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ETIOLOGY AND CONTROL OF DAMPING-OFF AND ROOT ROT
OF PEA (PISUM SATIVUM) IN IRAN

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Abstract

Pythium aphanidermatum is the cause of a serious damping-off and root rot of pea (Pisum sativum) in Southwestern Iran. High temperatures and excessive soil moisture favor disease development. Isolates from various food legumes (pulses) and different localities in Iran were highly pathogenic to pea and other pulse crops in greenhouse inoculation studies. In field soils heavily infested with P. aphanidermatum, emergence and yields of pea were increased significantly by treating seeds with certain fungicides.

Pythium spp. are important pathogens of many cultivated plants in Iran (1, 3, 4, 5). Different species of Pythium, including P. aphanidermatum (Edson) Fitzp. and P. ultimum Trow, were isolated from the necrotic roots or rotted seeds of several food legumes (pulses) in Iran (3, 4), the seeds of which are an important component in the daily diets of the people. These crops are: bean (Phaseolus vulgaris), broadbean (Vicia faba), chickpea (Cicer arietinum), cowpea (Vigna sinensis), lentil (Lens esculenta), mungbean (Phaseolus aureus), and pea (Pisum sativum).

Peas are a new cash crop in Southwestern Iran (Khuzestan Province) where the incidence and severity of pea diseases are greatly affected by the date of sowing (Kaiser, unpublished data). Diseases caused by bean yellow mosaic, cucumber mosaic, and pea leaf roll viruses were predominant in plantings made in late winter (3, 4). Plantings established in late summer or early fall, however, were relatively free from virus diseases, but were heavily damaged by damping-off and root rot organisms. In September 1969, emergence of several pea varieties in plantings at the Safiabad Experiment Station, Khuzestan Water and Power Authority, Dezful was poor and erratic. Pythium was consistently isolated from seeds and roots of diseased seedlings. This study reports on: (i) the relationship of Pythium to the etiology of the disease; (ii) pathogenicity of different isolates of the pathogen; and (iii) methods for controlling the disorder.

MATERIALS AND METHODS

Fungi were isolated by plating surface-sterilized (0.5% sodium hypochlorite for 5 minutes) pieces of seeds and roots on either water agar (WA), potato-dextrose agar (PDA), or acidified potato-dextrose agar, pH 4.0-4.5 (APDA) at 25°C. Pure cultures of all recovered microorganisms were maintained on PDA slants. Fungus isolates used in pathogenicity tests were grown on PDA or in a sterile mixture of cornmeal-sand (CS) (5%-95% v/v). Fungus inoculum, consisting of two coarsely macerated PDA slants or 330 cc of CS, was incorporated in the top layer of pasteurized soil contained in each 20-cm (8-inch) clay pot. Seeds were planted on the surface of the infested soil and then covered with a 1-cm layer of pasteurized soil. Controls were identical except that sterile PDA or sterile CS was added to pasteurized soil.

Soil was collected from pea fields at the Safiabad Experiment Station, which had a high incidence of damping-off and root rot. The soil in these areas was a clay loam with an alkaline soil reaction (pH 7.7-8.0). Pasteurized and nonpasteurized field soil was planted to nontreated seeds of different pulse crops in the greenhouse where temperatures varied from 12-28°C.

Nontreated, weighed samples of certified 'Rondo' pea seed were first sprayed with water mist from an aerosol spray bomb. While the seeds were still moist, fungicides were added separately to seed samples in plastic bags and shaken vigorously for 5 minutes before spreading to dry. Seeds were packaged in paper envelopes, stored in the dark at room temperature, and planted in the Field at Safiabad 7 to 10 days after treatment. Weather data for the period preceding and during our field trials at Safiabad are presented (Table 1). Seeds were treated with commercial wettable powder formulations of the following fungicides: 75% bis(dimethylthiocarbamoyl) disulfide (thiram); 65% 1,4-dichloro-2,5-dimethoxybenzene (chloroneb); 65% methyl 1-(butylcarbomyl)-2-benzimidazolecarbomate (benomyl); 70% sodium-p-(dimethyl-

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amino)benzenediazosulfonate (Dex.); a mixture of 35% sodium-p-(dimethylamino)benzenediazosulfonate and 35% pentachloronitrobenzene (PCNB)(Dex. -PCNB); 75% 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (carboxin); 75% 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide (oxycarboxin); 60% 2-(4-thiazolyl)-benzimidazole (thiabendazole); 75% pentachloronitrobenzene (PCNB); and 75% N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide (captan).

