

EVALUATION OF SOME POTENTIAL METHODS FOR  
CONTROL OF ROOT KNOT NEMATODES IN BLACK PEPPER  
(*PIPER NIGRUM*)

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ABSTRACT

In a field study different methods for controlling or suppressing populations of root-knot nematodes (*Meloidogyne* spp) on black pepper were compared. A nematicide, phenamiphos was more effective in controlling the number of nematodes but showed some phytotoxicity. The biological control agent *Pasteuria penetrans* showed promise by parasitizing female nematodes and preventing reproduction. Similarly, leaf mulches of *Gliricidia* used alone and in combination with *P. penetrans* could have some beneficial effects over the long-term by suppressing the population development of the root parasite.

KEY WORDS: *Gliricidia*, Nematode control, *Pasteuria*, Phenamiphos *Piper nigrum*

INTRODUCTION

The cultivation of black pepper (*Piper nigrum*) in Sri Lanka is long established and has earned handsome foreign exchange for several decades. During the 1970s the varieties, Panniyur 1 and Kuching, were introduced to achieve higher yields per unit area and their commercial cultivation was encouraged. Following this, several previously unrecorded

diseases appeared to be important. The incidence of slow wilt disease of pepper is considered to be one of the major diseases of pepper in Sri Lanka.

The first indication of slow wilt disease is the appearance of occasional wilted yellow leaves which increase in numbers at later stages. Growth ceases when yellowing becomes apparent and production of pepper rapidly declines. Defoliation, severe die-back and eventual death of the vines follow. Affected vines mostly have very few lateral roots and they are often damaged and decayed. Thus the main roots too become affected and devoid of laterals and feeder roots.

The association of nematodes, *Meloidogyne* spp and *Radopholus similis*, with slow wilt disease is recorded in Brazil, India, Indonesia and Malaysia (Hubert, 1957; Koshy and Bridge, 1990; Mustika, Unpublished, 1990; Ting, 1975). The predominant species found in the pepper growing areas of Sri Lanka were *Meloidogyne incognita* (Kofoid & White) Chitw., *M. javanica* (Traub) Chitw., *Radopholus similis* and *Rotylenchulus reniformis* Linford & Olivera (H. A. Ratnasoma, Unpublished, 1991).

Black pepper is mainly grown on live *Gliricidia sepium* supports which are maintained by trimming at a height of 3—4.5 m. In addition to its use as a live support and provider of shade, *Gliricidia* shows resistance to *Meloidogyne* spp. (Koshy *et al.*, 1977). The resistance of *Gliricidia* to *Meloidogyne* infestation is not understood, but it may be possible that it releases some repellent or nematicidal exudates from its roots as suggested by Ochse and Brewton (1954) for *Crotalaria*. Assuming that the same substance in roots could be available in the leaves of *Gliricidia*, leaves were used in this study as an organic amendment.

*Pasteuria penetrans* Sayre & Starr is a promising biological control agent for *Meloidogyne* spp. (Davies *et al.*, 1988). Spores of *P. penetrans* attach themselves to the cuticles of nematodes during their migration through soil and germinate after the entry of juveniles into roots where they begin feeding. Subsequent formation of microcolonies which sporulate result in spores, filling up the body of the female and preventing its reproduction (Sayre and Wergin, 1977).

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In this study the effect of *Gliricidia*, *P. penetrans*, and the nematocide, phenamiphos, were tested singly and in combination for control of *Meloidogyne javanica*.

### MATERIALS AND METHODS

A field established with *Gliricidia* live supports planted at a spacing of 2.4 m × 2.4 m was selected and each base of *Gliricidia* was sterilized by 1,3 dichloropropane and 1, 2 dichloropropane (4 ml/base). The field was kept fallow for one month before planting of pepper vines. Thereafter, pepper vines, Panniyur 1 (approx. 30 cm in height) were transplanted and left for 2 weeks to establish. The following treatments, replicated four times, were tested: Control, Nematodes only, *P. penetrans* (200 mg/kg soil), *Gliricidia* (18 g/kg soil), Phenamiphos (7 g/vine), *P. penetrans* (200 mg/kg soil) + *Gliricidia* (18 g/kg soil), Phenamiphos (7 g/vine) + *Gliricidia* (18 g/kg soil), and *P. penetrans* (200 mg/kg soil) + Phenamiphos (7 g/vine).

Each pepper vine excluding controls was inoculated with 3000 eggs of *Meloidogyne javanica*. The height of pepper vines was recorded immediately after nematode inoculation. Again after a period of 3 months, height of vines was recorded: thereafter each vine was uprooted, washed free of soil, roots excised, dipped in a pectinase solution (1×9 times diluted Pectinex 3 XL, NOVO Industri, Denmark) for 5—7 days and nematodes were extracted by maceration technique (Southey, 1986). Simultaneously, 250 g of soil from the base of each vine was collected and juveniles were extracted by the sieving technique (Southey, 1986). Numbers of females, juveniles, parasitized females and spore attached juveniles were then counted.

### RESULTS

Table 1 shows the height increase of pepper vines 12 weeks after nematode inoculation of *M. javanica*. Vines treated with only nematodes had the lowest height increase indicating probably the adverse effect of nematodes on the vine growth. Vines treated with *Gliricidia* achieved a significant height increase whereas in phenamiphos treated vines there was growth suppression.

