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PARASITISM OF *HETERODERA CAJANI* BY *FUSARIUM SOLANI* AND OTEHR SOIL FUNGI

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Abstract : *Heterodera cajani* is an important nematode pest of pegeonpea (*Cajanus cajan* (L.) Millsp.) in India. Forty-one *H.cajani* infested pegeonpea fields and *H.cajani* cultures maintained on pigeonpea at the ICRISAT Asia Center, Andhra Pradesh, were examined for presence of natural parasites of the nematode. The fungi *Cephalosporium* spp., *Fusarium solani*, *Fusarium* spp., *Glomus* spp., and gram positive bacterium, *Pasteuria penetrans* were found in cysts. *Fusarium solani* was the most commonly occurring fungus in eggs of *H. cajani*. On water agar, *F. solani* infected 70% of eggs in egg sacs. The numbers of eggs were reduced by 45-60% in presence of the fungus in greenhouse tests. Growth of pigeonpea cultivar ICPL 87 in *H. cajani* infested soil was improved by the presence of *F. solani*. The nematode reproduced in greater numbers in infested soil treated with 0.2% benomyl than in soil naturally infested with the nematode and the fungus but not treated with benomyl. The *F. solani* isolates recovered from *H. cajani* did not infect pigeonpea plants and were effective in suppressing *H. cajani* under natural conditions.

Key words : Biological control, cyst nematode, egg parasite, pigeonpea, soil fungi.

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most important legumes of subsistence farming systems in the semi-arid tropics. The cyst nematode, *Heterodera cajani* Koshy is an important pest of pigeonpea. It is widely distributed in the major pigeonpea producing states of India and in parts of Egypt (Sharma *et al.*, 1992). Nematode infection reduces foliage production and grain yield of pigeonpea. At sowing, population densities of 2-3 eggs and juveniles of *H. cajani* per cm^3 of soil may cause 25-30% reduction in plant biomass and seed yield (Saxena & Reddy, 1987; Sharma *et al.*, 1993). Resistant cultivars for the management of this nematode have not been developed and current options of chemical control are too expensive to be practical in subsistence agriculture.

Continuously monocropped systems have led to suppression of cyst nematodes by naturally-occurring antagonists in temperate agriculture (Kerry *et al.*, 1982). Many fungi such as *Allomyces anomalus, Catenaria auxiliaris, C. vermicola, Nematophthora* spp., *Olpidium* spp. and *Pythium* spp. have been found to be parasites of *H. cajani* (Sharma & Swarup, 1988). It is evident that management of naturally occurring antagonists for biological control of nematodes in subsistence agriculture is practical and environment friendly approach (Sikora, 1992). As egg parasites are considered to be more likely candidates for biological control than are fungi isolated from cyst (Jatala, 1986), the objective of the study, was to identify fungi parasitic on eggs of *H. cajani* in pigeonpea fields, and to

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evaluate the effect of the most frequently encountered fungi on nematode population density and the growth of pigeonpea.

MATERIALS AND METHODS

Isolation of fungi from cysts and eggs

Soil samples were collected from 33 pigeonpea fields on Vertisol (silty clay loam) and eight fields on Alfisol (sandy clay loam) at the ICRISAT, Patancheru, Andhara Pradesh, (Table 1). The cysts of *H. cajani* were extracted from these soil samples as also from pigeonpea culture pots. They were treated with 1% sodium hypochlorite for one minute, washed two times in sterile distilled water, and placed aseptically in petri dishes containing potato dextrose agar (PDA) or cornmeal agar (CMA). Both PDA and CMA media were amended with 0.05% dicrysticin. Eggs were released from cysts with sterile forceps onto the media and the petri dishes were incubated at 25 C. After two weeks of incubation, the fungi that had grown from the eggs were transferred to sterile agar to isolate the fungi. Axenic cultures of fungi were obtained by single spore isolations (Johnston & Booth, 1983.)

Bioassay tests

The isolates of *Fusarium solani* recovered from eggs collected from fields and greenhouse cultures of *H. cajani* were tested for their pathogenicity on *H. cajani* eggs on water agar plates in the laboratory and in pots in a greenhouse.

Laboratory tests- Egg sacs of *H. cajani* were extracted by processing the infested pigeonpea roots through 180 µm-pore size sieves. The eggs sacs were treated with 1% sodium hypochlorite solution for one minute and wahsed two times with sterile distilled water. The sterile eggs sacs were dipped in spore-hyphal suspension of fungi for two minutes and then transferred to 1.5% water agar plates and were incubated at 25C for two weeks. Egg sacs dipped in sterile distilled water before placing them on water agar served as a control. Each treatment was replicated five times. The incubated eggs sacs were placed in a drop of 0.1% cotton blue lactophenol and the eggs were observed for fungal infection. The eggs with fungal hyphae and /or spores were recorded as infected. Bioassay results were confirmed in repeat experiments.

