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Evaluation of Biocontrol Agents and Organic Amendments for the Management of Root Knot Nematode and Spiral Nematode in Banana

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A B S T R A C T

The effect of optimized dosage of bio-agents as soil application and as sucker treatment for the management of root knot nematode, *Meloidogyne incognita* and spiral nematode, *Helicotylenchus multicinctus* in banana was studied under field condition. Application of *Pseudomonas fluorescens* 100g as soil application resulted in 68% reduction of root knot nematode and spiral nematode populations over control. The *P. fluorescens* soil treatment also recorded enhanced the plant height (274.0 cm), number of leaves (14.0/plant), pseudostem girth (55.9 cm) at 270 days after planting. Bunch weight was also higher (25.5 kg/tree) in this treatment at harvest. Similarly, application of *P. fluorescens* at 20g/plant as sucker treatment resulted in 65% reduction of root knot nematode and spiral nematode population over control. Enhanced plant height, number of leaves, pseudostem girth and bunch weight was noticed in this treatment which resulted higher bunch weight (27.3 kg/tree) at harvest. Further sequential application of *P. fluorescens* at 20g as sucker treatment + farm yard manure at 12.5 ton/ha + press mud @ 1.5 ton/ha + growing antagonistic crop *Tagetus* in and around banana and ploughing *in situ* resulted in 66% reduction of root knot nematode and spiral nematode population over control. The treatments have also enhanced the plant height (276.7 cm), number of leaves (14.3 /plant) and pseudostem girth (53.2 cm) at 270 days of planting that caused higher bunch weight (26.3 kg) at harvest.

Keywords

Banana, *Meloidogyne incognita*, *Helicotylenchus multicinctus*, Eco-friendly control

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Introduction

Banana is the fourth ranked horticulture crop in the world and first among the fruits (Surya Prabha and Satheesh Kumar, 2015). A total of 132 species of nematode belonging to 54 genera have been reported to be associated with the rhizosphere of banana (Kumar *et al.*, 2014). The important nematode problem encountered in banana are the burrowing nematode, *Radopholus similis* followed by the root lesion nematode, *Pratylenchus coffeae* (Seenivasan, 2019). The other economically important nematode pests of banana includes spiral nematodes (*Helicotylenchus multicintus* and *H. dihystera*), root knot nematodes (*Meloidogyne incognita* and *M. javanica*), the cyst nematode (*Heterodera oryzicola*) and the reniform nematode (*Rotylenchulus reniformis*) (Das *et al.,* 2011; Das *et al.*, 2013; Seenivasan and Senthilnathan, 2018). The yield loss of banana due to *M. incognita* is 30 per cent with a similar loss estimated for *H. multicinctus* (Jonathan, 1994). The *R. similis* is a migratory endo-parasitic nematode that feeds on the root cortical tissue of bananas forming dark red lesions, which result in reduced bunch weights, increased vegetative cycling periods and may cause the plant to topple (Seenivasan, 2018). The root damage by nematodes results in lowering the uptake of water and nutrients that reduces average bunch weight by up to 25% (Devrajan *et al.*, 2003). The root damage caused by nematodes also entry points for other pathogens such as *Fusarium oxysporum* f.sp. *cubens* that result in destructive nematode disease complex (Das *et al.,* 2014; Selvaraj *et al.,* 2014). In India, *Radopholus similis* was first recorded in during 1966 from Kerala state. Systematic survey carried out in major banana growing districts of Tamil Nadu revealed the association of nematodes *viz*., *R. similis, H. multicintus, H. dihystera, P. coffeae* and *M. incognita* (Devrajan and Seenivasan, 2002; Seenivasn and Lakshmanan, 2002). Although several workers have reported its incidence in banana crop, observations made in and around major banana growing areas of Trichy district during 1992-93 revealed severe infestation of *M. incognita, H. multicintus* in almost all the banana garden (Jonathan, 1994; Das *et al.*, 2010). Application of chemical nematicide in soil causes the environmental problems like pollution, residual toxicity for longer period.

