Vol. 39, No. 2, pp. 201-206 Indian Journal of Nematology December, 2009

Effect of Bio-Control Agent, *Paecilomyces lilacinus* Along with Neemcake and Botanicals for the Management of *Meloidogyne incognita* on Banana

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ABSTRACT: Effect of bio-control agent (*Paecilomyces lilacinus*), neem cake and botanicals (*Tagetes erecta* and *Solanum torvum*) against root-knot nematode, *Meloidogyne incognita* in cv. Robusta was tested by applying individually and in combinations under pot condition. Results revealed that all the treatments were effective in increasing the plant growth with significant reduction in nematode populations. Among the treatments, the combined application of *P. lilacinus* + neem cake and *P. lilacinus* +*T. erecta* (flower extracts) resulted in maximum increase of plant height (5.3 cm each), number of leaves (26.6 & 25.0), pseudostem girth (11.7cm &11.0 cm), root length (36 & 26), number of healthy roots (42 & 43), root weight (45 each), root gall index (1) and nematode population from soils (30 & 50/250cc) and roots (110 & 115/5g) compared to the maximum root gall index (5) with the nematode population from soil (1020/250 cc) and roots (1760/5g) in nematode alone- inoculated control plants. The estimation of proteins, phenol, peroxidase and polyphenol oxidase detected from roots and leaves showed an increase in total orth-dihydroxy phenols and enhanced activities of polyphenol oxidase and peroxidase on inoculation of *M.* incognita.

Key words: Robusta, *Meloidogyne incognita*, *Paecilomyces lilacinus*,*Tagetes erecta*, *Solanum torvum*, neem cake, protein, phenol, peroxidase, polyphenol oxidase

Banana (*Musa* spp.) is one of the important commercial fruit crops grown widely in both tropical and sub-tropical conditions under different production systems. The root-knot nematode, *Meloidogyne incognita* considered to be the most economically important nematode pests of banana is widely distributed in South India (Sundararaju, 1996). Crop losses caused due to *M. incognita* in 'Poovan' was reported to be 30.9% (Jonathan & Rajendran, 2000). Management of nematode problems by indiscriminate use of nematicides has proved disastrous to the environment and human. Incorporation of organic amendment like neem cake, botanicals and bio-control agents have shown to be most effective ways to manage nematodes without affecting the produce (Jonathan *et al.* 1995; Sundararaju & Cannayane, 2003; Sundararaju & Kumar, 2003). Since, the nematodes are very well managed by using organic amendments/bio-control agents in different horticultural and plantation crops, an attempt was, therefore, made to evaluate the efficacy of bio-control agent (*Paecilomyces lilacinus*), organic amendment (Neem cake) and botanicals (*Tagetes erecta* and *Solanum torvum*) individually and in combination for the management of root-knot nematode problem on banana cv. Robusta.

MATERIALS AND METHODS

The pot culture experiment was conducted at the Research Farm, National Research Centre for Banana at Tiruchirapalli during 2006-07. Forty banana suckers from cultivar Robusta of uniform size with an average weight of 1.5 to 2 kg selected from the field were pared and dipped in hot water at a temperature range of 50-55^{0}C for 10 minutes. The suckers were planted individually in forty earthen pots containing 5 kg of soil mixture (Red soil; Sand; FYM) in the ratio of 2:1:1 arranged in a randomized manner and watered regularly under shade net condition. There were seventeen treatments replicated three treatment details are furnished below:

Treatment details

T-1 *P. lilacinus* alone	-10 g/plant
T-2 *Tagetes erecta* sp. (leaf extracts)	-10 g/plant
T-3 *Tagetes erecta* sp. (flower extracts)	-10 g/plant
T-4 Neem cake	-100 g/plant
T-5 *Solanum torvum*	-10 g/plant
T-6 *P. lilacinus* + *Tagetes erecta* (leaf extracts)	-each 10g/plant

T-7 *P. lilacinus* + *Tagetes erecta* (flower extracts)	-each 10g/plant
T-8 *P. lilacinus* + Neem cake	-each 10g + 100 g/plant
T-9 *P. lilacinus* + *Solanum torvum*	-each 10g/plant
T-10 *Tagetes erecta* (leaf extracts) + *Tagetes erecta* (flower extracts)	-each 10g/plant
T-11 *Tagetes erecta* (leaf extracts) + Neem cake	-each 10g + 100g/plant
T-12 *Tagetes erecta* (leaf extracts) + *Solanum torvum*	-each 10g/plant
T-13 *Tagetes erecta* (flower extracts) + Neem cake	-each 10g + 100g/plant
T-14 *Tagetes erecta* (flower extracts) + *Solanum torvum*	-each 10g/plant
T-15 Neem cake + *Solanum torvum*	-each 100g + 25 g/plant
T-16 Nematode alone	
T-17 Untreated Control (Check)	

Egg masses of root-knot nematode, *M. incognita* were collected from pure culture maintained on coleus plants. These egg masses were transferred into a beaker containing tap water. The top of the beaker was closed with a piece of polythene sheet with small holes for aeration. This was incubated at room temperature for 3-4 days. The water at the top was removed and then fresh water was added and aerated every day. After three days, freshly hatched juveniles (J2) of *M. incognita* were collected and used as a source of inoculum.

