

**BIOLOGICAL CONTROL OF *MELOIDOGYNE INCOGNITA* ON
BLACK PEPPER BY *PASTEURIA PENETRANS*
AND *PAECILOMYCES LILACINUS***

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ABSTRACT

Root-knot nematode, *Meloidogyne incognita* causes considerable damage to black pepper (*Piper nigrum* L.). Efficacy of two known bio-control agents namely *Pasteuria penetrans*, an endospore forming bacteria and *Paecilomyces lilacinus*, a parasitic fungus on eggs for the control of *M. incognita* on black pepper was studied in pots under greenhouse condition. *P. Penetrans* and *P. lilacinus* reduced nematode population, root-gall indices and improved the growth of the plants and root mass production significantly. Fifteen per cent reduction was noticed in the vine length of plants inoculated with nematode alone while it was only 8 to 15% in vines treated with either *P. lilacinus* or *P. penetrans* or both with nematodes. Number of leaves/nodes produced was maximum in control plants compared to a minimum in nematode inoculated vines. Fresh shoot and root weight were significantly greater in all treatments infested with *M. incognita* in the presence of one or both the bio-control organisms than *M. incognita* alone. Among the two biocontrol agents studied, *P. lilacinus* was more effective compared to *P. penetrans*, but combination of both the organisms (PI + PP) was found to be very effective in the management of root-knot nematode in black pepper. Both *P. lilacinus* and *P. penetrans* did not cause any adverse effect on plant growth and they were found to survive and effective even after two years of inoculation under greenhouse conditions. The natural occurrence of *P. penetrans* on *M. incognita* on cardamom from Vandanmedu, Idukki district, Kerala is reported for the first time. Black pepper being a perennial crop is a very good host for the maintenance of *P. penetrans* on *M. incognita*.

INTRODUCTION

Biological control is gaining greater importance throughout the world due to the increased cost of chemical pesticides, health hazards and environmental pollution due to indiscriminate use of the chemicals. The fungus, *Paecilomyces lilacinus* (Thom) Samson has been found to be a potential biocontrol agent for bringing down root-knot nematode population on various crops (Jatala *et al.* 1980; Jatala, 1986; Dube and Smart, 1987; Cabanillas and Barker, 1989; Cabanillas *et al.*; 1989; Ramana, 1994). Similarly an endospore forming bacterium, *Pasteuria penetrans* (Thorne) Sayre and Starr has been reported to be a promising biocontrol

agent of the root-knot nematode, *Meloidogyne* spp. (Mankau, 1980; Sayre, 1980; Stirling, 1984; Maheswari *et al.*, 1987). The root-knot nematode, *Meloidogyne incognita* is known to cause considerable damage to black pepper, *Piper nigrum* (Koshy *et al.*, 1979; Mohandas and Ramana, 1991). Hence, a pot culture experiment was conducted in the greenhouse to study the efficacy of *P. lilacinus* and *P. penetrans* alone and in combination in suppressing the nematode.

MATERIALS AND METHODS

Uniform sized two node cuttings of black pepper var. Panniyur-I were planted

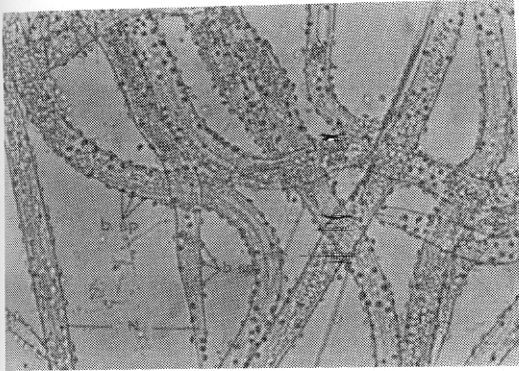


Fig. 1. *Pasteuria penetrans*

in 20 cm earthen pots containing fumigated sandy loam soil in the greenhouse. Thirty five rooted cuttings with uniform growth were selected. There were seven treatments replicated five times viz. T₁ - Untreated control, T₂ - Nematode (N) alone, T₃ - *P. lilacinus* (P1) alone, T₄ - *P. penetrans* (PP) alone, T₅ - Nematode + *P. lilacinus* (N + P1), T₆ - Nematode + *P. penetrans* (N + PP) and T₇ - Nematode + *P. lilacinus* + *P. penetrans* (N + P1 + PP).