Greenhouse test- Both the isolates of *F. solani* were grown on potato dextrose broth in liquid shake cultures at 25 C for one week. A 10ml fungal inoculum was added aseptically to 100g of steam sterilized sand and sorghum bran (1:1 v/v) medium in 250ml conical flasks and incubated at 25 C for two weeks. After two weeks, the colonized sand-sorghum bran at the rate of 1.0, 2.5, 5.0, and 10% w/w was added to a sterilized mixture of sand and black-cotton soil (3:1 w/w) and thoroughly mixed. The soil mixture (950 g) was added to 12.5-cm-dia. pots. Sand-sorghum bran without fungus inoculum was mixed at the same rate and added to pots as a control. Seeds of pigeonpea cv ICPL 87 were surface sterilized in 2.5% sodium hypochlorite solution for five minutes and then washed three times in sterile distilled water. Four seeds were sown in each pot containing the above mentioned soil treatments. Each treatment was replicated five times. The pots were arranged on a green house bench in a randomized block design and watered with 100ml sterile water every two days.

Nematode inoculations

Cysts and egg sacs of *H. cajani* were

collected from roots of four -week-old pigeonpea (cv. ICPL 87) plants. The cysts and eggs sacs were incubated at 25C for emergence of second-stage juveniles in petri dishes. Emerged juveniles were stored at 15C. One week after seedling emergence in pots, 600 J_2 were placed in depressions made near the plant roots in each pot. The pots were then irrigated with sterile water. Six weeks after the addition of nematodes pigeonpea shoot length, shoot and root weights were measured. The plant growth was regularly examined to check for any symptom of *F. solani* infection. The plant roots and the pot soil were washed on 180µm pore size (80mesh) and 38µm-pore size (400 mesh) sieves as described to assess the nematode population densities in soil and root. The average numbers of eggs produced per cyst and egg sac were estimated for each treatment by counting the number of eggs in 20 cysts and 20 egg sacs.

The proportion of eggs infected with fungus was estimated in 10 cysts taken at random. The cysts were placed separately in a drop of 0.1% cotton blue lactophenol solution on slides. Eggs were gently released from cysts to count healthy and infected eggs under x 1000 magnification. For reisolation of the fungus, cysts were treated with 1% sodium hypochlorite, placed on CMA in petri dish and eggs were released with sterile forceps. After two days at 25 C, eggs with fungal growth were subcultured on to another plate. The fungus was examined after one week, when it produced conidia, to confirm the presence of *F. solani*.

Suppression of *H. cajani* density in field soil

Soil samples in bulk were collected from a black watershed field naturally infested with *H.cajani* at the ICRISAT farm. Pigeonpea had been cultivated using traditional farming practices (summer fallow, rainfed, low nutrient input) every year for the last 15 years in this field and *F. solani* was frequently observed parasitic on *H. cajani* eggs (Singh, 1993). The soil was placed in 12.5 cm-dia. pots and treated with 0.2% benomyl, 0.2% banrot, or 0.1% streptomycin sulphate; soil not treated with these chemicals served as a control. Each treatment was replicated five times. Four seeds of pigeonpea cv. ICPL 87 were sown in each pot. The nematode population in the pots was enhanced by adding 2000 freshly emerged surface-sterilized *H. cajani* juveniles adjacent to the roots of one week old plants. The plants were watered with 100ml sterilized water per pot at two-day intervals. Numbers of females and eggs produced were recorded six weeks after the nematode inoculations.

RESULTS

Fungi recovered from *H. cajani*

The fungi *Cephalosporium* spp., *Fusarium solani, Fusarium* spp., *Glomus* spp., and a gram positive bacterium, *Pasteuria penetrans,* were recovered from *H. cajani* cysts from pigeonpea fields on Vertisol and greenhouse cultures (Table 1). No infected cysts or eggs were found in the samples from the Alfisols. *Fusarium solani* was found in 39% of locations surveyed on Vertisols and 5-7% cysts at each of these locations had *F. solani* infection. Cysts with spores of *Glomus* were also found in almost all the locations on Vertisols. *Cephalosporium* spp. and *Fusarium* spp. did not parasitize *H. cajani* eggs on water agar and were not included in further studies. *Pasteuria penetrans* is a known obligate parasite of *H. cajani* (Sharma & Swarup, 1988) and attempts were not made to culture it on artificial media. In laboratory tests, *F. solani* parasitized 28 to 100% of the eggs in egg sacs on water agar medium. Two isolates of *F. solani* collected from the field and

TABLE -1: Fungal parasites of *H. cajani* isolated from soils collected from pigeonpea fields at the research farm of ICRISAT, Patancheru.