Amending soil with fresh or decomposed organic matter alters the physical, chemical and biological properties of the soil. These changes are responsible for lowering nematode density (Nair *et al.,* 2015). Decomposition of organic matter like stable dung, green manure, compost and other organic material in soil was responsible for the reduction in nematode infestation in cultivated crops (Seenivasan, 2010). Seenivasan and Poornima (2010) observed that amending soil with FYM or pressmud or neamcake enhanced the predatory nematodes and reduced the infestation of *M. incognita* in jasmine. Under wet land conditions banana crop rotated with rice crop checked the nematode problems. Marigold can be grown as in intercrop incorporated around the plants, kills the nematodes (Seenivasan, 2011). Addition of organic amendments such as neem cake, farm yard manure and pressmud can be applied to encourage the predacious nematodes and antagonistic fungi which in turn kill the nematodes. Hence, the organic based technology involving the biocontrol agents and organic amendments / green manure/ intercrop for the management of banana nematodes were investigated in this study.

Materials and Methods

Three field trials was conducted one at farmers field of Sirugamani village of Trichy district, Tamil Nadu, India another two field trials at Sugarcane Research Station, Tamil Nadu Agricultural University, Sirugamani Tamil Nadu, India. All three fields were naturally infested with banana nematodes (mixed population of *M. incogntia* and *H. multicintus*). Banana cultivar of Poovan was used for all three trials. Suckers of uniform size, each weighing approximately 1.5 kg were selected, peeled to a depth of 2 cm and planted at a spacing of 2.1 x 2.1 m in randomised block design with four replication for each trial. The talc based formulation of isolates *Psuedomonas fluorescens* (Pf1) and *Trichoderma viride* (Tv1) were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. Pre-treatment soil samples were collected from the respective plots prior to planting, to a depth of 15 cm from 5 spots in each plot, mixed thoroughly and a representative sub-sample of 250 cm^3 used for nematode estimation. Field trial I composed of the following treatments; (i) *P. fluorescens* 100g as soil application, (ii) *P. fluorescens* 50g as soil application, (iii) *P. fluorescens* 10g as soil application, (iv) *Trichoderma viride* 100g as soil application, (v) *T. viride* 50g as soil application, (vi) *T. viride* 10g as soil application and (vii) Untreated control. Field trial II composed of the following treatments; (i) *P. fluorescens* @ 10g as sucker treatment, (ii) *P. fluorescens* @ 20g as sucker treatment, (iii) *T. viride* 10g as sucker treatment, (iv) *T. viride* 20g as sucker treatment and (v) Untreated control. Field trial III composed of the following treatments; (i) *P. fluorescens* @ 20g as sucker treatment, (ii) FYM @ 12.5 ton/ha, (iii) Pressmud @ 1.5 ton/ha, (iv) Growing antagonistic crop *Tagetes* in and around banana and ploughing *in situ*, (v) *P. fluorescens* @ 20g as sucker treatment + FYM @ 12.5 ton/ha + Pressmud @ 1.5 ton/ha + Growing antagonistic crop *Tagetes* in and around banana and ploughing *in situ* and (vii) Untreated control. Treatments were imposed as detailed above in all three field trials. Post treatment soil samples were collected on 90, 180, 270 and 360 days after planting, from the rhizosphere of five banana plants per plot, at a depth of 15 cm. The soil samples were mixed thoroughly and sub samples of 250 cm^3 were used for nematode estimation. Soil samples were processed by Cobb's sieving and decanting method (Cobb, 1918) and Modified Baermann funnel technique (Schindler, 1961). The pseudostem girth was recorded on 180 days after planting. The bunches were harvested on maturity at the end of 12^{th} month after planting and the yield was recorded. The data of each field trials were statistically analyzed using ANOVA and means were separated by DMRT using AGRES software (Gomez and Gomez, 1984).