Fresh leaves and flowers of *T. erecta* were collected in and around banana garden at NRCB Farm, Podavur. The leaves were first washed with tap water and dried under shade for 10 days. The dried leaves were then powdered and 10g each were used in all the treatments. Pure culture of the nematode egg parasitic fungus *P. lilacinus* was locally isolated from banana field. This was cultured on Potato Dextrose Agar (PDA). This isolate of *P. lilacinus* was used for the present investigation against *M. incognita.*

Thirty days after planting, the experimental plants were inoculated with root-knot nematode, *M. incognita* @ 5000 J2s/plant. One month after nematode inoculation, the required quantity of botanicals, neem cake and bio-control agent alone and in different combinations were incorporated in the experimental plants. All the treated plants were watered and fertilized as needed. The experiment was terminated three months after inoculation. The plants were removed carefully (with intact root system) and the roots washed thoroughly to remove the adhering soil particles. Growth characters including plant height, pseudostem girth, number of leaves, root length, number of roots, number of healthy roots, number of infected roots, and root weight were recorded. The root galling was assessed by examining the roots on the outside by visual observation of the whole root system based on the percentage of galled roots. The percentage was converted to a scale of 0-5 viz. 0=no galls; 1=trace infection with a few small galls; 2=25% roots galled; 3=25-50%; 4=50-75% roots galled; 5=75% roots galled. After this, roots were cut into small pieces, mixed thoroughly and samples of 5g each were stained in boiling acid fuschin-lacto phenol. These were blended and nematode populations (eggs, different stages of larvae and adult) were assessed. Soil samples (250cc) were also collected from each pot for estimating the nematode population. Nematode population from soil was estimated by using Cobb's sieving and decanting method followed by modified Baermann's funnel method.

Biochemical analysis of all the samples was carried out in roots since nematodes are associated with root system of banana. Root samples collected from both healthy and nematode inoculated plants were washed thoroughly in order to remove adhering debris and soil partihles. Later the samples were stored at 4°C for biochemical analysis. Estimation of the phenols, protein chlorophyll and assay of peroxidase and polyphenoloxidase were performed as per the method described by earlier workers (Malick & Singh, 1980; and Lowry's *et al.* 1951). The data were statistically analyzed (Gomez & Gomez, 1994) and treatment means were compared by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The data summarized in Table 1 & 2 clearly indicated that all the treatments either alone or in combination were effective in increasing the plant growth with significant reduction in nematode populations. Among

Table 1. Effect of promising botanicals, neem cake and biocontrol agent, *Paecilomyces lilacinus* against *Meloidogyne incognita* on plant growth parameters of banana

Treatments	Plant height (cm)	No. of leaves of roots	Pseudostem girth (cm)	Total number	Root length (cm)	Root weight (g)
T1 *P. lilacinus* alone	4.33	21.00	10.20	24	22	40
T2 *Tagetes erecta* (leaf extracts)	5.00	22.30	10.00	42	20	41
T3 *T. erecta* (flower extracts)	5.00	23.66	11.20	44	23	43
T4 Neem cake	5.00	19.16	9.50	37	23	30
T5 *Solanum torvum*	4.66	19.66	9.83	35	20	30
T6 T1+T2	5.00	24.66	11.00	30	22	40
T7 T1+T3	5.33	25.00	11.16	48	26	45
T8 T1+T4	5.33	26.66	11.66	46	36	45
T9 T1+T5	4.33	19.50	9.66	18	29	35
T10 T2+T3	4.00	18.00	7.16	34	27	45
T11 T2+T4	5.00	17.00	8.66	36	30	40
TI2 T2+T5	4.66	20.66	9.00	38	24	45
T13 T3+T4	4.33	18.66	9.00	32	32	35
TI4 T3+T5	4.00	19.66	8.66	34	35	40
T15 T4+T5	4.00	20.00	8.00	23	28	35
TI6 Nematode alone	3.33	11.66	6.16	31	15	20
T17 Untreated Control (Check)	4.00	18.75	10.25	40	21	45
SED	0.78	3.29	1.51	0.73	0.58	2.46
CD(P=0.05)	1.59	6.71	3.08	1.51	1.18	5.01
CD(P=0.01)	2.13	9.03	4.14	2.03	1.59	6.74
CV%	20.99	22.57	20.60	2.58	2.78	7.82

