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PRELIMINARY STUDIES ON THE EFFECT OF *PASTEURIA PENETRANS* ON *RADOPHOLUS SIMILIS* INFESTING ARECANUT SEEDLINGS

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ABSTRACT

The effect of the bacterium, *Pasteuria penetrans* on multiplication of the burrowing nematode, *Radopholus similis* infesting arecanut seedlings was studied. As reported earlier the threshold inoculum level of *R. similis* to cause significant reduction in growth parameters of arecanut was found to be 100 nematodes per plant. *P. penetrans* reduced the damaging effect of *R.similis* on arecanut seedlings on inoculation of both the organisms simultaneously. Inoculation of *P. penetrans* before or after inoculation with *R. similis* was not equally effective in reducing the damage.

INTRODUCTION

The present trend in nematological research is towards limited use of nematicides and increased use of biocontrol agents. The bacterium, *Pasteuria penetrans* (Thorne, 1940; Sayre and Starr, 1985) has been found to be a potent biocontrol agent for plant parasitic nematodes (Mankau, 1975; Mankau and Prasad, 1977; Sayre, 1980; Kerry, 1987; Maheswari, Mani and Rao, 1987).

The burrowing nematode, *Radopholus similis* has been found to cause severe damage to plantation crops like coconut, arecanut, banana and black pepper. Its pathogenic effect on arecanut has been proved and it was found that within three years at an initial inoculum level of 100 nematodes/seedling, visible damage and significant reduction in growth characters is caused (Koshy, 1986). There has been no attempt so far in controlling *R. similis* on arecanut using natural enemies. Moreover, ecological conditions such as monocropping system, continuous availability of moisture and organic matter rich soil prevailing in areca plantations are ideal for multiplication of biocontrol agents. Due to the above reasons an attempt was made to study the effect of *P.penetrans* on *R.similis* infesting arecanut.

MATERIALS AND METHODS

Arecanut seedlings var. Mangala grown in 20 cm earthen pots containing fumigated sandy loam soil were used for the experiment. There were six treatments viz., T1- uninoculated control, T2 - *P. penetrans* alone, T3 - *R. similis* alone, T4 - *R. similis* plus *P. penetrans*, T5 - *P. penetrans* followed by *R. similis* after seven days, T6 - *R. similis* followed by *P. penetrans* after seven days. There were five replications for each treatment. Culture of *P. penetrans* parasitizing root-knot nematode was obtained from Dr. A. Mani, APAU, Hyderabad. This culture was maintained on root-knot nematode infesting roots of *Coleus variegata* and *Solanum melongena*.

The bacterial inoculum used per treatment was 100 g of soil containing an average of ten *P. penetrans* infested second stage larvae of *Meloidogyne incognita* each of which had an average of 15 spores per larvae attached to their cuticle. The nematode suspension was obtained from axenic carrot culture and the inoculum consisted of 100 females and larvae. Each plant was inoculated at the four-leaf stage by exposing the root zone near the base and after inoculation covering the exposed area with fumigated soil. The plants were kept in the green house at a temperature ranging between 27 and 34°C and watered daily with boiled and cooled water. After ten months each plant was depotted and the root system washed thoroughly to remove the adhering soil particles. Plant growth parameters like shoot length, shoot weight, number of leaves, root weight, root volume and the total quantity of water absorbed within 48 hours (only one replicate each) were recorded.

Soil from each pot was placed on a plastic sheet and mixed well. An aliquot of 200 cm^3 was drawn out, processed and nematode population assessed. The root system was weighed and cut into small bits and mixed well. Three aliquots of one gram each were drawn out and stained separately for 60 seconds in boiling acid fuchsin lactophenol. The stained root bits were blended for 60 seconds and nematode population assessed.

RESULTS AND DISCUSSION

The data given in Tables I, II and III reveal that the uninoculated control plants recorded maximum growth closely followed by those which received *P. penetrans* alone. Among the different plants that received *P. penetrans* and *R. similis* in different combinations, maximum growth and least nematode multiplication were recorded in those which received both of them simultaneously. Eventhough the root system of these plants recorded lesions and rotting, lesser rotting was observed in tertiary roots compared to the roots in plants which received nematode alone. Introduction of *P. penetrans* before or after inoculation with *R. similis* did not have much effect on growth parameters as well as on nematode multiplication compared to plants that received *R. similis* alone. Lamina length and breadth did not show any significant difference in any of the treatments. *R. similis* alone caused maximum reduction in growth and recorded the maximum nematode multiplication. The lateral and feeder roots of the plants that received nematode alone recorded lesions and severe rotting.

It was seen that seedlings with higher root volume absorbed more water. Uninoculated seedling with a root volume of 44 cm^3 absorbed 110 ml water. Plants belonging to other treatments with root volumes in the range of 14 to 43 cm^3 absorbed 15 to 105 ml water.