Results and Discussion

Soil application of bio-agents

There were no significant differences between treatments in nematode soil population densities before planting. The statistical analysis of the experiment – I revealed that a significant reduction in the nematode population (*M. incognita* and *H. multicinctus*) treated with *P. fluorescens* @ 100 g per tree as soil application. The same treatment resulted in reduction of nematode population by 68% over control. Further it showed an enhanced plant height, number of leaves, pseudostem girth, bunch weight *viz.,* 274.0 cm, 14.0, 55.9 cm and 25.5 kg respectively at 270 days after planting. Soil application of *T. viride* 100g was the next best treatment with 67% nematode reduction over control and increased the bunch weight of 22.5 kg per plant. The lowest bunch weight of 11.7 kg per plant occurred in the untreated control (Table 1 and 2). These findings are in conformity with those of Seenivasan and Devrajan (2008) who also reported that the application of rhizobacteria *viz., Pseudomonas fluorescens* and *Trichoderma viride* to induce profused root development and reduced population of *M. incognita* in medicinal coleus. Similarly, Seenivasan (2018b) also noticed the plant growth due to *Pseudomonas fluorescens* in carrot infested with *Meloidogyne hapla*.

Sucker treatment of bio-agent

Nematode population density (*M. incognita* and *H.* multicinctus) were almost uniform in trial plots before planting. However, imposing of bio-agent sucker treatment resulted on significant change in nematode population i.e. significant reduction in the population of *M. incognita* and *H. multicinctus* in plots receiving *P. fluorescens* @ 20g as sucker treatment. The above treatment resulted in reduction of root knot and spiral nematode population by 73% over control. The same treatment have also enhanced the plant height, number of leaves, pseudostem girth and bunch weight *viz.*, 268.3 cm, 13.6, 53.4 cm and 27.3 kg at 270 days after planting. The next best treatment was suckers treated with *T. viride* at 20g/plant which had provided 65% reduction in population of root knot and spiral nematodes. The treatment has also enhanced the plant height, number of leaves, pseudostem girth and bunch weight *viz.*, 256.6 cm, 11.3, 47.6 cm, and 23.6 kg per plant respectively after 270 days of planting. It was followed by application of *P. fluorescens* and *T. viride* at 10g as sucker treatment (Table 3 & 4). The lowest bunch weight of 9.3 kg per plant was recorded in the untreated control. Our results are in agreement with Seenivasan (2011b) who reported that application of *P. fluorescens* has induced the systemic resistance in rice against rice root-knot nematodes. Seenivasan *et al.*, (2007) also observed considerable reduction in the potato cyst nematode population after seed tuber treatment with *P. fluorescens* in potato.

Influence of bio-agents and organic amendments on the nematode population

There were no significant differences between treatments in nematode soil population densities before planting. The statistical analysis of the experiment - III revealed that a significant reduction in the population of *M. incognita* and *H. multicinctus* was recorded in the combined application of *P. fluorescens* @ 20g as sucker treatment + farm yard manure @ 12.5 ton/ha + pressmud @ 1.5 ton/ha + growing antagonistic crop *Tagetus* in and around banana and ploughing *in situ*. The above treatments resulted in reduction of root knot and spiral nematode population by 66% over control. This treatment has also enhanced the plant height, number of leaves, pseudostem girth by 276.7 cm, 14.3 and 53.2 cm after 270 days of planting and bunch weight is 26.3 kg at harvest. Banana sucker treated with *P. fluorescens* at 20g was the next best treatment and was significantly differed from other treatments.

Table.1 Effect of soil application of bio-agents on nematode population (Mixed population of Meloidogyne incognita and *Helicotylenchus multicintus*)

Treatments	Initial nematode population	Nematode population 90 DAP	Nematode population 180 DAP	Nematode population 270 DAP	Nematode population 360 DAP	Per cent nematode reduction over control
T_1 - *P. fluorescens* 100g as soil application	576.6	145.3	173.0	205.0	229.6	68.34
T_2 - *P. fluorescens* 50g as soil application	578.3	163.6	259.3	270.0	298.0	59.03
T_3 - *P. fluorescens* 10g as soil application	569.3	212.3	323.3	335.0	357.3	50.11
T_4 - *T. viride* 100g as soil application	566.6	152.6	189.6	217.0	236.3	66.85
T_5 - *T. viride* 50g as soil application	572.3	168.0	284.0	293.3	325.6	54.77
T_6 - *T. viride* 10g as soil application	570.3	217.0	334.6	346.0	370.0	48.42
T_7 - Untreated control	545.6	367.6	395.6	546.3	686.3	0.0
CD (P=0.05)	--	36.9	44.28	33.03	90.53	--

