

## Identification and Pathogenicity of *Pythium* Species on Cantaloupe in Khorasan Razavi Province of IRAN

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**Abstract:** In order to study the role of *Pythium* species associated with cantaloupe root and crown rot, samples were collected from infected fields in different areas of Khorasan Razavi province during 2009-2010. The Root pieces were washed and cultured on CMA-PARP medium. The *pythium* isolates were then purified by hyphal tip method and identified based on van der Plaats-Niterink mycological key. The pathogenic species were identified as *Pythium aphanidermatum*, *P. ultimum* var. *ultimum*, and *P. deliense*. The pathogenicity of isolates on cantaloupe seedlings was tested under greenhouse conditions by using wheat grain inoculum. The results indicated that *P. aphanidermatum* was the most prevalent species and was recovered in all the regions. *P. ultimum* was isolated from Neyshabour, Fariman and Mashhad whereas *P. deliense* was detected in Khaaf. This to our knowledge, is the first report on occurrence and distribution of *Pythium* species causing root and stem rot on cantaloupes in Khorasan province.

**Keywords:** *Pythium* species, Root rot, Cantaloupes, Iran.

### Introduction

Cantaloupe (*Cucumis melo* L.) is grown in many parts of Iran. Khorasan Razavi province is the major area with more than 2000 hectares under cultivation of this crop. Damping-off and root rot caused by *Pythium* is considered as one of the devastating diseases of melon crops in Iran (Askari *et al.*, 2010). The causing pathogens affect nearly every crop grown in many parts of the world (Ben Yephet and Nelson 1999). In some circumstances, pathogenic species have been isolated from healthy looking roots, where their colonization causes a reduction in the plant growth without typical root rot symptoms (Martin and Loper 1999). The genus *Pythium* was established by Pringsheim in 1858 and at the

present time, more than 200 species have been described worldwide (Abdelghani *et al.*, 2004) but only 120 species have been given valid names and the rest have been placed in five groups known as F, T, G, P and HS groups (Tambong *et al.*, 2006).

The genus *Pythium* belongs to the class Oomycetes which have been separated from the true fungi and placed within kingdom Chromista. These microorganisms have aseptate mycelium and both molecular and biochemical studies suggest that they are closer to algae than to true fungi, but they infect plants in similar ways as do the true fungi (Alhussien *et al.*, 2011). *P. aphanidermatum* Edson (Fitzp.) can cause diseases on many plants such as root rot and damping off, stalk and rhizome rot, soft rot, fruit rot and cottony blight (Al-Sheikh 2010). This pathogen has been reported as a causal agent of the melon root rot and crown rot in Khorasan province of Iran (Jahanbakhsh 1998). *Pythium* species are common inhabitants of cultivated soils and *P. ultimum* Trow, *Pythium* Group HS

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and *P. oligandrum* Dreschsler have been isolated from vegetable crops in Khorasan Razavi province (Askari *et al.*, 2010). A sudden wilt of melons in California has been attributed to *P. ultimum*, *P. aphanidermatum* and *P. myriotylum* (Aegerter *et al.*, 2000). Root and crown rot of melons in Honduras have also been associated with *P. aphanidermatum* (De Cara *et al.*, 2008). In Oman *P. splendens* has been known as the causal agent of severe wilt of muskmelon (Al-Sadi *et al.*, 2008). *Pythium* spp. cause both pre- and post-emergence damping off, typically under wet soil conditions when zoospores can migrate to the plant surface (Scott *et al.*, 2005). Earlier studies in Hamedan province isolated and identified *P. pyriforme*, *P. perplexum*, *P. macrosporum* and *P. hydnosporum* in Iran (Abad *et al.*, 2011).

However, the pathogenicity of these species and other *Pythium* spp. associated with cultivated cantaloupes and melons in Iran and their impact on plant health are not well known. The objectives of this study were to isolate, and identify the *Pythium* fungi causing root and crown rot diseases of cantaloupes and to determine their distribution and pathogenicity in the east of Iran (Khorasan province).

## Materials and Methods

### Sampling and isolation of fungi

In order to obtain isolates of *Pythium* spp., sampling was carried out from cantaloupe fields in Khorasan Razavi province during 2009-2010. The sampling area included: Torbat jam, Khaaf, Kashmar, Neyshabour, Fariman, Mahvelat and Mashhad. Infected plants were collected on the basis of symptoms such as damping-off, yellowing and wilting and were carried to the laboratory. In the laboratory roots were washed under running tap water, cut into 1-cm segments, rinsed in sterile deionized water, crushed and blotted dry in sterile paper towels, and placed onto agar plates containing PARP medium (extract of 60 g ground maize, pimaricin 0.01 g, ampicillin 0.25 g, rifampicin 0.01 g, PCNB 0.1 g, agar 15 g, distilled water 1l) according to Jeffers and Martin (1968) procedure and incubated at 25 °C in dark.

