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EFFECT OF *PASTEURIA PENETRANS* ON THE PENETRATION AND MULTIPLICATION OF *HETERODERA CAJANI* IN *VIGNA UNGUICULATA* ROOTS

by
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Summary. The effect of different levels of spore load of *Pasteuria penetrans*, on penetration and multiplication of pigeon-pea cyst nematode on cowpea was studied. The penetration of spore encumbered second-stage juveniles (J2) of *Heterodera cajani* into the roots of cowpea plants was significantly reduced at and above levels of 20-40-spores/J2. Plant growth was positively correlated with bacterial spore load on J2, whereas the nematode multiplication was negatively correlated. The maximum decrease in final cyst formation and J2 population was 86.7 and 98.8 per cent, respectively at a level of >40 spores/J2.

Pasteuria penetrans (Thorne) Sayre *et* Starr is an obligate parasite of nematodes. Host specificity of different populations of *P. penetrans* has been reported (Sayre *et. al*., 1988a, 1988b; Singh and Dhawan, 1990). Work on the penetration of *P. penetrans* in root-knot nematodes, *Meloidogyne* species has demonstrated the bacterium to be a potential bioagent (Stirling, 1984; Raj and Mani, 1988; Somasekhar and Gill, 1990). However, very little information is available on the new strain of *P. penetrans* with respect to host range and bacterial spore attachment on the cuticle of *Heterodera cajani* second-stage juveniles (J2) (Singh and Dhawan, 1990). The present study was, therefore, initiated to ascertain the effect of different levels of *P. penetrans* spore load on the penetration and multiplication of *H. cajani* Koshy in cowpea roots.

Materials and methods

Spores of *P. penetrans* were obtained by crushing infected cysts of *H. cajani* collected from culture pots maintained at the Division of Nematology, I.A.R.I., New Delhi-12. Five different levels of bacterial spore load (1-5, 5-10, 10-20, 20-40, >40 spores/J2) were used in the trial. For determining the required spore load, a preliminary trial was done to standardise the technique in which half and one ml spore suspension with 3.5x10 spores/ml was added into two 5 cm Petri plates each containing 10 g sand (Singh and Dhawan, 1990). Approximately, 2000 active J2 were added separately in both the Petri plates and left for 24 h before they were extracted by inverting the sand from each Petri plate one by one on 400 mesh sieve, washed gently and the catch on the sieve was collected in a beaker. Thereafter, J2 were examined for attachment of spores under compound microscope. The observations made on the spore attachment revealed as many as 6-12 and 13-20 spores/J2 in 0.5 and 1.0 ml spore suspension. Based on this trial, required level of spore load were obtained by adding 0.2, 0.4, 1.0, 2.5 and 5.0 ml spore suspension to 10 g aliquots of sand. Approximately, 2000 active J2 were added in each Petri plate containing the different concentrations of spores. An equal number of J2 in sand without spores served as a control. All 24 Petri plates were then incubated at 25 C for 24h before spore encumbered J2 were extracted and used for inoculation.

Cowpea [*Vigna unguiculata* (L.) Walps., cv. Pusa Komal] seeds were sown singly in 10 cm earthen pots containing 500 cm^3 sterilized soil and sand in the ratio of 2:1. A week after germination, 200 J2 with each level of spore load were inoculated around roots of each plant. Eight replications were kept for each level of spore load. Also, two controls (with and without J2) were included. After one week of nematode inoculations four plants of each spore load were removed carefully and their roots stained by the procedure described by Byrd *et. al.* (1983). The number of J2 penetrated into each root was recorded using a binocular stereoscope.

Two months after inoculations, the plant tops were cut at the base and root and shoot lengths and their fresh weights were recorded. Dry shoot weights were recorded after drying them in an oven at 60 °C for 1 week. The number of J2 in soil were estimated by Cobb's modified sieving and decanting technique. Residues from the 60 mesh sieve were collected in a beaker and the number of cysts recorded using a stereoscopic binocular microscope.

