BIOLOGICAL CONTROL OF LESION NEMATODES IN BANANA

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Summary. Field trials were conducted to assess the efficacy of biological agents on the control of root lesion nematodes in banana in comparison with the nematicide carbofuran. The bio-agents were *Pseudomonas fluorescens*, *Trichoderma viride*, *Glomus fasciculatum, Bacillus subtilis* and *Paecilomyces lilacinus*. Application of *P. fluorescens* at 20 g/plant gave the greatest bunch length (95 cm), bunch weight (24 kg), number of hands per bunch (10) and number of fingers per bunch (176). The increases in yield parameters ranged from 59 to 110%. *Pseudomonas fluorescens* was also the most effective to control nematodes until harvest as the populations of *Radopholus similis, Pratylenchus coffeae* and *Helicotylenchus multicinctus* were reduced by 48.7, 46.3 and 44.3%, respectively. Soil application of *T. viride* or carbofuran were the next best treatments followed by the application of *G. fasciculatum*, *B. subtilis* and *P. lilacinus*. The lowest bunch weight (15 kg) was obtained from the control plants. Carbofuran was the most effective to control nematodes until three months after planting, but its efficacy thereafter was reduced and remained at a level similar to that of *P. fluorescens*. Root colonization by *P. fluorescens, T. viride, G. fasciculatum*, and *B. subtilis* was 105×10^8cfu/g, 60×10^6 cfu/g, 44% and 58×10^8 cells/g of root.

Key words: *Bacillus subtilis, Glomus fasciculatum, Musa* sp., *Paecilomyces lilacinus, Pseudomonas fluorescens*, *Trichoderma viride.*

Banana (*Musa* sp.) is cultivated in the humid tropics and sub-tropics in the central and southern states of India, including Tamil Nadu. Its cultivation is predominantly polyclonal and the choice of cultivars is determined by regional preferences. The crop is well adapted to a variety of soil conditions and yield is high under a favourable climate and adequate irrigation. However, it is prone to attack by pathogens such as fungi, viruses, bacteria and nematodes. Among these, plant parasitic nematodes constitute one of the major factors limiting banana production because of the extensive root damage they cause. The burrowing nematode, *Radopholus similis* (Cobb) Thorne, root lesion nematode, *Pratylenchus coffeae* (Zimmermann) Filipjev *et* Schuurmans Stekhoven, and spiral nematode, *Helicotylenchus multicinctus* (Cobb) Golden, are reported to be the predominant and most severe nematode pests in Tamil Nadu (Rajendran *et al.*, 1980). Biological control has long been considered a good alternative to nematicides for controlling nematodes, due to the great adaptability and multiplication of biological agents in soils rich in organic matter as are those in the banana growing areas of India. Among these agents, the plant growth promoting rhizobacteria, fluorescent pseudomonads, constitute a major bacterial group and *Bacillus* spp. were found to be effective in suppressing phytonematodes in many crops (Oostendorp and Sikora, 1990; Shanthi and Sivakumar, 1995; Cannayane and Rajendran, 2001). *Trichoderma viride* (Pers. ex. S.F. Gray), *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerd. *et* Trappe and the egg parasite, *Paecilomyces lilacinus* (Thom.) Samson, were also found effective in suppressing phytonematodes (Harish and Gowda, 2001; Umesh *et al.*, 1988; Jonathan and Rajendran, 2000). Considering the above problems and prospects, the present investigation was undertaken to test the efficacy of several biocontrol agents against lesion nematodes in banana.

