimes intercalary, 16-29 μm diameter; oogonia globose, smooth, intercalary, 23-27 μm diameter; antheridia 1 per oogonium, paragynous, monoclinous sometimes diclinous, terminal or intercalary, 7 μm wide; oospores nearly aplerotic, 23-27 μm diameter, wall 3 μm thick (Fig. 2, k-m). Daily growth rate on PCA 8 mm at 25°C.

12. *P. vexans* de Bary: isolates produced submerged colonies with indistinct patterns on CMA, PCA, and HAS; main hyphae 5 μm wide, sporangia globose 12-25 μm or pyriform 30-20 X 10-15 μm , terminal sometimes intercalary, occasionally with proliferation; oogonia globose, smooth, terminal or intercalary, 14-19 μm diameter; antheridia 1 per oogonium, paragynous, monoclinous, 7 μm wide; oospores nearly aplerotic, 12.5-17.5 μm diameter, wall 2.5 μm thick (Fig. 2, n-p). Daily growth rate on PCA 12 mm at 25°C.

13. *Pythium* Group "G": isolates could not form oogonia in dual cultures and only produced (sub)globose sporangia without proliferation; variable in their morphological characters and temperature relationships.

14. *Pythium* Group “HS”: isolates could not form oogonia in dual cultures and neither sporangia; produce single hyphal swellings, less than 30µm diameter; variable in their morphological characters and temperature relationships.

