FIELD APPLICATION OF BIOCONTROL AGENTS FOR THE MANAGEMENT OF SPIRAL NEMATODE, *HELICOTYLENCHUS MULTICINCTUS*, IN BANANA

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Summary. Field experiments were conducted for two years to assess the efficacy of *Pseudomonas fluorescens, Trichoderma viride* and *Glomus fasciculatum* for management of the spiral nematode, *Helicotylenchus multicinctus*, infesting banana. Soil application of *P. fluorescens* at the rate of 2 g or *G. fasciculatum* at 25 g mixed in 3 kg pressmud (sugar factory waste)/plant significantly reduced the infestation of *H. multicinctus*. The treatments increased plant height, pseudostem girth, leaf area, number of leaves and fruit yield. The effectiveness of the biocontrol agents was comparable with that of carbofuran. A significant reduction in nematode infestation was observed in the combined application of *P. fluorescens* and pressmud up to harvest of the crop.

Banana is an important fruit crop grown in India. The spiral nematode, *Helicotylenchus multicinctus* (Cobb) Golden, is often associated with the crop and causes serious damage (Rajendran, 1984). The plant growth promoting rhizobacterium, *Pseudomonas fluorescens* Migula, the vesicular arbuscular mycorrhizal fungus, *Glomus fasciculatum* (Thaxter Senscu. Gerd.) Gerd. Trappe and *Trichoderma* spp. have been reported to reduce the infestation of nematodes in many crop plants (Jonathan *et al.*, 2000; Sankaranarayanan and Rajeswari Sundara Babu, 1994; Windham *et al.*, 1989). The role of pressmud and neem cake in the management of nematodes in banana has been proved by Jonathan *et al.* (2000). Therefore, an investigation was undertaken for the management of spiral nematode infesting banana by rational integration of the three biocontrol agents, *P. fluorescens, Trichoderma viride* Pers. Ex Fr. and *G. fasciculatum* with the organic amendments, pressmud and neem cake.

MATERIALS AND METHODS

The experiment was conducted for two years (2000-2001 and 2001-2002) in a field at the Sugarcane Research Station, Sirugamani, Tamil Nadu, India, severely infested with *H. multicinctus*. The experimental design was a completely randomized block with thirteen treatments (Tables I-IV) replicated three times. The talc based formulations of *P. fluorescens* (Strain PF1) and *T. viride*, prepared for commercial use, were obtained from the Department of Plant Pathology and the soil based formulation of *G. fasciculatum* was obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore, India. The effects of these bioagents were compared with that of Carbofuran (40 g/plant) and pressmud and neem cake. Pressmud (sugar factory waste) and neem cake (residue obtained after extracting oil from neem seed) were obtained from a private sugar factory and commercial oil mill, respectively. These organic amendments were mixed with the biocontrol agents and applied to the rhizosphere of banana one month after planting. Controls were plants with no treatment. The population densities of *P. fluorescens* and *T. viride* in the formulations were estimated as 7 x 10^8 and 2 x 10^8 cfu/g, respectively. The soil based formulation of *G. fasciculatum* contained 750 infective propagules/g at the time of application.

Banana (*Musa* spp.) corms of cv. Poovan (Syn: Mysore, AAB) of uniform size, each weighing approximately 1,250 g, were selected, peeled to a depth of 1 cm, treated with hot water (50-55 °C) for 10 minutes and planted in the experimental field at a spacing of 2.1 x 2.1 m. 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. The biocontrol agents were applied at a depth of 15 cm, at the rates shown in the tables, around the sucker 30 days after planting and covered with soil.

The crop was irrigated once every ten days with underground water, hand weeded every 30 days, and farmyard manure was applied at the rate of 25 t/ha 60 days after planting. Fertilizer was applied at the rates of 160, 50 and 390 g of N, P and K per plant, respectively. The entire P dose was applied 90 days after planting, while N and K were applied in three splits at 90, 150 and 210 days after planting.