Isolates of fungi recovered from pea and other pulse crops were included in the pathogenicity tests: Fusarium oxysporum Schlecht., pea; F. solani (Mart.) Appel & Wr., pea; Rhizoctonia solani Kuehn, pea; Pythium aphanidermatum, pea; P. aphanidermatum, lentil; P. aphanidermatum, lentil; P. aphanidermatum, mungbean; and P. ultimum, lentil.

RESULTS

Disease symptoms and fungus isolations: Pea seeds that had damped-off prior to emergence were discolored and rotted. At times, decayed seeds were covered with a white mycelial growth. Diseased seedlings were usually stunted and dull green to yellow in color. The root system of infected plants was usually poorly developed, with numerous dark, necrotic lesions forming on the primary and secondary roots (Fig. 1). A Pythium sp., subsequently identified as P. aphanidermatum, was consistently isolated from discolored, necrotic and rotted pea seeds and roots. Fusarium oxysporum, F. solani, and Rhizoctonia solani were occasionally recovered from necrotic pea tissues.

Greenhouse studies: In greenhouse studies using nonpasteurized field soil from heavily diseased pea plantings, emergence of 'No. 40', 'Progress', and 'Rondo' pea was 20% or less. Most seeds that failed to germinate were discolored and mushy. Surviving seedlings remained stunted and dull green in color; however, in pasteurized field soil emergence of these pea varieties exceeded 75% (Table 2, Fig. 2). Emergence of chickpea, cowpea, and mungbean was reduced 14-33% in nonpasteurized field soil, but that of bean, broadbean and lentil was unaffected (Table 2, Fig. 2). P. aphanidermatum was isolated most frequently from seeds and roots of diseased plants in nonpasteurized field soil, but fungi were seldom isolated from nongerminated seeds in the pasteurized series.

Pathogenicity studies: Pathogenicity tests were carried out in the greenhouse with Rondo pea and isolates of P. aphanidermatum, F. oxysporum, F. solani and R. solani from pea, P. aphanidermatum from lentil and mungbean, and P. ultimum from lentil (Table 3). All isolates of P. aphanidermatum and P. ultimum were highly pathogenic to pea; and 75-100% of the seedlings were attacked prior to emergence. Roots of most surviving seedlings were discolored and necrotic (Fig. 1) while the plants remained stunted and pale green in color (Fig. 3). In repeated tests, F. oxysporum, F. solani, and R. solani were nonpathogenic to pea (Table 3).

Isolates of both Pythium spp. were reisolated from the seeds and roots of diseased peas. Symptoms of the disease reproduced in the greenhouse inoculation tests with isolates of P. aphanidermatum were similar to those observed in the field (Figs. 1 and 3). There was no apparent difference in the pathogenicity of P. aphanidermatum or P. ultimum to pea when soil was infested with PDA or CS inoculum of each isolate.

Pythium aphanidermatum reduced emergence of cowpea and mungbean and was pathogenic to the roots of surviving seedlings, but did not reduce the emergence of bean, broadbean, chickpea, or lentil (Table 2). This fungus could occasionally be isolated from the roots of these plants when growing in soil heavily infested with the pathogen.

Control of the disease in the field by fungicide seed treatment: Rondo pea seed was treated with 10 fungicides individually, including four systemics, and planted in replicated trials in a pea field in Southwestern Iran with a history of pea damping-off and root rot. Seedling survival in the nontreated control was reduced by 90%, 75 days after planting (Table 4). Seeds treated with captan and thiram produced the highest stand counts of 63 and 53%, respectively, and the largest seed yields. There was little or no control of the disease by several systemic fungicides included in these trials.

DISCUSSION

Pythium aphanidermatum, an important and serious pathogen of pea in Iran, could become a limiting factor in the cultivation of this crop in Southwestern Iran. Isolates of this fungus from pulse crops grown in different areas of Iran were all highly pathogenic to peas and other edible legumes in greenhouse studies. P. ultimum, a common and widely distributed root-rot pathogen of pulses in Iran, was also found to be pathogenic to peas, although it has not been isolated from peas in Khuzestan.