DAP- Days after planting

Table.2 Effect of soil application of bio-agents on plant growth characters

Treatments	Plant height (cm)			Pseudostem girth (cm)			Number of leaves			Bunch weight (Kg / plant)
	90 DAP	180 DAP	270 DAP	90 DAP	180 DAP	270 DAP	90 DAP	180 DAP	270 DAP	
T_1 - *P. fluorescens* 100g as soil application	93.5	178.3	274.0	42.5	53.4	55.9	7.6	11.0	14.0	25.5
T_2 - *P. fluorescens* 50g as soil application	92.2	175.3	268.3	38.8	47.3	54.5	7.0	8.6	12.6	21.2
T_3 - *P. fluorescens* 10g as soil application	88.5	171.0	250.3	34.3	40.2	47.9	6.3	8.3	9.3	18.5
T_4 - *T. viride* 100g as soil application	93.3	182.3	277.6	40.7	50.3	56.2	7.3	10.3	14.0	22.8
T_5 - *T. viride* 50g as soil application	91.6	173.6	262.0	37.0	45.8	52.3	6.6	8.6	13.0	21.1
T_6 - *T. viride* 10g as soil application	86.5	168.3	245.6	33.9	38.2	45.4	6.0	8.6	10.0	19.6
T_7 - Untreated control	80.8	137.6	220.3	29.6	33.9	38.3	5.3	7.0	7.6	11.7
CD (P=0.05)	9.0	15.66	15.40	8.17	9.12	8.2	1.17	1.23	1.39	3.08

DAP- Days after planting

Table.3 Effect of sucker treatment of bio-agents on nematode population (Mixed population of Meloidogyne incognita and *Helicotylenchus multicintus*)

Treatments	Initial nematode population	Nematode population 90 DAP	Nematode population 180 DAP	Nematode population 270 DAP	Nematode population 360 DAP	Percent nematode reduction over control
T_1 - *P. fluorescens* @ 10g as sucker treatment	568.0	157.4	253.9	262.6	290.6	57.23
T_2 - *P. fluorescens* @ 20g as sucker treatment	560.7	139.1	167.6	197.6	222.2	72.64
T_3 - *T. viride* @ 10g as sucker treatment	545.5	163.8	278.6	285.9	318.2	52.32
T_4 - *T. viride* @ 20g as sucker treatment	564.3	146.4	184.2	209.6	228.9	65.32
T_5 - Untreated control	574.3	361.6	390.3	536.3	680.3	--
CD (P=0.05)	--	31.7	42.2	30.06	78.62	--

DAP- Days after planting

Table.4 Effect of sucker treatment of bio-agents on plant growth character

Treatments	Plant height (cm)			Pseudostem girth (cm)			Number of leaves			Bunch weight (Kg / plant)
	90 DAP	180 DAP	270 DAP	90 DAP	180 DAP	270 DAP	90 DAP	180 DAP	270 DAP	
T_1 - *P. fluorescens* @ 10g as sucker treatment	79.6	158.4	248.3	32.3	37.6	41.6	7.0	8.3	10.6	21.3
T_2 - *P. fluorescens* @ 20g as sucker treatment	90.0	174.6	268.3	40.4	46.3	53.4	7.3	11.0	13.6	27.3
T_3 - *T. viride* @ 10g as sucker treatment	75.2	155.3	240.4	30.6	34.4	39.6	6.0	7.3	9.3	20.6
T_4 - *T. viride* @ 20g as sucker treatment	85.3	163.3	256.6	36.3	41.3	47.6	6.3	9.3	11.3	23.6
T_5 - Untreated control	68.4	108.4	180.3	25.4	29.6	31.4	5.0	6.6	7.3	9.3
CD (P=0.05)	8.7	16.32	21.40	7.56	8.81	9.21	1.45	1.67	1.82	3.23