The plant growth parameters, *viz.* plant height, pseudostem girth, number of leaves and leaf area, were recorded 180 days after planting. Post-treatment soil and root samples were collected 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 and root samples

were mixed thoroughly and sub-samples of 250 cm^3 and 5 g, respectively, were used for nematode estimation. Nematodes were extracted from the soil using Cobb's sieving gravity method and a Baermann funnel technique and from root samples by the mistifier technique.

The data were statistically analysed and standard error and critical differences determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Significant increases in plant height, pseudostem girth, number of leaves and total leaf area occurred in banana plants treated with *P. fluorescens* at 2 g or *G. fasciculatum* at 25 g along with 3 kg pressmud per plant when compared to the untreated control plants (Tables I and II).

Table I. Effect of *Pseudomonas fluorescens, Trichoderma viride* and *Glomus fasciculatum*, alone and in combination with pressmud and neem cake, on plant growth and yield of banana in soil infested by *Helicotylenchus multicinctus* in the 2000-2001 period.

Treatment and dosage per sucker	Plant height (cm)	Pseudostem girth (cm)	Leaves/plant (number)	Total leaf area (m^2)	Bunch weight (kg)
Pseudomonas fluorescens 2 g (Pf)	191.5	40.3	7.9	0.91	12.6
Trichoderma viride 1 g (Tv)	190.3	39.5	7.6	0.89	11.9
Glomus fasciculatum 25 g (Gf)	191.3	41.0	7.5	0.89	12.0
Pressmud 3 kg (Pm)	194.5	42.3	8.0	0.93	12.9
Neem cake 100 g (Nc)	192.6	41.9	7.5	0.86	11.6
Pf + Pm	199.3	46.5	8.9	1.06	14.9
Pf + Nc	193.0	42.5	8.0	0.96	12.9
Tv + Pm	193.5	42.9	7.9	0.92	12.3
Tv + Nc	192.3	42.3	7.6	0.87	11.9
Gf + Pm	198.5	45.3	8.6	1.03	14.7
Gf + Nc	193.6	42.9	8.0	0.94	12.6
Carbofuran 40 g	197.3	44.6	8.5	1.01	14.5
Control	153.5	30.3	5.9	0.69	9.3
SE	1.3	0.6	0.1	0.02	0.3
CD at 5 %	3.6	1.7	0.3	0.05	0.7

Table II. Effect of *P. fluorescens, T. viride* and *G. fasciculatum* alone and in combination with pressmud and neem cake on plant growth and yield of banana in soil infested by *H. multicinctus* in the 2001-2002 period.

Treatment and dosage per sucker	Plant height (cm)	Pseudostem girth (cm)	Leaves/plant (number)	Total leaf area (m^2)	Bunch weight (kg)
Pseudomonas fluorescens 2 g (Pf)	194.3	39.9	8.0	0.96	12.9
Trichoderma viride 1 g (Tv)	192.9	39.3	7.9	0.93	12.3
Glomus fasciculatum 25 g (Gf)	194.9	40.0	8.0	0.95	12.6
Pressmud 3 kg (Pm)	195.3	43.3	8.3	1.06	13.3
Neem cake 100 g (Nc)	193.5	40.6	7.9	0.89	11.9
Pf + Pm	203.5	47.3	8.9	1.09	15.6
Pf + Nc	198.5	43.9	8.3	1.03	13.6
Tv + Pm	199.9	43.6	8.3	1.03	13.9
Tv + Nc	199.6	42.9	8.0	0.95	12.6
Gf + Pm	202.3	46.6	8.9	1.11	15.3
Gf + Nc	199.6	43.3	8.3	1.01	13.3
Carbofuran 40 g	203.9	45.9	8.6	1.09	14.9
Control	165.6	32.6	5.6	0.65	9.9
SE	1.2	0.8	0.1	0.01	0.4
CD at 5 %	3.5	2.3	0.3	0.03	1.1

Table III. Effect of *P. fluorescens, T. viride* and *G. fasciculatum* alone and in combination with pressmud and neem cake on the infestation of *H. multicinctus* on banana in the 2000-2001 period.

