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Field evaluation of *Trichoderma harzianum*, *Pochonia chlamydosporia* and *Pasteuria penetrans* in a root knot nematode infested black pepper (*Piper nigrum* L.) garden in India

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Abstract

Two fungal bioagents (*Trichoderma harzianum* and *Pochonia chlamydosporia*) and a bacterial endoparasite (*Pasteuria penetrans*) were evaluated for biological control of nematodes in a black pepper garden in Waynad District of Kerala, India for five years (1998-2001) in a randomized block design with six treatments replicated thrice. The yellowing of vines in the experimental plot has decreased in all the treatments after the first year onwards. After four years, the lowest incidence of yellowing (15.25 %) was noticed in plots treated with phorate followed by plots treated with *P. chlamydosporia* (20.78 %) and *P. penetrans* (24.13 %). The highest mean yield (1.83 kg vine^{-1}) was obtained in *P. chlamydosporia* treated plots followed by combined application of phorate and potassium phosphonate (1.50 kg vine^{-1}). The lowest mean population of root-knot nematodes in black pepper roots was observed in phorate + potassium phosphonate treated plants followed by *P. penetrans* treated plants. The final nematode level was the lowest in *P. chlamydosporia* treated plots. The study has clearly proved the efficacy of these bioagents, especially *P. chlamydosporia* for managing root knot nematodes in black pepper gardens.

Keywords: Biological control, black pepper, *Pasteuria penetrans*, *Pochonia chlamydosporia*, root-knot nematode, *Trichoderma harzianum*

Introduction

Black pepper (*Piper nigrum* L.) is an important export earning spice crop of India. Of the several plant parasitic nematodes infesting this crop, root knot nematodes (*Meloidogyne* spp.) are the most widely distributed one. It has been reported as one of the primary agents for the slow decline disease of black pepper. Several management options viz., application of nematicides, organic cakes, use of resistant plants etc. are available to ward off the damage caused to black pepper by these nematodes. Because of their potential health and environmental risks, use of nematicides is currently not preferred worldwide. The search for environmentally friendly alternatives to manage plant parasitic nematode populations has, therefore, become increasingly important.

Biological control is defined as the management of plant diseases and pests with the aid of living organisms. A variety of soil microorganisms have been isolated from soil, host-plant tissues and nematodes infesting spice crops by earlier workers (Eapen *et al*., 2005; Sreeja *et al*., 1996). Several of them have been proved to be antagonistic to root knot nematodes in laboratory and greenhouse studies (Eapen *et al*., 2005). However, their efficacy under field conditions is yet to be proved. The present study is an attempt to evaluate two fungal bioagents *viz*. *Trichoderma harzianum* and *Pochonia chlamydosporia* (syn. *Verticillium chlamydosporium*) and a bacterial endoparasite, *Pasteuria penetrans* for their efficacy to suppress root knot nematodes infesting a perennial crop such as black pepper under field conditions.

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Materials and Methods

Experimental plot: A root-knot nematode infested (mean population – 1615.97 per g of root) five year-old Panniyur-5 black pepper garden at Mullankolli, Wyanad District, Kerala, India was selected for this study in 1998. Each vine in the garden was visually indexed for yellowing and defoliation (common symptoms of nematode infestation) using a 0 - 4 scale (0 - healthy and no yellowing, 1 - slight yellowing, 2 - moderate yellowing, 3 - severe yellowing with defoliation and 4 - severe yellowing, defoliation and mortality). The experimental site was divided into plots consisting of 12 - 30 vines.