DAP- Days after planting

Table.5 Effect of bio-agents and organic amendments on nematode population (Mixed population of Meloidogyne incognita and *Helicotylenchus multicintus*)

Treatments	Initial nematode population	Nematode population 90 DAP	Nematode population 180 DAP	Nematode population 270 DAP	Nematode population 360 DAP	Per cent nematode reduction over control
T_1 - *P. fluorescens* @ 20 gram as sucker treatment	588.0	162.2	199.2	226.6	245.9	62.35
T_2 - FYM @ 12.5 ton/ha	581.3	173.2	268.9	279.6	307.3	60.42
T_3 - Pressmud @ 1.5 ton/ha	562.6	177.6	293.6	302.9	335.2	57.21
T_4 - Growing antagonistic crop *Tagetes* in and around banana and ploughing *in situ*	543.6	221.9	332.9	344.6	366.6	53.73
T_5 - T_1 + T_2 + T_3 + T_4	552.6	154.9	182.6	214.6	239.2	65.84
T_6 - Untreated control	568.6	377.2	407.2	555.9	695.6	--
CD (P=0.05)	--	38.32	46.18	35.07	87.26	--

DAP- Days after planting

Table.6 Effect of bio-agents and organic amendments on plant growth characters of banana

Treatments	Plant height (cm)			Pseudostem girth (cm)			Number of leaves			Bunch weight (Kg /plant)
	90 DAP	180 DAP	270 DAP	90 DAP	180 DAP	270 DAP	90 DAP	180 DAP	270 DAP	
T_1 - *P. fluorescens* @ 20 gram as sucker treatment	96.0	185.0	280.3	40.3	47.4	50.6	7.0	10.6	12.6	24.6
T_2 - FYM @ 12.5 ton/ha	94.9	178.0	271.1	37.6	44.2	48.3	7.0	10.0	12.2	24.0
T_3 - Pressmud @ 1.5 ton/ha	94.3	176.2	264.7	36.3	42.8	46.7	6.7	9.6	11.6	23.3
T_4 - Growing antagonistic crop *Tagetes* in and around banana and ploughing *in situ*	91.2	173.6	253.2	32.6	40.6	44.6	6.3	9.3	11.3	21.6
T_5 - $T_1 + T_2 + T_3 + T_4$	96.2	186.0	276.7	42.2	49.3	53.2	7.4	11.2	14.3	26.3
T_6 - Untreated control	64.3	140.3	223.6	28.3	32.6	34.3	5.2	7.3	8.6	9.6
CD (P=0.05)	10.12	16.53	18.31	9.45	11.6	13.1	1.36	1.62	1.81	2.86

DAP- Days after planting

There was 62% nematode reduction over control and increased the bunch weight of 24.6 kg per plant was noticed. The lowest bunch weight of 9.6 kg per plant was observed in the untreated control (Table 5 and 6). The improved nematode control achieved in trial III attributed to combined application of bio-agent, organic amendment and enemy plants. The *P. fluorescens* sucker treatment with *P. fluorescens* might lead to root colonization of the bacterium. The *P. fluorescens* colonized roots are reported to protect the early penetration of nematodes (Seenivasan and Rajeswari Sundarababu, 2007). Simultaneous nematode control also might be achieved through nematotoxic organic acids released from farm yard manure and press mud organic amendments (Seenivasan and Senthilnathan, 2017). Sustainable control of banana nematodes through *Tagetus* cover crop is earlier established by Seenivasan *et al.,* (2013). Seenivasan (2017) also reported that application of *P. fluorescens*, organic amendment and *Tagetus* cover crop gave the greatest bunch length, bunch weight, number of hands per bunch, number of fingers per bunch with most effective control of nematodes until harvest.

It is concluded that the strategies such as *P. fluorescens* @ 20g as sucker treatment or soil application of *P. fluorescens* @ 100 g per tree or combined application of *P. fluorescens* @ 20g as sucker treatment + farm yard manure @ 12.5 ton/ha + pressmud @ 1.5 ton/ha + growing antagonistic crop *Tagetus* in and around banana and ploughing *in situ* can be recommended for the effective management of nematode menace in banana cropping systems.

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