Table I. Effect of *P. penetrans* and *R. similis* alone and in combination on shoot growth parameters of arecanut seedlings (mean of five replications)

Sl. No.	Treatment *	Shoot length (cm)	Percent reduction over control	Shoot weight (g)	Percent reduction over control	No. of leaves	Percent reduction over control	Lamina length (cm)	Percent reduction over control	Lamina breadth (cm)	Percent reduction over control
1.	C	86.0	—	96.6	—	6.2	—	32.0	—	4.0	—
2.	B	84.0	2.33	92.6	4.14	5.0	19.35	31.8	0.63	4.0	0.00
3.	B f M	81.2	5.58	77.0	20.29	4.8	22.58	29.8	6.87	3.0	25.00
4.	B + N	82.8	3.72	87.6	9.32	5.0	19.35	30.2	5.62	3.0	25.00
5.	N f B	80.0	6.98	78.2	19.05	4.8	22.58	29.6	7.50	3.0	26.00
6.	N	78.8	8.37	74.4	22.98	4.6	25.81	29.6	7.50	3.0	25.00
	C.D	4.45	—	10.28	—	0.63	—	—	—	—	—

C.Control, B. Bacteria, B-f-N. Bacteria followed by nematode, B+N. Bacteria and nematode together, N-f-B. Nematode followed by bacteria, N. Nematode

Analysis of Variance

Source	df	Mean sum of squares: Shoot length	Shoot weight	No. of leaves	Lamina length
Treatment	5	35.41	419.52	1.65	4.94
Error	24	11.60	62.07	0.23	1.95

Table II. Effect of *P. penetrans* and *R. similis* alone and in combination on root growth parameters of arecanut seedlings (mean of five replications)

Sl.No.	Treatment	Root weight (g)	Percent reduction over control	Root volume (cm^3)	Percent reduction over control
1.	C	34.60	—	44.20	—
2.	B	34.40	0.58	42.80	3.17
3.	B-f-N	12.40	64.16	20.60	53.39
4.	B + N	14.20	58.96	26.40	40.27
5.	N-f-B	12.00	65.32	18.00	59.28
6.	N	10.00	71.10	14.20	67.87
	C.D	2.62	—	5.13	—

Analysis of variance

Source	df	Mean sum of squares	
		Root weight	Root volume
Treatment	5	674.96	828.70
Error	24	4.02	15.45

Table III. Effect of *P. penetrans* on the multiplication of *R. similis* infesting arecanut seedlings (mean of five replications)

Sl. No.	Treatment	Total population
1	B→N	58000.6016 (4.7630)
2	B+N	26711.8008 (4.4216)
3	N→B	58981.9492 (4.7704)
4	N	59620.9570 (4.7752)
	C.D	0.0458

Figures in parentheses are the log transformed values

Analysis of variance

Source	DF	Mean sum of squares
Treatment	3	0.15158
Error	16	0.00168

When different stages of *R. similis* extracted from plants treated with *P. penetrans* and *R. similis* were observed under 100x objective, some glistening granulations were found in the oesophageal region, especially around the median bulb. No pustule formation was encountered. Similar granulations were absent in *R. similis* extracted from plants that received nematodes alone. Such granulations other than pustules were observed in second stage larvae of *Meloidogyne incognita* infested with *P. penetrans*.

Results reveal that *P. penetrans* did not have any deleterious effect on plant growth. *P. penetrans* could suppress the damaging effect of *R. similis* on the growth of plants to a limited extent when both of them were inoculated simultaneously. Presence of *P. penetrans* before or after inoculation with *R. similis* was not very effective in reducing the damage. The present study has confirmed the damage potential of the burrowing nematode on arecanut and the threshold inoculum level as 100 nematodes per plant (Koshy and Sundararaju, 1989). Ten-fold increaase in yield was reported with application of aldicarb @ 10 g a.i./palm in arecanut (Sundararaju and Koshy, 1986). But under the high density multispecies cropping system there is limitation for control of nematodes through chemicals in view of the residues left in raw arecanut.

Therefore, studies are to be intensified on biological control of the burrowing nematode on arecanut.

P. penetrans has been found to be effective in reducing populations of *Pratylenchus scribneri* on bean roots (Mankau, 1975) and root-knot nematode or various crops (Maheswari *et al.*, 1987 and Raj and Mani, 1988). *P. penetrans* readily multiplied on *Tylenchulus semipenetrans* parasitizing *Citrus aurantifolia* proving to be a potent biocontrol agent for this nematode (Mani 1988). It was found to be more effective than *Paecilomyces lilacinus*, a fungal biocontrol agent, in reducing *Meloidogyne javanica* on tomato plants (Maheswari and Mani, 1988). Hence, this preliminary investigation carried out has opened up possibilities for control of the burrowing nematode using *P. penetrans*. However, intensive studies are warranted to standardize the mode and frequency of inoculation and quantum of inoculum of *P. penetrans* and to study the abnormalities consequent to *P. penetrans* infection in *R. similis*.

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