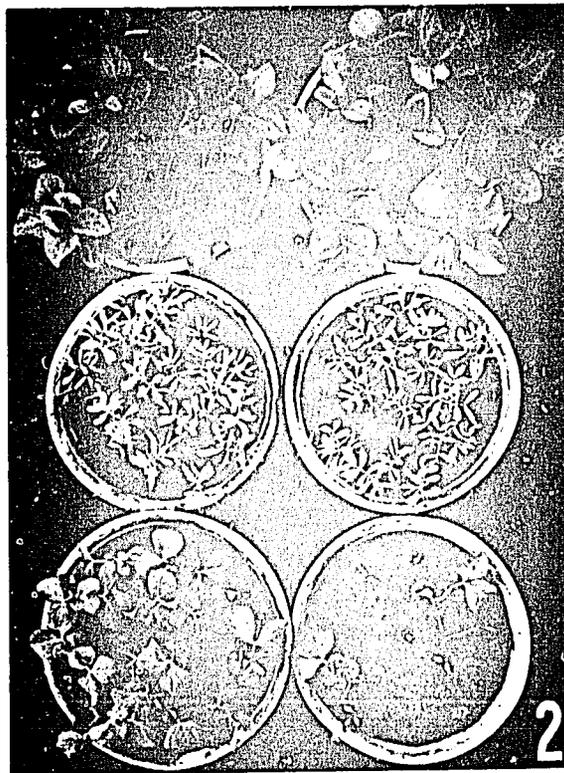
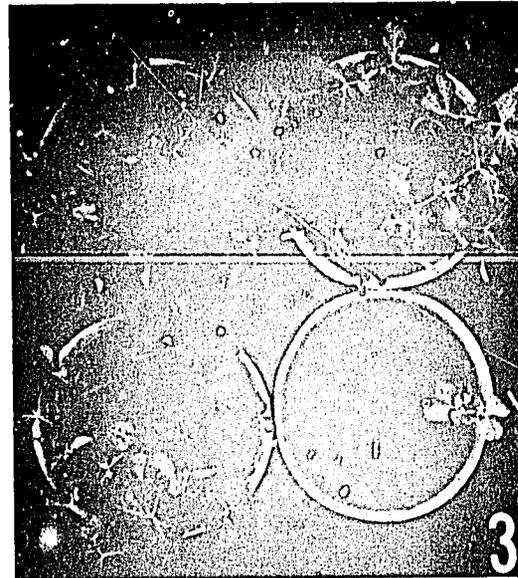
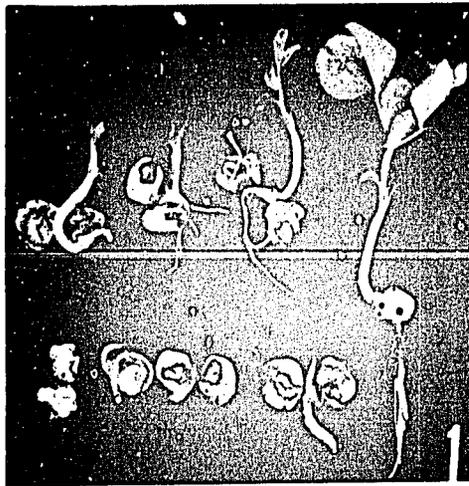


FIGURE 1. Symptoms of rotting, discoloration, and necrosis in seeds and roots of 'Rondo' pea from soil infested with *Pythium aphanidermatum*.

FIGURE 2. Emergence of broad-bean (top row), lentil (middle row), and pea (bottom row) in field soil (from a pea planting in Southwestern Iran with a history of root rot) which had been pasteurized (left column) and non-pasteurized (right column).

FIGURE 3. Pathogenicity test with three fungi isolated from the roots or seed of diseased peas in Southwestern Iran. The inoculum of each fungus consisting of macerated potato-dextrose agar (PDA) was incorporated in pasteurized greenhouse soil. Pots were planted with 15 'Rondo' pea seed. Treatments were: Control (sterile PDA), upper left; *Rhizoctonia solani*, upper right; *Pythium aphanidermatum*, lower right; and *Fusarium solani*, lower left.

Peas were planted at Safiabad in September and October 1969 when average maximum and minimum air temperatures ranged from 18.0-41.1°C. High temperatures accompanied by excessive soil moisture, probably resulting from over-irrigation of the clay loam soils in this area, appear to have been important factors contributing to the disease epiphytotic caused by *P. aphanidermatum*. Optimum temperature for mycelial growth on PDA of pea isolates of the pathogen from Safiabad was 35°. High soil temperatures and excessive moisture also were reported by Hine and Ruppel (2) to favor root rot of sugarbeet by *P. aphanidermatum* in Arizona.

Observations which were made on the reaction of over 50 pea cultivars to natural infection by *P. aphanidermatum* in Southwestern Iran indicated that most varieties had little resistance to the pathogen in soils with a high inoculum level. In the absence of disease, however, several varieties appeared to be well adapted to the soils and environmental conditions of Khuzestan

Table 1. Average monthly maximum and minimum air temperatures and total monthly rainfall at the Safiabad Experiment Station, Dezful, Iran from January 1969 to January 1970.