Experimental treatments: There were six treatments i) *Trichoderma harzianum* (IISR1292), ii) *Pochonia chlamydosporia* (IISR1568), iii) *Pasteuria penetrans* (Pp. 1), iv) phorate @ 3 g a.i. vine^{-1} + 0.3% potassium phosphonate @ 3-5 l vine^{-1}, v) phorate @ 3 g a.i. vine^{-1} and vi) Check (no biocontrol agent or chemical). Each treatment was replicated three times in a randomized block design. *T. harzianum* (~10^8 cfu g^{-1}) and *P. chlamydosporia* (~10^6 cfu g^{-1}) were multiplied on sorghum and applied @ 50 g vine^{-1}. *P. penetrans* multiplied by the method described by Stirling and Wachtel (1980) was applied to the basins @ 10 g vine^{-1} after mixing with dry sand. Each plant, irrespective of the treatment, received five kg of dry farm yard manure. All other farm operations were uniform for all the plants in the plot. All the treatments were made twice in a year, during May-June and after monsoon during October-November for four consecutive years (1998-2001). The mortality and yellowing of vines were recorded every year at the time of application of biocontrol agents and pesticides. The yield of experimental vines in each plot was recorded for the last two years (2000 and 2001).

Estimation of nematodes: Every year the nematode population in soil and roots were estimated during the post monsoon period just before the second round of treatment imposition. For estimating the nematode population, plant parasitic nematodes were extracted from soil samples by sieving and a modified Baermann's funnel method (Pitcher and Flegg, 1968). The roots were washed and the nematode population in roots was estimated by staining with acid-fuchsin, blending and sampling the suspension to count nematodes (Byrd *et al*., 1983).

Estimation of bacteria/fungi: The nematodes were checked for bacterial/fungal colonization by methods already described (Eapen *et al*., 2005). Fungi and bacteria from rhizosphere soil were isolated by the standard dilution plate method (Waksman, 1922).

Statistical analysis: The data on visual scoring of yellowing (0-4 scale) was converted to yellowing severity as follows:

$$\text{Yellowing severity (\%)} = \frac{[(y1 * 1) + (y2 * 2) + (y3 * 3) + (y4 * 4)] \times 100}{T}$$

where,

y1 = No. of vines having an yellowing index of 1

y2 = No. of vines having an yellowing index of 2

y3 = No. of vines having an yellowing index of 3

y4 = No. of vines having an yellowing index of 4

T = Total no. of vines in a plot

The incidence of yellowing was also worked out as given below:

$$\text{Incidence of yellowing (\%)} = \frac{Y}{T} \times 100$$

where,

Y = Total no. of vines showing yellowing in a plot

T = Total no. of vines per plot

The percentage data was transformed using angular transformation before analysis while logarithmic transformation was used for nematode data. Means given in the results were back-transformed values. ANOVA or two-way analysis of variance was used for data analysis (Gomez and Gomez, 1984). Means were separated by Least Significant Difference (LSD).

Results and Discussion

The yellowing of vines in the experimental plot has steadily declined in all the plots treated with biocontrol agents or chemicals (Table 1). The mean incidence of foliar yellowing in the plot at the start of the experiment was 72.92 %. The yellowing started reducing within one year in all the plots, irrespective of the treatments. The yellowing in the control plot also had reduced over a period of time. During the final year the lowest incidence of yellowing was noticed in plots treated with phorate (15.25 %) followed by plots treated with *P. chlamydosporia* (20.50 %) and *P. penetrans* (22.82 %). Similarly maximum reduction in incidence of yellowing was also recorded in phorate treated plots. However, statistically significant reduction in the pooled mean (yellowing) was observed only wherever biocontrol agents were applied.

Table 1. Crop stand and yield of black pepper in a biocontrol field trial at Pulpally, Kerala (Mean of three replications)