Sites/ Locations	Number of fields surveyed	% fields with fungal parasites	Fungi recovered	% cysts infected
Black	5	60	*Glomus* spp.	0.9
Watershed[1]			*Fusarium solani*	6.8
			Cephalosporium spp.	1.2
Black	10	50	*Glomus* spp.	3.4
Unsprayed[1]			*F. solani*	6.9
Black	4	50	*Glomus* spp.	7.4
Manmool[1]				
Black	7	14	*Glomus* spp.	4.5
Precision[1]				
Black	2	50	*Glomus* spp.	3.0
ICRISAT Lake[1]			*Fusarium* spp.	7.4
Black Reserve[1]	3	0	None	0.0
Black Lowlying[1]	2	50	*F. solani*	4.8
Red Manmool[2]	5	0	None	0.0
Red Campus[2]	1	0	None	0.0
Red Precision	2	0	None	0.0
Greenhouse	-	-	*F. solani*	6.0

[1]Vertisol [2]Alfisol

Pasteuria penetrans populations were observed in females collected from black watershed, black unsprayed, black ICRISAT lake sites.

greenhouse cultures (designated as isolates 1 and 2, respectively) were used in subsequent studies. These isolates produced abundant loose aerial white mycelium (2.5-4.0 µm dia.) on the egg sacs and cysts. Both micro-and macro conidia were produced. Sometimes conidia and chlamydospores were produced within eggs. The fungus-infected females were light coloured and sometimes filled with chlamydospores.

Bioassays

On water agar, the two isolates of *F. solani* did not differ (P = 0.05) in their virulence; isolate 1 infected 72% and isolate 2 infected 69% of the eggs. Infected eggs contained fungal hyphae and stained blue when placed in lactophenol cotton blue. The surface of diseased eggs appeared rough while healthy eggs had smooth surfaces.

Sand-sorghum bran medium alone had an adverse effect on cyst and egg numbers of *H. cajani*. With increase in sorghum bran quantity in the soil, there was a corresponding reduction in cyst and egg numbers. However, fewer (P = 0.05) *H. cajani* females were

produced six weeks after addition of nematodes on plants grown in soil treated with *F. solani* colonized sand-sorghum bran medium (Table 2). Similarly, the number of eggs produced per plant and reproductive rate (number of eggs produced after six weeks initial population density) decreased in the fungus inoculated pots. Isolate 2 was more virulent than isolate 1 in reducing the egg number as well as the reproductive rate. The average egg number was reduced by 12 and 34% by isolate 1 and 2, respectively in comparison with egg number in the sand-sorghum bran treatment. Isolate 1 reduced the average reproductive rate of *H. cajani* by 11% and isolate 2 by 33%. The two isolates were similar (P= 0.05) in their ability to infect *H. cajani* eggs and the number of eggs parasitized by the two isolates ranged between 5and 36% of the total eggs produced.

Suppression of *H. cajani* in field soil

The field soil infested predominantly with *F. solani* had a suppressive effect on *H. cajani* density. Numbers of *H. cajani* cysts producted were 1.3 to 1.9 times greater in field soil treated with 0.2% benomyl than in non-treated field soil (Table 3). The effect of soil treatment with 0.1% streptomycin sulphate on cyst and egg numbers was not significant (P = 0.05). Benomyl treated soil had about 2-times more

TABLE -2: Effect of *F. solani* isolates on *H. cajani* population and growth of pigeonpea cv. ICPL 87.

Treatment	Sand sorghum bran medium (% w/w)*	No. cysts /root	No. eggs/ root	Repro-ductive factor	Shoot length (cm)	Shoot weight (g)	Root weight (g)
Control	10.0	18.0	3549	23.7	14.1	0.41	0.85
(No Fungus)	5.0	19.8	4668	31.1	12.7	0.41	0.89
	2.5	24.5	6156	41.0	14.7	0.44	0.93
	1.0	29.0	6602	44.0	15.1	0.45	1.01
Isolate I	10.0	8.5	1681	11.2	16.8	0.63	1.15
	5.0	16.7	3820	25.5	16.1	0.51	1.08
	2.5	21.7	6113	40.8	16.0	0.49	0.98
	1.0	25.5	6990	46.6	16.0	0.53	1.05
Isolate 2	10.0	9.9	1446	9.6	17.5	0.71	1.20
	5.0	16.4	2652	17.7	15.6	0.56	1.15
	2.5	25.5	5004	33.4	15.8	0.60	1.04
	1.0	24.4	4742	31.6	14.5	0.46	0.95
LSD (P = 0.05)		7.3	1613.7	10.8	1.4	0.10	0.20

Sand- sorghum bran medium with and without fungus inoculum was mixed with soil in 12.5-cm-dia pots.
Reproductive factor = No.of eggs producted per root/initial inoculum.

eggs than in non-treated soil. Pigeonpea plant growth was poor, and egg and cyst numbers of *H. cajani* were 51-62% lower in soil treated with banrot than in non-treated soils.