Results and discussion

The data reveal a negative correlation between number of spores/J2 and penetration of J2 into the roots. Significantly fewer juveniles with 20-40 spores/J2 attached invaded roots compared to those J2 without spores. The maximum reduction in penetration (35.4%) was observed with > 40 spores/J2 and the minimum (0.7%) at the lowest level of 1-5 spores/J2 (Table I). Our results corroborate with the findings of several workers (Prasad, 1971; Stirling 1984; Somasekhar and Gill, 1990), who also observed less penetration by spore encumbered juveniles of root-knot nematodes into the roots than the healthy ones. Data in table II show that all plant growth characters, except root length, were significantly increased at a level of 10-20 spores/J2 and above as compared to control (J2 without spores), while there were no significant differences obtained between levels of 20-40 spores/J2 and > 40 spores/J2 which were similar to uninoculated control (without J2 and spores). These findings are in conformation with Raj and Mani (1988) who got increased fresh weights of shoot and root and dry weights of shoot in tomato plants when different concentrations of *P. penetrans* infected root powder were added in *Meloidogyne javanica* infested soil in pots.

TABLE I - *Effect of different levels of spore load of* Pasteuria penetrans *on penetration of second-stage juveniles (J2) of* Heterodera cajani *into cowpea roots.*

Spore load/J2	Number of J2 penetrated	Per cent decrease in penetration
0 (Control)	103 (10.1)	0.0
1-5	102 (10.0)	0.7
5-10	101 (10.0)	2.2
10-20	94 (9.7)	8.5
20-40	72 (8.4)	29.8
>40	66 (8.0)	35.4
C.D. at 5%	(1.4)	

Figures in parentheses are square root transformed values.

The data on cyst multiplication showed a corresponding decrease with an increase in the levels of spore/J2 (Table III). Significant reduction in number of cyst was observed at a level of 5-10 spores/J2 and above as compared to control (J2 without spores). The per cent reduction in cyst production at a level of 5-10, 10-20, 20-40, and > 40 spores/J2 was in the order of 32.8, 49.2, 81.7 and 86.7 respectively. Further, the percentage of infected cysts increased with the increase in level of spores with maximum being 97% at a level of > 40 spores/J2 followed by 90, 50 and 24% at a level of 20-40, 10-20 and 5-10 spores/J2 respectively. The reduction in population of free J2 in the soil showed a similar trend as that of cyst multiplication.

The egg contents in cysts were significantly reduced at a level of 10-20 spores/J2 and above compared to the control (Table III). Similar results were also observed by Stirling (1984) who reported decrease in galling of tomato roots and soil population of *M. javanica* by adding spore powder @ 212-600 mg/kg soil.

It can be, therefore, concluded that the indigenous strain of *P. penetrans* has potential to contain the population of *H. cajani* by reducing its penetration and multiplication in the host plant.

TABLE II - *Effect of different levels of* P. penetrans *encumbering second-stage juveniles (J2) of* H. cajani *on plant growth characters of cowpea.*

Spore load	Length (cm)		Fresh weight (g)		Dry shoot weight (g)
	Shoot	Root	Shoot	Root	
0 (Control)	9.9	18.8	4.5	1.7	0.8
1-5	10.4	19.6	4.7	1.8	0.9
5-10	11.1	20.0	5.1	2.2	0.9
10-20	12.9	26.5	5.8	2.8	1.1
20-40	14.9	28.0	6.6	3.2	1.2
>40	15.3	31.3	6.8	3.3	1.2
Uninoculated Control (without J2 and spores)	15.8	32.0	7.2	3.3	1.3
C.D. at 5%	2.2	NS	1.0	0.7	0.1

TABLE III *Effect of different levels of* P. penetrans *on the multiplication of* H. cajani.

Spore load/J2	Number of cyst in soil	% Reduction	% Infection	Number of J2 in soil	% Reduction	Number of eggs/cyst	% Reduction
	462.8 (21.5)*	0.0	0.0 (0.0)**	662.8 (25.7)*	0.0	74.6 (8.5)*	**0.0**
1-5	433.3 (20.6)	6.4	4.0 (9.9)	586.00 (24.2)	11.6	56.2 (6.7)	24.7
5-10	311.0 (17.6)	32.8	24.0 (29.0)	389.3 (19.6)	41.3	43.8 (5.6)	41.3
10-20	235.0 (12.4)	49.2	50.0 (45.0)	178.3 (13.2)	73.1	27.2 (3.9)	63.5
20-40	84.8 (9.1)	81.7	90.0 (72.0)	23.3 (4.5)	96.5	11.6 (1.5)	84.5
>40	61.5 (7.7)	86.7	97.0 (84.6)	8.0 (2.9)	98.8	8.0 (1.3)	89.3
C.D. 5%	(3.0)		(8.7)	(2.5)		(4.3)	

* Square root transformed values; ** Angular transformed values.

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