Fungal colonies recovered after 36 hours were plated on water agar (agar 15 g, distilled water 1l) and purified with the hyphal tip method. Colony morphology was studied on CMA (extract of 60 g ground maize, agar 15 g, distilled water 1l) and PCA (extract of 20 g carrots and 20 g potatoes, agar 15g, distilled water 1l) at 25 °C according to Tuite (1969) procedure. Growth rate was measured on CMA at 5, 15, 20, 25, 30, 35, 40 and 45 °C. To induce the formation of sporangia, 5 mm pieces of autoclaved grass leaf (*Poa annua*) were placed on PCA plates of *pythium* 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. The sexual organs were studied on HSA (extract of 20 g ground hemp seeds, agar 15 g, distilled water 1l). Identification of *Pythium* strains were based on the keys of van der Plaats-Niterink (1981) and Dick (1989).

### Pathogenicity of *Pythium* spp. isolated from cantaloupe plants

Isolates of *Pythium* spp. were evaluated for pathogenicity on cantaloupe. Infested wheat seed (hard red winter wheat) was used as the inoculum source. A mixture of 25 ml of deionized water and 20 g of wheat seed was allowed to soak for 24 h in each of two 250-ml flasks for every isolate. The flasks were then autoclaved twice during two consecutive days. Each flask was inoculated with five 5-mm disks from a 5-day-old culture grown on CMA. Flasks were incubated for 2 to 4 weeks in the dark at 25 °C and shaken periodically to ensure uniform growth of inoculum. The inoculum was added to the soil in pots containing 14-day-old cantaloupe seedlings under greenhouse condition (temp: 25 °C at day and 18 °C at night, photoperiod: 12 h light/ 12 h dark). Symptoms were recorded every day from first symptom appearance on the plants up to 14 days after inoculation. Symptoms on roots were rated as follows: 1 = no symptom, 2 = slight root rot and discoloration, 3 = moderate root rot, 4 = extensive root rot. Flasks inoculated with non-infested agar plugs acted as control inoculum source. Infected roots were plated on selective medium to verify the identification of the pathogen, as described previously (Abdelzaher 2003).

## Results and Discussion

A total of 85 *Pythium* isolates were recovered from the 35 fields in which three *Pythium* species were identified as, *P. aphanidermatum*, *P. ultimum* var. *ultimum* and *P. deliense*. The most prevalent *Pythium* species recovered was *P. aphanidermatum* (71.8 %) followed by *P. ultimum* (22.3 %) and *P. deliense* (5.9 %) respectively. Table 1 and figure 1 show the morphological characteristics of the three *Pythium* species isolated from cantaloupes in different locations in Khorasan Razavi province. The characteristics of the specimens agree with those described by van der Plaats-Niterink (1981), and Baptista *et al.*, (2004). Geographical origins of the isolates from cantaloupes are listed in Table 2.

On the basis of number of isolates collected from the different districts, the results indicated that *P. aphanidermatum* was the most widely distributed species in cantaloupe fields in Khorasan Razavi province and was recovered

from all samples. In this survey, *P. deliense* was detected only in Khaaf area (Table 2). *P. ultimum* was isolated from Neyshabour, Fariman and Mashhad.

### Cardinal temperature studies

The effect of minimum, optimum and maximum temperatures on three species of *Pythium* are listed in Table 3. *P. aphanidermatum* and *P. deliense* had the same daily growth rates on CMA, 30 mm at 25 °C. The cardinal temperatures of *P. aphanidermatum* were similar to those of *P. deliense* but van der Plaats-Niterink (1981) pointed out that optimum temperature for these species were 35-40 and 30 °C respectively. In Egypt, Abdelzaher studied many isolates of *P. aphanidermatum* and *P. deliense* and found that they had the same cardinal temperatures (Gherbawy *et al.*, 2005).