TABLE -3:Influence of soil treatment with benomyl, banrot, and streptomycin sulphate on *Heterodera cajani* population in field soil infested naturally with *F. solani* and *H.cajani*.

Treatment	Number of cyst /plant	Number of eggs /plant
Control	213	30.5 (4.42)
Benomyl (0.2)	407	60.8 (4.77)
Banrot (0.2%)	81	14.7 (4.15)
Streptomycin sulphate (0.1%)	284	37.4 (4.47)
LSD (P = 0.05)	133.3	(0.33)

Non-treated field soil.
Data were $\log_{10}$ transformed for statistical analysis
Mean $\log_{10}$ number of eggs in different treatments are in parentheses.

DISCUSSION

F. solani was the most frequently occurring fungus isolated from *H. cajani* eggs. This fungus is a parasite of *Meloidogyne* spp. (Ansari, 1992; Owino, 1992), *H. glycines* (Kabana & Morgan-Jones, 1988), *H. schachtii* (Lopez & Romero, 1988; Saleh & Quadri, 1989), *Globodera pallida* and *G. rostochiensis* (Crump & Flynn, 1992). The reduction in cyst as well as egg numbers in the *F. solani* infested soils are in confirmation with Crump & Kerry (1987) who also found that *Fusarium* spp., traditionally termed egg parasites were also effective female parasites. Parasitism of females and eggs by microorganisms was not observed in pigeonpea fields on Alfisol. Population densities of *H. cajani* were always found to be much greater on Vertisols than on Alfisols in peninsular India (Sharma & Nene, 1992; Sharma *et al.*, 1992) and this study indicated that natural antagonists might not be the cause of low *H. cajani* densities on Alfisols. In general, the level of natural infection was low, one-time sampling provided only a snap shot of the composition of associated mycoflora and data on seasonal changes, influences of cropping patterns, and climatic conditions will be needed to assess the potential of these antagonists.

The average effect of sand-sorghum bran medium on cyst and egg number could be attributed to influence of decompostion products of the sorghum bran on the nematode. De Leij & Kerry (1991) found similar adverse effects of wheat bran on nematodes infecting tomato It is difficult to separate the degree of nematode control achieved by fungal colonisation from the one brought about by the addition of organic matter of soil (Kabana & Morgan-Jones, 1988). The organic matter might directly contribute to suppression of the nematode density or indirectly by enabling the natural antagonist to parasitize greater number of eggs. Such problems in separating the effects of the fungi from the effect of the culture media are known (Baker *et al.*, 1984) and probably will be resolved by developing methods which enable fungus-alone to be added to soil (Crump & Flynn, 1995).

Fungicide application have been used to determine the degree of natural suppression of nematode-populations by fungal parasites (Crump & Kerry, 1987; Kerry *et al.*, 1980). In the present studies, *H. cajani* reproduction was 50% lower in non-treated soils naturally infested with *F. solani* than in the soils treated with benomyl. Although accurate quantitative prediction of influence of fungal parasitism

on *H. cajani* cysts and eggs was not possible, comparisons of *H. cajani* final densities in benomyl, and streptomycin treated soils with those in non-treated soils presumably reflect suppressive influence of *F. solani* and other soil fungi. Benomyl has also been reported to suppress growth of two other nematode parasitic fungi, *Verticillium chlamydosporium* and *Cylindrocarpon destructans* (Crump & Kerry, 1986). Reduction in number of cysts and eggs in banrot treated soil may be either due to its toxic effects on the *H.cajani* population or due to poor plant growth with fewer roots to support nematode population.

These investigations have identified *F. solani* as antagonistic pathogen of *H. cajani*. Natural control of *H.cajani* to a certain level is apparently active in pigeonpea fields on Vertisols. Further research to manage and amplify this naturally occurring antagonistic potential in the traditional pigeonpea based production systems on Vertisols deserves attention. This study also highlights the potential side effects of soil application of fungicides on non-target soil fungi that are natural antagonists of plant parasitic nematodes.

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