MATERIALS AND METHODS

Two field trials were conducted in farmers' holdings at Telungupalayam, Coimbatore, during 2001-2003, where severe incidence of nematodes *viz., R. similis, P. coffeae* and *H. multicinctus* had beeen recorded in the previous crop. The field was selected because its soil nematode population density was larger than the economic threshold level (> one juvenile/g soil (Rajendran *et al.*, 1980). It was divided into twenty eight plots of 30 m^2 each, and initial nematode soil population density was assessed. In each plot, soil samples were collected from the top 30 cm soil and pooled to form a composite sample of 250-500 cm^3 soil. Nematodes were then extracted from 250 cm^3 soil using Cobb's sieving and decanting method followed by a modified Baermann's funnel technique (Schindler, 1961), and counted. The treatments were: soil application of *Pseudomonas fluorescens* Migula at 20 g/plant with a spore load of 2.5×10^8 cfu/g, *Trichoderma viride* at 20 g/plant (20×10^6 cfu/g), Vesicular Arbuscular Mycorrhiza (VAM), *Glomus fasciculatum*, at 250 g/plant (2 spores/g), *Bacillus subtilis* (Ehrenberg) Cohn at 20

g/plant (10×10^8 cells/g), *P. lilacinus* at 20 g infested sorghum grains/plant (2.4×10^6 spores/g), carbofuran 3G at 40 g/plant and an untreated control. The biological agents were local isolates and are available as commercial formulations from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, as *Pseudomonas fluorescens* (Pf-1), *Trichoderma viride*, VAM, *Bacillus subtilis* and *Paecilomyces lilacinus*. These agents and carbofuran were incorporated into the soil at planting. The commercial formulations of *P. fluorescens* (Pf 1), *T. viride* and *B. subtilis* were talc-based products and the commercial formulation of *G. fasciculatum* was prepared on vermiculite flakes, but *P. lilacinus* was cultured on sorghum grains *in vitro*.

Nine nematode-free planting materials (trimmed suckers) of triploid banana (Musa AAA) cv. Robusta were selected and planted per plot at a spacing of 1.8 m × 1.8 m on 03.06.2001 (Trial I) and 14.06.2002 (Trial II). The trials were laid out in a randomised block design with seven treatments and four replications.

Nematode populations in soil (250 cm^3) and root (5 g) were assessed three, six and nine months after planting, and at harvest (13.06.2002, Trial I; 26.06.2003, Trial II). Soil and root samples were collected from the top 30 cm soil from each plot and pooled to form composite samples of 250-500 cm^3 soil or 5-10 g feeder roots. Soil samples were processed as mentioned before and nematodes counted. The nematodes were extracted from the roots using a root maceration technique (Fallis, 1943) followed by a modified Baermann's funnel method (Schindler, 1961).

At harvest (12 months after planting), yield parameters and microbial colonization were assessed. The populations of the nematode antagonists in the rhizosphere soil were estimated by following standard methods: *P. fluorescens* according to Papavizas and Darey (1961) using King's B medium; *T. viride* by the method of Elad and Chet (1983) using the *Trichoderma* selective medium (TSM); *B. subtilis* by the method of Papavizas and Darey (1961) using nutrient agar medium; VAM fungus by recovering the spores by the wet sieving and decanting method of Gerdemann and Nicolson (1963). The roots of bananas inoculated with VAM fungi, were examined for the colonization and presence of VAM hyphae, arbuscules and vesicles by clearing and staining the roots by a modified method of Phillips and Hayman (1970). The spores of *P. lilacinus* were extracted by the following method. Pure culture of the fungus *P. lilacinus* maintained at the Department of Nematology, TNAU, Coimbatore was subcultured in test tubes with potato dextrose agar (PDA) and incubated at room temperature (28±1°C) for 10 days. Commercial sorghum grains were washed and soaked in water for 12 hours. These grains were then filled in half of 500 cc. Erlenmeyer flasks and the flasks were autoclaved for 30 minutes at 15 lb pressure. The fungus was then inoculated to the sorghum grains in a laminar flow-chamber. The flasks were tumbled daily to maintain uniform growth of fungus and to prevent the seeds from sticking together. After 15 days one gram of seed was taken, washed in distilled water and the number of spores present was counted in a haemocytometer. One gram of grain was found to contain more than 10^6 spores . Cultural practices were as suggested by a crop production guide.

The data were first analysed from each trial individually and then, as they were similar, they were pooled, reanalysed and the means compared using Duncan's Multiple Range Test.