Month	Temperature °C		Rainfall (mm)
	Maximum	Minimum	
January 1969	17.1	6.5	158.3
February	18.4	6.7	28.0
March	25.1	12.6	54.5
April	27.2	13.2	71.0
May	37.2	18.8	24.5
June	43.1	21.4	0
July	45.1	23.0	0
August	44.2	22.9	0
September	41.1	19.8	0
October	35.8	18.0	8.0
November	24.6	9.3	59.5
December	20.9	7.5	25.0
January 1970	16.2	6.4	91.5

Table 2. Emergence of different food legumes in pasteurized or nonpasteurized field soil from pea plantings in Southwestern Iran, and in greenhouse soil infested with a cornmeal-sand inoculum of *Pythium aphanidermatum*.

Test plant	Field soil									Infested greenhouse soil		
	Pasteurized			Nonpasteurized			Emergence ^a			Emergence ^a		
	Emergence ^a			Emergence ^a			Emergence ^a			Emergence ^a		
	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%
Bean 'Contender'	50	39	78.0	75	58	77.3	75	57	76.0			
Broadbean 'Algerian'	45	41	91.1	60	58	96.6	75	75	100			
Chickpea 'Ghazvin'	50	49	98.0	75	63	84.0	75	70	93.3			
Cowpea 'Early Ramshorn'	50	47	94.0	75	49	65.3	75	33	44.0			
Lentil 'Ghazvin'	50	43	86.0	50	45	90.0	75	65	86.7			
Mungbean 'Berken'	50	50	100	75	64	85.3	75	47	62.7			
Pea 'No. 40'	45	34	75.5	60	6	10.0	45	3	6.7			
Pea 'Progress'	45	39	86.6	60	12	20.0	45	2	4.4			
Pea 'Rondo'	45	41	91.1	60	5	8.3	125	2	1.6			

^aEmergence in pasteurized and natural field soil, and infested greenhouse soil, was recorded 14 and 21 days after sowing, respectively.

Province, which are similar to those of the Imperial Valley of California. Before peas can be grown in *Pythium*-infested soils of this region it will be necessary to develop economical control measures such as fungicide seed treatments. Preliminary results indicate that pea stands and seed yields can be increased 4.3 to 5.3 times in heavily infested field soils by treating seeds with either thiram or captan, respectively.

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Table 3. Pathogenicity of eight fungus isolates from different pulse crops grown in Iran to pea (*Pisum sativum* 'Rondo') in greenhouse inoculation tests^a.

Fungi	Host	Location	No. seeds planted	Emergence ^b	
				No. Seedlings	%
<i>Fusarium oxysporum</i>	Pea	Dezful	45	41	91.1
<i>F. solani</i>	Pea	Dezful	45	44	97.8
<i>Rhizoctonia solani</i>	Pea	Dezful	45	39	86.6
<i>Pythium aphanidermatum</i>	Pea	Dezful	45	1	2.2
<i>P. aphanidermatum</i>	Lentil	Dezful	45	0	0
<i>P. aphanidermatum</i>	Lentil	Shiraz	45	0	0
<i>P. aphanidermatum</i>	Mungbean	Dezful	45	11	24.4
<i>P. ultimum</i>	Lentil	Karaj	45	0	0
Control (noninoculated)	--	--	45	42	93.3

^aPasteurized greenhouse soil was infested with fungus inoculum in the form of macerated potato-dextrose agar. ^bEmergence was recorded 19 days after planting.

Table 4. Effect of seed treatment fungicides on control of damping-off and root rot of pea (*Pisum sativum*) in field trials at Dezful (Khuzestan Province), Iran.

Fungicide	Dosage (g/kg)	Mean % Stand ^a		Average Yield ^c (g)
		22 Days after planting	75	
captan 75 W	2.5	69	63 ab	508
thiram 75 W	1.9	65	53 ab	557
Dex. 70 W	1.2	52	41 bc	405
Dex. -PCNB 35-35 W	2.5	43	37 c	308
carboxin 75 W	3.7	41	34 cd	368
chloroneb 65 W	3.7	29	22 de	219
oxycarboxin 75 W	3.7	16	14 ef	171
thiabendazole 60 W	3.7	18	12 ef	169
Control (nontreated)	---	11	10 ef	156
PCNB 75 W	1.9	5	5 f	63
benomyl 65 W	4.0	7	4 f	83

^aThe trial was planted October 1, 1969. Mean stand counts based on four replications. ^bThe small letters indicate Duncan's multiple range groupings of treatments which do not differ significantly at the 1% level. ^cPlots were harvested in January 1970 from four, 4-row plots, 4 m long.

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