**Table 1** Morphological characteristics of *Pythium* species isolated from cantaloupe plants.

Characteristic	<i>P. aphanidermatum</i>	<i>P. ultimum</i>	<i>P. deliense</i>
<b>Sporangia</b>	Filamentous and inflated	Not formed	Inflated, filamentous
<b>Oogonia</b>	Terminal, globose, smooth 24.9 (22-27)	Terminal, sometimes intercalary, globose, smooth 21 (16-18) µm	Globose, smooth, terminal or intercalary, 20.3 (17-23) µm diameter; oogonial stalk bending towards the antheridium
<b>Antheridia</b>	Broadly sac-shaped, mostly intercalary, 9-14 µm wide	Terminal, one or rarely two per oogonium, sac-like, and mostly close monoclinal	Mostly 1 per oogonium, with a straight stalk or sessile, terminal or intercalary, paragynous, diclinous
<b>Oospore</b>	Aplerotic 20 (17-24) µm diam.	Aplerotic, globose 17.5 (10- 22) µm diam.	Aplerotic, 16.5 (13-18) µm diam.
<b>Colony on CMA</b>	Cottony aerial mycelium	Cottony aerial mycelium	Little loose aerial mycelium

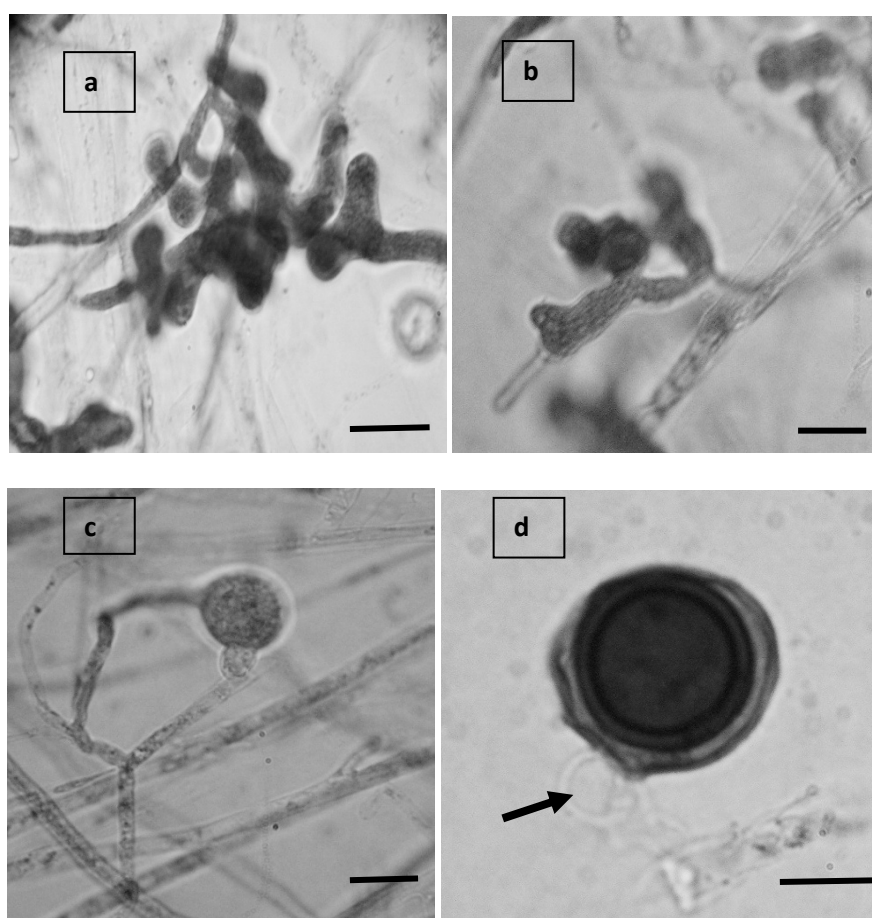
**Table 2** Geographical origin of the *Pythium* isolates from cantaloupe plants in Khorasan Razavi province.

species	Districts						
	Kashmar	Mahvelat	Fariman	Neyshabour	Torbatjam	Khaaf	Mashhad
<i>P. aphanidermatum</i>	15	12	6	6	5	10	7
<i>P. ultimum</i>	-	-	8	6	-	-	5
<i>P. deliense</i>	-	-	-	-	-	5	-
<b>Total</b>	15	12	14	12	5	15	12

**Table 3** Linear growth of *Pythium* spp. at various temperatures.

<i>Pythium</i> sp.	Average growth (mm) after 24 h on corn meal agar							
	5 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	45 °C
<i>P. aphanidermatum</i>	NG <sup>b</sup>	12 <sup>a</sup> ± 0.3 <sup>c</sup>	24 ± 0.5	30 ± 0.5	45 ± 0.8	49 ± 0.5	38 ± 0.6	3 ± 0.1
<i>P. deliense</i>	NG	10 ± 0.3	23 ± 0.5	30 ± 0.5	43 ± 0.6	46 ± 0.5	40 ± 0.5	3 ± 0.1
<i>P. ultimum</i>	2.5 ± 0.1	13 ± 0.3	20 ± 0.3	22 ± 0.5	28 ± 0.5	3 ± 0.0	NG	NG

<sup>a</sup> Mean of 5 replicates; <sup>b</sup> NG, no growth; <sup>c</sup> Standard error.