Table I. Effect of biocontrol agents on bunch performance of banana cv. Robusta infested with lesion nematodes under field conditions.

Treatment	Bunch length (cm)	Bunch weight (kg)	No. of hands/ bunch	No. of fingers/ bunch
Pseudomonas fluorescens at 20 g/plant	95.6 a	24.3 a	10.1 a	176.0 a
Trichoderma viride at 20 g/plant	86.3 b	22.1 b	9.2 b	147.2 b
Glomus fasciculatum at 250 g/plant	78.5 c	20.3 c	8.5 c	115.5 c
Bacillus subtilis at 20 g/plant	73.2 c	19.3 c	8.0 c	112.1 c
Paecilomyces lilacinus at 20 g/plant	71.3 c	18.1 c	8.0 c	111.6 c
Carbofuran 3G at 40 g/plant	83.1 b	21.1 c	9.0 b	144.2 b
Untreated control	60.1 d	15.3 d	6.3 d	83.5 d

Means in the same column sharing a common letter do not differ significantly according to Duncan's Multiple Range Test (P = 0.05)

RESULTS

Effects on yield parameters. Bunch length, bunch weight, number of hands per bunch and number of fingers per bunch in the plots treated with *P. fluorescens* were 95.6 cm, 24.3 kg, 10.1 hands and 176 fingers, respectively, significantly superior to all the other treatments (Table I). They represented increases over the control of 59.1, 58.9, 60.3 and 110.8%, respectively. Soil applications of *T. viride* or carbofuran 3G were the next best treatments and were not significantly different from each other, with increases in the same yield parameters of 43.6, 44.1, 46.0, 76.3 and 38.3, 37.9, 42.9 and 72.7%, respectively. Increases of yield parameters, after application of *G. fasciculatum*, *B. subtilis* and *P. lilacinus*, were similar to each other but significantly greater than the control. The lowest bunch weight of 15.3 kg per plant occurred in the untreated control (Table I).

Effects on nematode soil populations. There were no significant differences between treatments in nematode soil population densities before planting. Three months after planting, the application of carbofuran 3G resulted in the lowest population densities per 250 cm^3 of soil of *R. similis* (157.3), *P. coffeae* (66.3) and *H. multicinctus* (136.3), representing decreases of 60.2, 57.5 and 50.5% over the untreated control (Table II). The next best treatment was the application of *P. fluorescens*, which provided 48.4, 46.2 and 42.3% reductions in populations of the three lesion nematode species. It was followed by application of *T. viride* and *G. fasciculatum*, which also differed significantly from each other. Applications of *G. fasciculatum*, *B. subtilis* and *P. lilacinus* reduced populations of the three nematodes similarly. Six months after planting, there was little change in the efficacy of the treatments in reducing nematode populations. At this time, the smallest nematode population densities occurred in the plots that received soil application of *P. fluorescens*, with decreases of 51.6, 49.0 and 46.5% relative to the control. A similar efficacy was observed with the application of carbofuran 3G. Application of *T. viride* and *G. fasciculatum* also significantly reduced nematode populations, but this reduction was intermediate between that for the first-mentioned treatments and that achieved with the application of *B. subtilis* or *P. lilacinus*, which was, however, still significant. Similar trends were observed nine months after planting when the decreases in populations of *R. similis*, *P. coffeae* and *H. multicinctus* by different treatments ranged from 49.1 to 35.1, 47.5 to 30.6 and 42.7 to 28.7%, respectively (Table II). At harvest, the application of *P. fluorescens* was also the most effective treatment in reducing the population densities of the three nematode species. Treatment with *T. viride* was the next best, with 46.1, 42.0 and 40.5% decreases, and it was comparable with application of carbofuran 3G. The lowest reduction in nematode populations was given by application of *P. lilacinus* (34.3, 30.4 and 26.0% decrease), which was on par with *B. subtilis* but still significant.