The fungus *P. lilacinus* was cultured on autoclaved wheat grains in 250 ml Erlenmeyer flasks. The flasks were incubated in the laboratory at a temperature range of 79-91° F. After 21 days, sterile water was added to the flasks, shaken and the spore suspension having a spore load of 4×10^6 was added to the root zone in all plants with *P. lilacinus* treatment.

Root-knot nematodes infested with *P. penetrans* (Fig. 1) were collected from roots of caradamom plants in Vandanmedu, Idukki district, Kerala which incidentally is the first report of the natural occurrence of this bacterial hyperparasite from Kerala and multiplied on brinjal (*Solanum melongena*). Infested brinjal roots were collected, washed and air-dried in the laboratory and ground into powder as described by Stirling and Wachtel (1980). The soil collected from the culture pot was also air dried and mixed with the root powder. Two grams each of

this mixture (soil and root powder) was mixed with the soil in all treatments having *P. penetrans*. Freshly hatched second stage larvae (J₂) of *M. incognita* (100 each) collected from egg masses of infected black pepper roots of pure culture maintained in the greenhouse served as nematode inoculum. One hundred J₂ in 10 ml water was introduced into the soil at the root zone of the pepper vines of all treatments having nematode as treatment. All the plants were maintained under even light at an ambient temperature ranging from 27 to 34°C and watered daily with boiled and cooled water. After two years, the plants were depotted and washed thoroughly to remove the adhering soil particles. The root gall index was recorded using the root gall index scale of 1 to 5 (Taylor and Sasser, 1978). Plant growth parameters such as vine length, shoot weight, number of nodes/leaves, maximum length and breadth of leaf lamina, root length and root weight were recorded. Leaf area index was calculated from lamina length into breadth. Nematode population in 250 cm³ of soil was assessed from each pot by modified Cobb's sieving and sifting method. The root system of each vine was cut into small pieces, mixed well and an aliquot of 1 gm was drawn. The root samples were stained for 30 seconds in boiling acid - fuchsin lactophenol, blended for 30 seconds and nematode population (eggs, different stages of larvae, males and females) were assessed. Ten each of egg masses and nematode infested root bits of *P. lilacinus* treated vines were plated on PDA in petri-plates for reisolation of the fungus.

RESULTS AND DISCUSSION

Significant differences between treatments were observed in all the growth parameters studied (Table 1). Maximum vine length was recorded in uninoculated control plants (1078 cm) followed by plants receiving *P. lilacinus* (1024 cm) and *P. penetrans* (1021 cm) and minimum in nematode

Table 1. Effect of *P. lilacinus* and *P. penetrans* alone and in combination with *M. incognita* on growth parameters of black pepper.

Treatment	Vine length (cm)	No. of nodes/leaf	Leaf area index	Shoot wt. (g)	Root length (cm)	Root wt. (g)
Control	1078	201	162	329	74	69
<i>P. lilacinus</i> (P1)	1024 (6)	204 +(1)	151 (7)	284 (14)	66 (11)	71 +(2)
<i>P. penetrans</i> (PP)	1021 (7)	179 (11)	164 +(1)	278 (16)	68 (8)	64 (7)
Nematode (N)	466 (57)	110 (46)	131 (20)	193 (41)	45 (39)	37 (46)
N+P1	975 (10)	157 (22)	157 (4)	266 (19)	64 (14)	56 (19)
N+PP	917 (15)	150 (25)	130 (20)	233 (29)	61 (18)	54 (22)
N + P1 +PP	993 (8)	187 (7)	161 (1)	288 (12)	74 (0)	62 (10)
Gen. Mean	924.9	169.7	151.7	267.3	64.7	59.1
Cv(%)	14.54	27.35	14.05	17.68	20.67	22.31
F. ratio	12.02*	2.58*	2.63	4.19**	2.72*	3.72*
CD (P=0.05)	174.1	60.09	27.02	61.0	17.31	17.0

Note: Values in parenthesis indicate percentage reduction over control.
+ indicate percentage increase over control.

inoculated vines (466 cm) with 57 per cent reduction. The reduction was only 8 to 15 per cent in vines treated with either *P. lilacinus* or *P. penetrans* or both along with the nematode. In the case of total number of nodes/leaves, leaf area index, shoot weight, maximum root length and root weight also a similar trend was observed. Maximum percentage reduction in all growth parameters was recorded in vines inoculated with nematode alone. Number of nodes/leaves was maximum in *P. lilacinus* treated vines (204) with a minimum in nematode inoculated vines (110).