**Figure 1** Morphological characteristics of *Pythium* spp. a) Sporangia of *P. aphanidermatum*. b) Sporangia of *P. deliense*. c) Immature antheridium and oogonium of *P. deliense*. d) Oogonia with close monoclinus antheridium of *P. ultimum* var *ultimum*. (scale bar = 10µm).

*P. aphanidermatum* and *P. deliense* are common species and typical plant pathogens of warm regions (van der Plaats-Niterink 1981). The high frequency of isolated *P. aphanidermatum* in this study may be attributed to environmental conditions during cultivation

season in Razavi Khorasan that is suitable for this species. Rahimian and Banihashemi (1979) reported *P. aphanidermatum* as causal agent of cucurbit root rot in the Fars province of Iran for the first time.

Isolation of *P. ultimum* var. *ultimum* and *P. aphanidermatum* from soil and infected plants of vegetable crops have previously been reported in Razavi province (Askari et al., 2010). *Pythium deliense* has not been reported previously in Iran on cucurbits and differs from *P. aphanidermatum* by its oogonial stalks which curve towards the antheridia (Fig. 2) and smaller oogonia as declared by van der Plaats-Niterink (1981).

*P. ultimum* in contrast to *P. aphanidermatum* is active in cool to moderately warm environments. The optimum temperatures for mycelial growth on CMA medium of *P. aphanidermatum* and *P. ultimum* isolated from cantaloupe seedlings were 35 to 40 °C and 30 °C respectively. In previous studies, Kuo and Hsieh (1991) reported that the diseases caused by *P. aphanidermatum* were most serious during the summer when average daily temperatures reached 23-32 °C, whereas *P. ultimum* var. *ultimum* caused the greatest damage at cooler temperatures 12-22 °C. In addition, it is confirmed that *P. aphanidermatum* was the most predominant pathogen during the warm summer, with an air temperature higher than 24 °C, whereas *P. ultimum* played a more important role during the cool winter when air temperature was lower than 20 °C (Ho 2009).

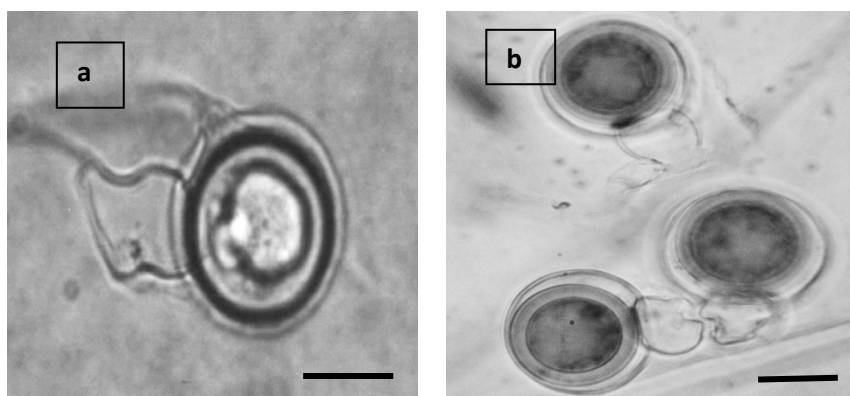
Tremendous economic loss due to the infection with *P. ultimum* has previously been reported (Riad et al., 2009). This pathogen causes damping-off and root rot diseases on many plants such as cucumber, tomato, melon, pumpkin, watermelon and muskmelon (Askari et al., 2010). Broders et al., (2009) reported that there was a strong association between abiotic soil components and the structure of *Pythium* communities, as well as the diversity of *Pythium* spp. collected from agronomic production fields in Ohio.

The three *Pythium* species were successfully recovered from the inoculated root and stem rot which indicated that all of the three species were able to cause root and stem rot of cantaloupes. Based on results of

pathogenicity tests, the isolates of *Pythium* varied in virulence. The most common symptoms on cantaloupe seedlings were discoloration and necrosis of root and crown tissues or loss of root (Fig. 3). *P. ultimum* var. *ultimum* was isolated from cantaloupe seedlings while *P. aphanidermatum* was recovered from both seedlings and mature cantaloupe plants. It was also observed that *P. deliense* was recovered from both seedling and fruiting stages (Table 4).