Effects on nematode population in the roots. All treatments decreased the number of lesion nematodes in the roots throughout the crop cycle. However, application of carbofuran 3G and *P. fluorescens* resulted in the lowest nematode infection until nine months after planting (47.1-52.4% reduction) (Table III). At harvest (12 months after planting), the greatest reduction in nematode population (50.4-53.7%) was observed in the roots of bananas treated with *P. fluorescens*. Soil treatments with the other bio-control agents gave intermediate root protection with *T. viride* and *G. fasciculatum* generally performing significantly better than *B. subtilis* and *P. lilacinus* (Table III).

Root colonization by the bio-control agents. Root colonization by *P. fluorescens, T. viride, G. fasciculatum* and *B. subtilis* was 105×10^8cfu/g, 60×10^6 cfu/g, 44% and 58×10^8 cells/g, respectively. In the soil, the spore counts were 213/50 cm^3 for *G. fasciculatum* and 16×10^6/g for *P. lilacinus* (Table IV).

DISCUSSION

In general, the application of the bio-control agents enhanced yield. Among them, application of *P. fluorescens* at planting was the most effective treatment. Increase in yield by *P. fluorescens* may be attributed to the production of plant growth regulators, including gibberellins, cytokinins and indole acetic acid (IAA) (Brown, 1974; Lifshits *et al.*, 1987). Carbofuran was the most effective treatment in reducing nematode population only until three months after planting. Thereafter, the effectiveness of this nematicide was reduced, although it remained as effective as the most effective bio-control agent, *P. fluorescens*. Our findings confirm earlier results reported by Shanthi *et al.* (1998) and Mani *et al.* (1998), who observed that application of formulations of *P. fluorescens* at 1, 2 and 4 g per vine significantly reduced the severity of root knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., infection in grapevine compared with application of carbofuran 3G at 1.8 g a.i./vine. Also, application of *P. fluorescens* at 10 and 20 kg/ha reduced parasitization of potato roots by *Globodera* spp. by 47.7 and 62.6%, respectively (Shanthi *et al.*, 2004).

The persistent effect of *P. fluorescens* throughout the crop growth period might be due to the multiplication of the bio-control agent on the organic substrates in the soil and production of antibiotic compounds which are toxic to nematodes. This agent certainly multiplied in the present study as the density of propagules of *P. fluorescens* in the soil at harvest was greater than that inoculated. Several mechanisms may be involved in the suppression of phytonematodes by *P. fluorescens*. This

Table II. Effect of biocontrol agents on soil population densities of lesion nematodes in banana cv. Robusta under field conditions, three, six, and nine months after planting and at harvest.

Treatment	Initial nematode population/250 cm³ of soil			Number of nematodes/250 cm³ of soil					
				Third month (3.9.2001, 14.9.2002)			Sixth month (3.12.2001, 14.12.2002)		
	R. similis	*P. coffeae*	*H. multicinctus*	*R. similis*	*P. coffeae*	*H. multicinctus*	*R. similis*	*P. coffeae*	*H. multicinctus*
Pseudomonas fluorescens at 20 g/plant	278.0	130.0	211.0	204.3b (48.4)	84.0b (46.2)	159.0b (42.3)	221.5a (51.6)	97.5a (49.0)	175.3a (46.5)
Trichoderma viride at 20 g/plant	283.0	135.0	223.0	236.3c (40.3)	96.5c (38.1)	175.3c (36.4)	264.3b (42.3)	115.3b (39.7)	206.0b (37.1)
Glomus fasciculatum at 250 g/plant	292.0	132.0	229.0	256.0d (35.3)	108.0d (30.8)	192.3d (30.2)	286.5c (37.4)	123.0c (35.6)	218.5c (49.9)
Bacillus subtilis at 20 g/plant	302.0	131.0	226.0	267.8d (32.3)	109.0d (30.1)	197.0d (28.5)	302.3d (34.0)	131.0d (31.4)	230.0[d] (29.8)
Paecilomyces lilacinus at 20 g/plant	309.0	135.0	228.0	272.0d (31.2)	110.5d (29.2)	200.5d (27.2)	305.0d (33.4)	132.8d (30.5)	235.5d (28.1)
Carbofuran 3G at 40 g/plant	311.0	138.0	236.0	157.3a (60.2)	66.3a (57.5)	136.3a (50.5)	228.5a (50.1)	98.3a (48.6)	180.0a (45.0)
Untreated control	329.0	142.0	241.0	395.5e	156.0e	275.5e	458.0e	191.0e	327.5e