Yellowing of the interveinal areas of leaves was observed in vines inoculated with nematode alone, whereas it was not very prominent in nematode inoculated vines treated with *P. lilacinus* and *P. penetrans* alone and together in combination with nematode (Table 1). This showed that both organisms can be used simultaneously as they did not inhibit each other. Maximum root weight was recorded in *P. lilacinus* treated vines followed by uninoculated control plants and *P. penetrans* treated vines with minimum root weight in nematode inoculated vines.

Table 2. Effect of *P. penetrans* and *P. lilacinus* on the growth characters of black pepper-percentage improvement over nematode inoculated plants.

Treatments	Vine length	No. of nodes/leaf	Leaf area index	Shoot wt. (g)	Root length	Root wt. (g)
N + P1	109	43	20	38	42	54
N + PP	98	36	-0.7	21	36	46
N + P1 + PP	112	70	23	49	64	68

N - Nematode, P1 - *Paecilomyces lilacinus*, PP - *Pasteuria penetrans*

Table 3. Effect of *P. lilacinus* and *P. penetrans* alone and in combination on *M. incognita* population in black pepper.

Treatments	Gall index	Nematode population/g root	
		Original Values	Transformed values
Control	1.0	0	1.0
<i>P. lilacinus</i> (P1)	1.0	0	1.0
<i>P. penetrans</i> (Pp)	1.0	0	1.0
Nematode (N)	4.6	6148	77.3
N + P1	2.4	918	30.1
N + Pp	2.8	1452	37.9
N + P1 + Pp	2.5	652	24.7
Gen. mean	2.19	13.0	24.7
Cv (%)	17.72	65.68	26.75
'F' ratio	58.71**	32.82**	88.8**
CD (p = 0.05)	0.50	1110.00	7.24

A perusal of the data in Table 1 showed that the fungus *P. lilacinus* or the bacterium *P. penetrans* caused little reduction in growth parameters compared to uninoculated plants. Vines inoculated with nematode along with biocontrol agents (N + P1 + PP) recorded 23 to 112% increase in different growth parameters over the vines inoculated with nematode alone. Similarly vines that received *P. lilacinus*(P1) or *P. penetrans*(PP) alone also resulted in similar growth increase over vines inoculated with nematode alone. However, combined inoculation of P1 + PP was not significantly different from P1 or PP (Table 2).

Maximum gall index (4.6) was recorded in vines inoculated with nematode alone. Gall index was significantly reduced in the vines treated with two biocontrol agents either alone or in combination and were at par. Similar trend was noticed in nematode population in different treatments (Table 3). Further, 70 per cent of the egg masses collected from roots of fungus treated vines were infected with *P. lilacinus*. More than 60 per cent of the eggs in the egg masses were infected and the fungus could be reisolated from egg masses.

The above results confirmed the earlier reports that *P. penetrans* had suppressed root galling and egg mass production by *M. incognita* on tomato (Mankau, 1980; Mankau and Prasad, 1972). Hence, reduction of a nematode population could be correlated with increased healthy root and shoot growth. In plants treated with *P. penetrans* more than 80 per cent of larvae were loaded with spores. Infected adult females were eventually filled with as many as two million spores. Number of females developing in the roots was also found to be reduced in plants treated with nematode plus *P. penetrans* compared to plants inoculated with nematode alone indicating that *P. penetrans* affected the reproduction of the nematode.

The fungus *P. lilacinus* is the most promising biocontrol agent for management of root-knot nematode when compared to *P. penetrans*. Black pepper vines inoculated with *M. incognita* plus *P. lilacinus* showed more improvements in growth characters and reduction in nematode population density than in vines treated with nematode plus *P. penetrans*. However, *P. lilacinus* and *P. penetrans* together reduced population densities of *M. incognita* resulting in better

plant growth than would occur with either of the organisms acting alone. Similar reduction in nematode population was reported in tomato by Dube and Smart (1987) and in brinjal by Zaki and Maqbool (1992). Both biocontrol agents were found to be effective even after two years of inoculation

which is very encouraging in view of the fact that black pepper is a perennial crop. The inocula can easily be introduced to nursery potting mixture as well as to the planting pits, thereby reducing the need for application of nematicides. However, this need to be tested under field conditions.

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