**Table 1.** Height of pepper vines 12 weeks after inoculation with *M. javanica* (mean of four replicates)

<i>Treatment</i>	<i>Height (cm)</i>
Control	4.76
Nematodes only	1.59
<i>P. penetrans</i>	4.13
<i>Gliricidia</i>	6.35
Phenamiphos	1.91
<i>P. penetrans</i> + <i>Gliricidia</i>	5.08
Phenamiphos + <i>Gliricidia</i>	3.18
<i>P. penetrans</i> + Phenamiphos	2.22
SED	0.49
	P < 0.01

Table 2 illustrates the effect of different treatments on total number of females and parasitized females (on treatments having *P. penetrans*) recovered from pepper roots. Treatments giving the lowest total number of females and/or highest number of parasitized females were considered as the most effective treatments. The treatments, phenamiphos alone and its combinations resulted in lower

**Table 2.** Total number of females and parasitized females of *M. javanica* in roots of pepper vines 3 months after inoculation (mean of four replicates)

<i>Treatment</i>	<i>Total number of females</i>	<i>Parasitized females</i>
Control		
Nematodes only	77 (8.7)	
<i>P. penetrans</i>	61 (7.1)	37 (6.1)
<i>Gliricidia</i>	34 (5.7)	
Phenamiphos	11 (3.3)	
<i>P. penetrans</i> + <i>Gliricidia</i>	29 (5.3)	10 (3.0)
Phenamiphos + <i>Gliricidia</i>	22 (4.7)	
<i>P. penetrans</i> + Phenamiphos	13 (3.6)	4 (2.1)
SED	0.5	0.5
	P < 0.001	P < 0.001

Square root transformations of means are given in parentheses.

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total number of females compared to the other treatments. In the treatment containing *P. penetrans* alone more nematodes were parasitized than in other combinations of *P. penetrans*.

The number of juveniles in soil and the levels of spore attachment on those treatments having *P. penetrans* are shown in Table 3. *P. penetrans* + phenamiphos had the lowest number of

**Table 3. Total number of juveniles and spore attached juveniles (mean of four replicates)**

<i>Treatment</i>	<i>Total number of juveniles/kg of soil</i>	<i>Spore attached juveniles/kg of soil</i>
Control		
Nematodes only	1920 (43.8)	
<i>P. penetrans</i>	440 (20.7)	207 (13.9)
<i>Gliricidia</i>	843 (28.8)	
Phenamiphos	268 (16.3)	
<i>P. penetrans</i> + <i>Gliricidia</i>	620 (24.8)	232 (15.1)
Phenamiphos + <i>Gliricidia</i>	552 (23.4)	
<i>P. penetrans</i> + Phenamiphos	149 (12.4)	68 (8.1)
SED	2.1	2.1
	P < 0.001	P < 0.05

Square root transformations of means are given in parentheses

juveniles whereas *P. penetrans* + *Gliricidia* had highest number of spore attached juveniles. In order to find out the best treatment or treatments a ranking system was developed by giving scores of 1—7 to each of the treatments for plant height, total number of females and total number of juveniles (Table 4). Based on this scoring system, all treatments except nematodes only treatment can be considered effective for nematode control. The order was *P. penetrans* + phenamiphos > phenamiphos > *P. penetrans* + *Gliricidia* = Phenamiphos + *Gliricidia* > *P. penetrans* = *Gliricidia*.

Table 4. The relative effectiveness of nematode control treatments according to plant height and nematode populations in roots and soil based on their ranking

<i>Treatment</i>	<i>Height increase</i>	<i>Total number of females</i>	<i>Total number of juveniles</i>	<i>Overall score</i>
Nematodes only	1	1	1	03
<i>P. penetrans</i>	5	2	5	12
<i>Gliricidia</i>	7	3	2	12
Phenamiphos	2	7	6	15
<i>P. penetrans</i> + <i>Gliricidia</i>	6	4	3	13
Phenamiphos + <i>Gliricidia</i>	4	5	4	13
<i>P. penetrans</i> + Phenamiphos	3	6	7	16

## DISCUSSION

It is a normal practice for Sri Lankan farmers to lop the *Gliricidia* supports twice a year with the onset of yala and maha rains. These lopped branches placed near the base of pepper vines act as a mulch and also as a source of green manure. Results of this experiment indicated that *Gliricidia* increased the crop growth and controlled nematode population. With little extra labour, farmers could incorporate the branches into soil thus saving the expenses of using nematicides.

Despite the fact that phenamiphos adversely affected the growth of pepper vines, overall, it has become a better treatment either singly or in combinations with other main treatments. However, the use of nematicides is costly; therefore it is desirable to look for alternative treatments.

Results also indicated that *P. penetrans* could be used as an effective treatment for nematode control when combined with other treatments. It is also reported to be compatible with several nematicides such as phenamiphos, carbofuran and ethoprop (Sayre, 1980).

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Although treatments having only *P. penetrans* do not have immediate effects on populations, it does seem a more promising technique if its influence on the reproductive potential of root-knot nematodes is considered.

Thus, of those treatments, nematicide-free combination of *P. penetrans* and *Gliricidia* seems to be a promising control measure of root-knot nematodes.

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