Treatment	Vines showing yellowing (%)					Yield (kg-dry vine-1)		
	1998	1999	2000	2001	Mean	2000	2001	Mean
Trichoderma harzianum @ 50 g vine^{-1}	46.67	31.69	17.30	36.93	32.44	1.23	1.41	1.32
	(43.09)	(34.26)	(24.60)	(36.91)	(34.72)			
Pochonia chlamydosporia @ 50 g vine^{-1}	58.18	33.69	24.82	20.50	33.72	1.66	2.00	1.83
	(49.71)	(35.48)	(29.88)	(26.92)	(35.50)			
Pasteuria penetrans @ 10 g vine^{-1}	59.41	47.98	37.27	22.82	41.42	1.22	1.13	1.18
	(50.43)	(43.84)	(37.63)	(28.34)	(40.06)			
Phorate @ 3 g a.i. vine^{-1} + 0.3% Potassium	83.81	66.29	50.51	34.44	59.53	1.08	1.91	1.50
phosphonate @ 3-5 l vine^{-1}	(66.27)	(54.51)	(45.29)	(35.90)	(50.49)			
Phorate @ 3 g a.i. vine^{-1}	86.40	67.43	32.86	15.25	50.06	1.00	1.70	1.35
	(68.36)	(55.20)	(34.98)	(21.60)	(45.03)			
Control	92.41	66.95	41.04	32.39	60.15	0.75	1.30	1.02
	(74.01)	(54.91)	(39.84)	(34.67)	(50.86)			
Mean	72.92	52.38	33.51	26.10	-	1.16	1.58	-
	(58.64)	(46.37)	(35.37)	(30.72)				
$LSD_{0.05}$		Years (Y)- 8.22; Treatments (T) - 9.93					Y - 0.36; T - 0.42	
		Y x T - N.S.					Y x T - N.S.	

Figures in parentheses are arc sine transformed values. N.S. - Not significant

The highest mean yield (1.83 kg vine^{-1}) was obtained in *P. chlamydosporia* treated plots followed by combined application of phorate and potassium phosphonate (1.50 kg vine^{-1}), which were significantly higher than that of control plots (Table 1).

The root-knot nematode population decreased in all the treatments compared to the initial population (Fig.1A), but the reduction was not statistically significant in any of the treatment. However, the lowest mean population of root-knot nematodes in black pepper roots was observed in phorate + potassium phosphonate treated plants followed by *P. penetrans* treated vines. The reduction in nematode population in alternate years very well illustrated the density-dependant multiplication of root-knot nematodes. After four years of field evaluation, the nematode level was the lowest in *P. chlamydosporia* treated plots. The microbial load in these plots also varied widely (Fig. 1B). Both *P. chlamydosporia* and *P. penetrans* could be reisolated from different life stages of root knot nematodes extracted from black pepper roots.

The field experiment laid out in an established black pepper garden at Wynad, Kerala was very unique in several respects. This long-term experiment was conducted with the objective of evaluating the remedial action of three promising biocontrol agents *viz*. *P. chlamydosporia*, *T. harzianum* and *P. penetrans* in comparison to application of pesticides on root-knot nematode infestation in black pepper vines (Panniyur 5). The study has proved that *P. chlamydosporia* was highly effective compared to *T. harzianum* in suppressing nematodes and increasing the yield of black pepper.