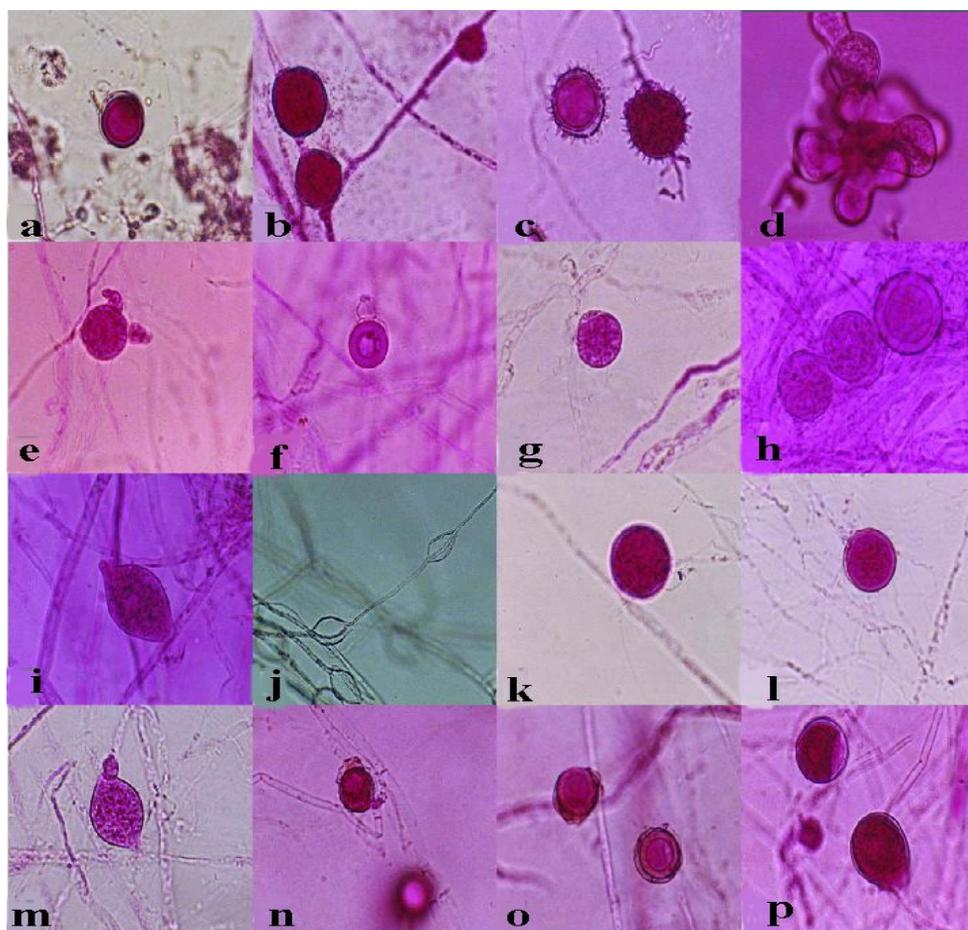


Fig. 2. Sexual and asexual organs of *Pythium* species isolated in Fars province of Iran. a-b: *P. okanoganense*, a: oospore and antheridium, b: sporangium; c-d *P. oliganderum*, c: oogonium and oospore, d: sporangium; e-g: *P. orthogonon*, e: oogonium and antheridium, f: oospore and antheridium, g: sporangium; h-j: *P. ostracodes*, h: oogonium and oospore, I: sporangium, j: proliferation of sporangia [417X]; k-m: *P. rostratum*, k: oogonium and antheridium, l: oospore, m: sporangium; n-p: *P. vexans*, n: oogonium and antheridium, o: oospore, p: sporangium (1050X)

4. DISCUSSION

It seems that variation among the location of origin is due to plant variety at the site, however, some species such as *P. aphanidermatum* and *Pythium* Group “HS” were found in a wide range of habitats.

In the case of *P. aphanidermatum*, *P. deliense*, *P. diclinum*, *P. echinulatum*, *P. oligandrum*, *P. orthogonon*, and *P. vexans*, morphological characters of identified isolates from Fars province did not show any major disagreements with species characterized in van der Plaats monograph [5], and even with those species recorded and characterized as isolates from Iran [7, 12, 14, 15]. Nevertheless in contrast with *P. deliense* isolates reported earlier from sugarbeets in the province, the oogonium and

oospore of the isolates in the present study were 4 and 2 μm smaller, respectively [7]; in addition, average sporangial length of Fars isolates of *P. vexans* were 12 μm more than isolates previously recorded from Iran [12].

The average oospores wall thickness in *P. aquatile* isolates was 7 μm compared to 2-3 μm in the descriptions of van der Plaats-Niterink [5].

Both *P. inflatum* and *P. rostratum* have been recorded to have monoclinal antheridia [5], while in Fars isolates beside monoclinal antheridia diclinal ones were also observed, and oospores of *P. inflatum* were to some extent aplerotic.

In Fars, isolates of *P. okanoganense* intercalary sporangia were observed compared to terminal sporangia which were recorded previously for this species [5].

The average diameter of oogonium of Fars isolates of *P. ostracodes* was 20 μm , while it was 30 μm in isolates recorded in van der Plaats-Niterink [5].

Isolates of *Pythium* Group "G" are incongruous in many respects, and each isolate was placed in this group based only on its inability for sexual reproduction and globose sporangium production. It requires more morphological and physiological characterization for the precise grouping of the isolates of this group.

Since there were no mating types, most of the isolates which were incapable of sexual reproduction and did not produce sporangia, were placed in *Pythium* Group "HS". It is probable that some of these isolates may belong to species such as *P. heterothallicum* and *P. sylvaticum* which needs further studies.

P. aquatile, *P. diclinum*, *P. echinulatum*, *P. inflatum*, *P. okanoganense*, *P. oliganderum*, *P. orthogonon*, *P. ostracodes*, *P. rostratum*, and *P. vexans* are a new record for Fars province [7, 9, 12, 5], and *P. aquatile*, *P. echinulatum*, *P. inflatum*, *P. okanoganense*, *P. orthogonon*, *P. ostracodes*, and *P. rostratum* are new for Iran flora [1, 7-20, 27]; and this is the first record from soil of isolating *P. deliense*, *P. diclinum*, *P. okanoganense*, *P. orthogonon*, and *P. ostracodes* from soil [5].

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