Treatment	Number of nematodes/250 cm³ of soil					
	Ninth month (3.3.2002, 14.3.2003)			At harvest (13.6.2002, 24.6.2003)		
	R. similis	*P. coffeae*	*H. multicinctus*	*R. similis*	*P. coffeae*	*H. multicinctus*
Pseudomonas fluorescens at 20 g/plant	265.0a (49.1)	121.0a (47.5)	215.0a (42.7)	308.8a (48.7)	158.0a (46.4)	234.0a (44.4)
Trichoderma viride at 20 g/plant	316.0b (39.3)	145.3b (37.0)	242.3b (34.6)	324.0b (46.1)	171.0b (42.0)	250.3b (40.5)
Glomus fasciculatum at 250 g/plant	320.3c (38.5)	149.5c (35.1)	247.5c (33.2)	367.5c (38.9)	185.3c (37.1)	273.5c (35.0)
Bacillus subtilis at 20 g/plant	332.5d (36.1)	155.3d (32.7)	258.0d (30.4)	390.0d (35.2)	201.3d (31.7)	303.0d (28.0)
Paecilomyces lilacinus at 20 g/plant	338.0d (35.1)	160.0d (30.6)	264.3d (28.7)	395.3d (34.3)	205.0d (30.4)	313.0d (26.0)
Carbofuran 3G at 40 g/plant	270.3a (48.1)	126.0a (45.3)	220.0a (40.6)	328.0b (45.5)	171.5b (41.8)	254.5b (40.0)
Untreated control	520.5e	230.5e	370.5e	601.5e	294.5e	420.5e

Figures in parentheses are per cent decrease relative to control. Means in the same column sharing a common letter do not differ significantly according to Duncan's Multiple Range Test (P = 0.05).

Table III. Effect of bio-control agents on root population densities of lesion nematodes in banana cv. Robusta under field conditions, three, six, and nine month after planting and at harvest.

Treatment	Number of nematodes/5 g root					
	Third month (3.9.2001, 14.9.2002)			Sixth month (3.12.2001, 14.12.2002)		
	R. similis	*P. coffeae*	*H. multicinctus*	*R. similis*	*P. coffeae*	*H. multicinctus*
Pseudomonas fluorescens at 20 g/plant	84.0a (54.2)	39.0a (52.2)	47.0a (50.5)	96.0a (54.0)	50.5a (52.6)	71.0a (43.4)
Trichoderma viride at 20 g/plant	92.3b (48.1)	46.3b (43.3)	52.5b (44.7)	109.0b (47.7)	57.0b (46.5)	79.5b (36.7)
Glomus fasciculatum at 250 g/plant	101.0b (45.1)	48.5b (40.5)	57.3b (39.7)	115.5b (44.6)	58.3b (45.3)	83.8b (33.3)
Bacillus subtilis at 20 g/plant	108.0b (41.1	50.0b (38.7)	59.0b (37.9)	120.8b (42.1)	64.5b (39.4)	87.5b (30.3)
Paecilomyces lilacinus at 20 g/plant	110.3b (39.9)	52.3b (35.9)	62.3b (34.5)	125.3b (39.9)	66.0b (38.0)	91.5b (27.1)
Carbofuran 3G at 40 g/plant	64.0a (65.1)	32.3a (60.4)	39.5a (58.4)	92.5a (55.6)	52.3a (50.9)	65.0a (48.2)
Untreated control	183.5c	81.5c	95.0c	208.5c	106.5c	125.5c