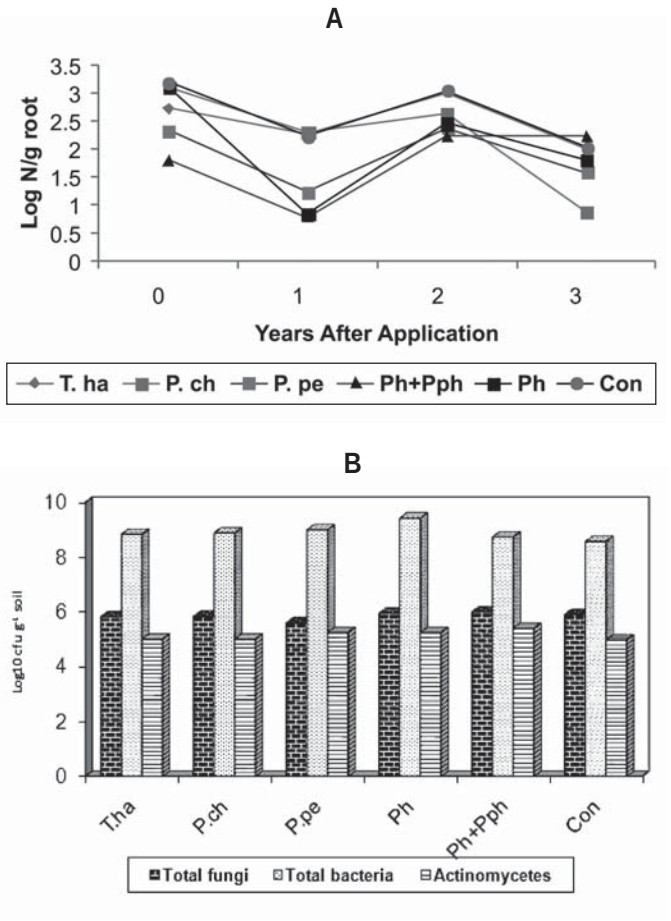

Fig. 1. Effect of biological control agents on nematodes and soil microbes of the field trial at Pulpally, Kerala. A. Mean root- knot nematode population in roots of black pepper plants (T. ha - *Trichoderma harzianum*, P. ch - *Pochonia chlamydosporia*, P. pe - *Pasteuria penetrans*, Ph - Phorate, Ph+Pph - Phorate + Potassium phosphonate and Con - Control). B. Mean number of colony forming units of fungi, bacteria and actinomycetes in soil

T. harzianum is reported to have a limited capacity to grow in the rhizosphere (Ahmad and Baker, 1987). Moreover, it is suggested that the main anti-nematode activity caused by *T. harzianum* takes place in soil and not within roots (Sharon *et al*., 2001). In a crop like black pepper which is highly susceptible to root-knot nematodes and produce very large compound galls, the soil phase of this nematode is only for a very brief spell.

Though the parasitism of eggs by these microorganisms significantly reduced multiplication of nematodes, their level in roots was still very high. When the nematode levels are very high, galls are larger in size, and fewer egg masses are present externally. Therefore, it may take more time for the applied fungi to reduce nematode populations to non-damaging levels. *P. chlamydosporia* did not colonize cells of tomato roots in an experiment and hence *M. arenaria* egg masses that formed inside galls were protected from parasitism by the fungus (Leij and Kerry, 1991). In spite of this, the yellowing in black pepper plants has come down and there was a steady increase in yield, wherever *P. chlamydosporia* was applied. The yield increase was almost similar to that obtained with nematicides.

P. chlamydosporia established and survived in the organic soil during the course of the experiment as the reisolation studies proved. This was expected because organic soils have been reported to be a better substrate for the growth of *P. chlamydosporia* than mineral soils (Kerry *et al*., 1993). The tritrophic interaction between root-knot nematodes, *P. chlamydosporia* and the host plant is very complex (Kerry, 2001). Leij *et al*. (1993) showed that some isolates of this fungus were good root colonizers. In addition, the robust chlamydospores ensure the better survival and rhizosphere colonization of this fungus.

P. penetrans, since deployed as a single control measure, failed to give consistent and durable control of nematodes in perennials. It has been evaluated in perennial crops like kiwi (Verdejo, 1992). Under a continuous crop it may require more time to suppress the nematodes (Oostendorp *et al*., 1991).

Determination of the exact prerequisite conditions for successful infection of the nematode by the applied biocontrol agents is clearly difficult given the number of possible variables such as quality of inoculum supply, condition of plant host, colonization potential, rhizosphere competence, condition of the nematodes and the complexity of the soil habitat (Morgan-Jones *et al*., 1981). Microbial communities are spatially discrete. They tend to occupy the same favoured sites and therefore interactions occur between them. Therefore, these interactions determine the fate of the introduced inoculants that are applied for the biological control of pests and pathogens. Many attempts to establish biocontrol agents in field soil have failed because of the antagonistic interaction of endemic components of these microflora (Stirling, 1991).

In conclusion, the long term field study has proved the efficacy of all the three biocontrol agents in suppressing root knot nematodes. However, considering the ease of mass multiplication, saprophytic nature and the resilience of chlamydospores produced, only *P. chlamydosporia* is advocated for biological management of root knot nematodes infesting black pepper. More studies are needed at different locations using varying dosages and frequency of applications of *P. chlamydosporia* to realize sustainable nematode management in black pepper gardens.

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