the individual treatments, significant increase in plant height (5.0 cm) number of leaves (23.7), pseudostem girth (11.2 cm), root length (23cm), number of healthy roots (38) and root weight (43) was recorded from plants treated with flower extracts of *T. erecta* followed by leaf extracts of *T. erecta* and neem cake alone over untreated control that medium root gall index (2) and significant reduction in nematode population from soils (70/250cc) and roots (190/5g) were recorded from the same treatment compared to the maximum root gall index (5) with the nematode population from soil (1020/250cc) and roots (1760/5g) in nematode alone inoculated control plants. However, results obtained from all the other individual treatments were on par with each other. Among the combined treatments, the combined application of *P. lilacinus* + neem cake and *P. lilacinus* + *T. erecta* (flower extracts) resulted maximum increase in plant height (5.3 cm each), number of leaves (26.6 & 25.0), pseudo stem girth (11.7 cm & 11.0 cm), root length (36 & 26), number of healthy roots (42 & 43) and root weight (45 each) over nematode alone inoculated control plants. Minimum root gall index (1) and nematode population from soils (30 & 50/250cc) and roots (110 & 115/5g) were recorded compared to the maximum root gall index (5) with the nematode population from soil (1020/250 cc) and roots (1760/5g) in nematode alone-inoculated control plants. The other combined treatments were on par with each other. These findings were in agreement with Mojumdar and Mishra (1999) who reported that aqueous extracts of leaf, stem and roots of *T. erecta* and neem leaves from autumn fall have nematicidal property against *M. incognita*.

Table 2. Effect of promising botanicals, neem cake and biocontrol agent, *Paecilomyces lilacinus* on host infestation and nematode multiplication in soil and root system of banana

Treatments	Number of healthy roots	Number of infected roots	Root-knot nematodes	Total number of nematodes	
				Soil (250cc)	Root (12m)
T1 *P. lilacinus* alone	17	21.2	4	90	320
T2 *Tagetes erecta* (leaf extracts)	34	19.5	3	60	315
T3 *T. erecta* (flower extracts)	38	13.5	2	70	190
T4 Neem cake	33	28.8	4	260	690
T5 *Solanum torvum*	29	29.5	4	200	625
T6 T1+T2	26	15.5	1	40	125
T7 T1+T3	43	12.5	1	50	115
T8 TI+T4	42	10.5	1	30	110
T9 TI+T5	15	18.5	3	65	285
T10 T2+T3	30	20.0	3	45	240
T11 T2+T4	33	16.5	3	55	200
TI2 T2+T5	35	17.5	4	70	450
T13 T3+T4	28	12.5	3	60	280
TI4 T3+T5	26	18.5	2	50	140
T15 T4+T5	17	16.1	4	90	375
TI6 Nematode alone	16	71.5	5	1020	1760
TI7 Untreated Control (Check)	37	19.5	1	0	0
SED	0.95	0.72	1.12	5.68	8.36
CD(P=0.05)	1.93	1.47	2.28	11.58	17.03
CD (P=0.01)	2.59	1.97	3.06	15.57	22.89
CV%	3.95	4.12	39.93	5.18	2.79

The results of the study clearly indicated that though the individual treatment has proved to be the effective treatment against *M. incognita* but over all performance was noticed better in plants treated with *P. lilacinus* + neem cake followed by *P. lilacinus* + *T. erecta* (flower extracts) and *P. lilacinus* + *T. erecta.* (leaf extracts). The present results are in confirmitive with earlier worker (Nagesh *et al.*, 2003) who reported that the combined use of *P. lilacinus* formulation with neem cake enhanced fungal propagules in rhizosphere (700-1070), fungal infectivity (32-52%) and yield (23-28%) in chrysanthemum depending on the dose of formulation and reduced *M. incognita* population. Similar results were obtained by Munoz *et al.* (1982) identified the nematicidal root exudates of *T. erecta,* as terthienyl and other alkaloids. Anti nematode effect of neem cake may be attributed to the phenolic compounds released during its degradation apart from its stimulatory effect on root growth and predaceous fungi and also to small amount of azadirachtin present in it.

The estimation of proteins, phenol, peroxidase and polyphenol oxidase detected from roots and leaves of above experiments are given in Table-3. Significant variation in protein concentration was noticed among the treatments. The highest concentration of protein was recorded in 1.40 mg/g and 7.32 mg/g with respect to root and leaf samples in the treatment combination of *P. lilacinus* + neem cake. However, the lowest concentration of protein was recorded in 0.4 mg/g and 3.36 in nematode infected plants. Similar to Protein, the highest concentration of phenol (2.11 mg/g) was also

Table 3. Concentration of protein, phenol, polyphenol oxidase, peroxidase and chlorophyll content in roots and leaves of banana