Treatment	Number of nematodes/5 g root					
	Ninth month (3.3.2002, 14.3.2003)			At harvest (13.6.2002, 24.6.2003)		
	R. similis	*P. coffeae*	*H. multicinctus*	*R. similis*	*P. coffeae*	*H. multicinctus*
Pseudomonas fluorescens at 20 g/plant	120.0a (52.4)	67.5a (50.2)	75.0a (48.5)	147.0a (50.4)	73.0a (53.7)	90.3a (51.7)
Trichoderma viride at 20 g/plant	134.3b (46.5)	74.0b (45.4)	83.0b (43.0)	161.3b (45.6)	85.3c (45.9)	111.5b (40.2)
Glomus fasciculatum at 250 g/plant	138.5b (44.8)	77.3b (43.0)	86.5b (40.1)	168.3c (43.3)	89.5c (43.2)	117.3c (37.1)
Bacillus subtilis at 20 g/plant	146.5c (41.6)	81.0c (40.2)	96.0c (34.0)	177.5d (40.1)	95.0d (39.7)	123.0d (34.1)
Paecilomyces lilacinus at 20 g/plant	152.3c (39.3)	85.3c (37.1)	94.3c (35.2)	181.0d (39.0)	101.3d (35.7)	125.3d (32.8)
Carbofuran 3G at 40 g/plant	124.3a (50.5)	69.0a (49.1)	77.0a (47.1)	156.3b (47.3)	83.5b (47.0)	96.0b (48.5)
Untreated control	251.0d	135.5d	145.5d	296.5e	157.5e	186.5e

Figures in parentheses are per cent decrease relative to control. Means in the same column followed by the same letter do not differ significantly according to Duncan's Multiple Range Test (p= 0.05)

Table IV. Colonization of roots of banana cv. Robusta by the bio-control agents at harvest under field conditions.

Treatment	Unit
Pseudomonas fluorescens at 20 g/plant	105×10^8 cfu/g
Trichiderma viride at 20 g/plant	60×10^6 cfu/g
Glomus fasciculatum at 250 g/plant	213 spores/50 cm^3 soil (44% root colonization)
Bacillus. subtilis at 20 g/plant	58×10^8 cells/g
Paecilomyces lilacinus at 20 g/plant	16×10^6 spores/g
Carbofuran 3G at 40 g/plant	-
Untreated control	-

rhizobacterium induces systemic resistance to nematode pests (Oostendorp and Sikora, 1990) and inhibits early root penetration of phytonematodes by alteration of specific root exudates such as polysaccharides and amino acids, which modify nematode behaviour. Moreover, the bacterium has the ability to envelop or bind the root surface with carbohydrate-lectin, thereby interfering with normal host recognition by plant parasitic nematodes (Oostendorp and Sikora, 1990; Racke and Sikora, 1992). As shown by Becker *et al.* (1988), production of secondary metabolites that exhibit antibiotic or phytotoxic activity, such as phenazines, pyrrolnitrin, tropolone, pyocyanin and 2,4-diacetylphloroglucinol, by *P. fluorescens* may be lethal to lesion nematodes in the rhizosphere of banana. Hence, the reduction in population of lesion nematodes might have resulted in increased growth of plants treated with *P. fluorescens* compared to the untreated control, thus making the application of this bio-agent an alternative to nematicides for controlling root lesion nematodes in bananas.

It is evident that the application of *P. fluorescens* had significant suppressive effects on nematode populations. However, the degree of nematode population reduction was to just above the economic threshold level. A single application of *P. fluorescens* may not be sufficient to keep the nematode population below the economic threshold level, but it was found to be more effective against lesion nematodes than other biocontrol agents. Hence, it is suggested that additional applications of *P. fluorescens* should be tested in further studies.

The egg parasitic fungus *P. lilacinus* may not have any effect on nematodes that lay eggs inside plant tissue. However, it has been reported that culture fitrates of *P. lilacinus* have effects on nematode populations and, therefore, it was considered in our trials.

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