*P. aphanidermatum* and *P. deliense* have been reported to cause damping off and root rot on sugar beet in Khorasan province (Afzali and Ershad 2006). In Oman, *P. deliense* has been isolated from muskmelon and has been reported to cause sudden collapse of whole crop (Deadman et al., 2007).

The present study showed that *Pythium* populations are diverse in Khorasan Razavi province and could be potential inoculum source for infection of cucurbit crops. To effectively design *Pythium* disease management strategies, it is important to accurately identify the species, and to determine how many different species are present in the field and their relative abundance. This observation showed that *P. ultimum* var. *ultimum*, *P. aphanidermatum*, and *P. deliense* are capable of causing reductions in emergence and maturation of cantaloupes. Our results also indicate that *P. aphanidermatum* and *P. ultimum* var. *ultimum* may be two of the most important pathogens associated with cantaloupe in Khorasan Razavi and the most aggressive parasites under the given experimental conditions. However, the frequency at which these two species were recovered may have more to do with their ability to grow well under climatic conditions in Khorasan Razavi. Therefore, studying the occurrence of *Pythia* in cultivated fields is important for reducing their damage on cucurbits in the area.



**Figure 2** Antheridium and oogonium: a) *Pythium deliense* b) *P. aphanidermatum* (scale bar = 10 µm).



**Figure 3** Ten-day-old cantaloupe seedlings inoculated with *P. aphanidermatum*.

**Table 4** Grouping of isolates in pathogenicity test.

Isolate <sup>a</sup>	Symptom <sup>b</sup>	Time of isolation	Region
Pa <sub>1</sub>	3	Fruit set	Mashhad
Pa <sub>2</sub>	4	Flowering stages	Kashmar
Pa <sub>3</sub>	3	Seedling stages	Neyshabour
Pa <sub>4</sub>	4	Fruit set	Mahvelat
Pa <sub>5</sub>	3	Flowering stages	Torbat jam
Pu <sub>1</sub>	3	Seedling stages	Neyshabour
Pu <sub>2</sub>	3	Seedling stages	Mashhad
Pu <sub>3</sub>	4	Seedling stages	Neyshabour
Pu <sub>4</sub>	3	Seedling stages	Mashhad
Pu <sub>5</sub>	3	Seedling stages	Fariman
Pd <sub>1</sub>	4	Seedling stages	Khaaf
Pd <sub>2</sub>	3	Flowering stages	Khaaf

<sup>a</sup> Isolate naming is on the basis of the first letter of the name of fungus.

<sup>b</sup> Symptom of diseases: 1 = no symptom, 2 = slight root rot and discoloration, 3 = moderate root rot, 4 = extensive root rot.

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## شناسایی و بیماری‌زایی گونه‌های پی‌تیوم روی طالبی در استان خراسان رضوی

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**چکیده:** به‌منظور شناسایی گونه‌های پی‌تیوم مولد پوسیدگی ریشه و طوقه طالبی در استان خراسان رضوی در سال‌های زراعی ۱۳۸۸-۸۹ از گیاهان بیمار نمونه‌برداری صورت گرفت. قطعاتی از حاشیه بافت سالم و آلوده پس از شستشوی سطحی با آب معمولی و خشک کردن بدون ضدعفونی سطحی روی محیط کشت CMA-PARP کشت گردیدند. خالص‌سازی جدایه‌ها به روش تک‌ریسه انجام شد و شناسایی براساس کلید فارچ‌شناسی van der Plaats -Niterink صورت گرفت. گونه‌های بیماری‌زای شناسایی شده شامل *Pythium aphanidermatum*، *P. ultimum* var. *ultimum* و *P. deliense* بودند. بیماری‌زایی جدایه‌ها روی طالبی در شرایط گلخانه و با استفاده از مایه پی‌تیوم روی بذر گندم در مرحله گیاهچه‌ای طالبی به اثبات رسید. نتایج نشان می‌دهد که *P. aphanidermatum* بیشترین پراکنش در سطح مزارع استان را دارا بوده و از همه مناطق جداسازی گردید. *P. ultimum* از نیشابور، فریمان و مشهد جداسازی شد. *P. deliense* در خواف ردیابی شد و این‌گونه قبلاً در ایران از روی صیفی‌جات گزارش نشده بود. براساس اطلاعات ما، این تحقیق اولین گزارش از شناسایی و پراکنش گونه‌های پی-تیوم مولد پوسیدگی ریشه و طوقه طالبی در ایران است.

**واژگان کلیدی:** گونه‌های پی‌تیوم، پوسیدگی ریشه، طالبی، ایران