Treatments	Phenol mg/g of sample		Protem mg/g of sample		Polyphenol oxidase units/ mg of protein		Peroxidase units/ mg of protein		Chlorophyll mg/g of sample
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	
TI *P.lilacinus* alone	0.51	1.15	1.04	5.16	0.20	9.98	11.87	1.76	0.53
T2 *Tagetes erecta* (leaf extracts)	0.21	0.69	0.40	3.36	0.04	2.71	6.07	0.80	0.87
T3 *T. erecta* (flower extracts)	0.59	1.20	1.00	5.12	0.18	9.54	8.24	1.92	0.43
T4 Neem cake	0.41	0.79	0.80	5.04	0.03	3.34	5.37	1.20	0.62
T5 *Solanum torvum*	0.34	0.68	0.76	5.00	0.80	1.47	6.07	1.60	0.85
T6 T1+T2	0.76	1.58	1.32	6.32	0.06	11.86	13.60	2.32	0.79
T7 T1+T3	0.88	1.81	1.36	7.02	1.00	12.40	18.30	2.64	0.53
T8 TI+T4	0.91	2.11	1.40	7.32	1.21	14.77	28.20	2.80	0.31
T9 T1+T5	0.60	1.00	0.88	5.16	0.09	5.23	6.14	1.44	0.42
T10 T2+T3	0.59	1.10	0.96	4.96	0.07	4.49	7.32	2.00	0.50
T11 T2+T4	0.42	0.11	0.60	4.20	0.10	1.52	4.50	0.80	0.40
TI2 T2+T5	0.39	0.78	0.72	4.52	0.11	2.50	9.07	0.98	0.46
T13 T3+T4	0.48	0.92	1.08	5.04	0.17	7.88	8.37	1.52	0.48
T14 T3+T5	0.61	1.49	1.12	5.56	0.26	11.02	11.87	2.08	0.51
T15 T4+T5	0.72	1.50	1.16	5.66	0.27	11.67	12.50	2.24	0.42
TI6 Nematode alone	0.32	0.92	0.65	3.64	0.06	2.46	6.14	1.00	0.59
TI7 Untreated Control	0.58	1.09	0.96	4.24	0.15	6.91	8.43	1.60	0.98
SED	11.09	0.14	0.04	0.08	0.06	0.14	0.009	132.77	0.19
CD (P=0.05)	22.56	0.29	0.029	0.16	0.13	0.38	0.019	270.46	0.39
CD (P=0.01)	3.03	0.39	0.04	0.22	0.17	0.28	0.026	363.61	0.53
CV%	6.74	15.14	7.33	7.90	80.85	23.32	3.98	537.27	42.59

accumulated in the leaves from the treatment combination of *P.lilacinus* + neem cake whereas the lowest concentration of phenol was accumulated in 0.21 mg/g in nematode infected plants. The present results are in agreement with Devarajan (1995) who reported that highest phenol activity was recorded in *P. lilacinus* inoculated plants in banana. Application of *P. lilacinus* increased chlorophyll content. Several researchers have emphasized the role of phenols as an expression of defence mechanism by the host plants (Bajaj *et al.*, 1983; Bleve-zacheo *et al.*, 1990). In roots, the highest concentration of peroxidase 28.20 units/min/mg was recorded in the treatment of *P. lilacinus* + neem cake. However, the minimum concentration of peroxidase 4.50 units/min/mg of protein was recorded in the treatment of *T. erecta* (leaf extracts) + neem cake.

In leaves, the highest concentration of peroxidase 2.80 units/min/mg of protein was recorded in the treatment of *P. lilacinus* + neem cake. The lowest concentration of peroxidase 0.80 units/min/mg of was recorded in treatment of *T. erecta* (leaf extracts) and neem cake than other treatments. Similar results were obtained for poly phenol oxidase and chlorophyll content from root and leaf samples. These results are in conformity with the earlier research workers who reported that increase in total and ortho-dihydroxy phenols and enhanced activities of polyphenol oxidase and peroxidase was

recorded with the inoculation of *M. incognita* on different agricultural crops (Ganguly & Dasgupta, 1984; Bajaj *et al.*, 1985; Gapasin *et al.*, 1988; Sundararaju & Pandisubha, 2006).

Thus, the present investigation clearly concluded that integration of *P. lilacinus* with neem cake or anyone of the botanicals namely viz., *Tagetes* spp., (leaf or flower extracts), *S. torvum* can be effectively used in the management of root-knot nematode in banana since the use of single bioagent or botanical cannot be very effective in the management of nematode induced disease complex.

ACKNOWLDEGEMENT

Authors are thankful to Dr. M.M. Mustaffa, Director, National Research Centre for Banana, Trichy, Tamil Nadu for providing necessary facilities and encouragement during the conduct of this study. Thanks are also due to Mr. T. Sekar, Technician for his technical assistance rendered in carrying out the work.

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Accepted for publication : September, 2009