Treatment and dosage per sucker	Initial nematode population (250 cm^3 soil)	Post treatment nematode population in 250 cm^3 soil or 5 g roots							
		90 DAP[1]		180 DAP		270 DAP		360 DAP	
		Soil	Root	Soil	Root	Soil	Root	Soil	Root
Pseudomonas fluorescens 2 g (Pf)	572.6	215.3	8.9	222.0	13.9	232.6	19.3	254.0	23.9
Trichoderma viride 1 g (Tv)	566.0	225.3	10.3	229.6	16.3	252.0	21.5	269.0	26.3
Glomus fasciculatum 25 g (Gf)	554.6	220.6	9.5	228.0	15.3	257.0	20.9	277.6	24.3
Pressmud 3 kg (Pm)	581.3	201.3	8.5	220.5	14.0	225.3	19.5	249.5	23.5
Neem cake 100 g (Nc)	577.9	210.5	10.5	230.3	15.3	235.5	20.0	260.3	25.3
Pf + Pm	560.6	153.3	5.9	165.3	9.3	191.6	13.6	197.5	16.3
Pf + Nc	538.6	209.6	7.5	215.3	13.3	238.3	16.9	259.3	21.3
Tv + Pm	561.6	223.3	8.5	225.6	12.3	251.3	14.5	272.6	19.0
Tv + Nc	571.3	235.0	8.3	240.3	12.6	261.6	16.9	285.6	21.3
Gf + Pm	571.0	169.6	5.5	183.6	9.9	208.0	14.3	226.3	19.9
Gf + Nc	552.6	236.6	8.9	241.6	12.5	279.3	18.3	303.3	24.0
Carbofuran 40 g	567.0	154.0	6.3	162.6	11.9	195.3	16.3	223.3	20.9
Control	570.8	625.6	15.9	649.0	20.3	662.0	25.9	770.0	30.3
SE	-	7.2	0.2	8.2	0.3	9.9	0.5	10.3	0.6
CD at 5 %	-	21.5	0.6	25.2	0.8	27.2	1.14	30.1	1.18

[1] DAP = Days After Planting.

Pseudomonas fluorescens at 2 g and *G. fasciculatum* at 25 g, along with 3 kg pressmud per plant, caused significant reductions in spiral nematode infestation. The untreated control plots had the largest soil and root populations of the nematode. Reduction in nematode population in soil and roots was significant up to harvest in plots treated with *P. fluorescens* along with pressmud (Tables III and IV). The effects of the two biocontrol agents, along with pressmud, were comparable with carbofuran treatment. *Trichoderma viride*, along with pressmud, was found to be the third best treatment in reducing the infestation of the spiral nematode and as effective as *P. fluorescens* or *G. fasciculatum*, along with pressmud in improving plant growth.

The results of the experiment repeated during 2001-2002, in a separate field, were similar to those obtained in 2000-2001. The combined treatments of *P. fluorescens* or *G. fasciculatum* along with pressmud were synergistic in reducing the nematode infestation and enhancing plant growth and yield. The increase in plant growth and reduction in nematode infestation that we found may be due to induced systemic resistance or multiple potential defense mechanisms (Wei *et al.*, 1996; Jonathan *et al.*, 2000; Anita and Rajendran, 2002). Earlier studies proved the efficacy of pressmud in containing the nematode infestation in banana (Jonathan *et al.*, 1999). Moreover, the effectiveness of *P. fluorescens* against root-knot nematode in banana (Jonathan *et al.*, 2000) and spiral nematode in tomato (Rajeshwari *et al.*, 1997) has also been demonstrated. The experimental data show the potential of *P. fluorescens* and *G. fasciculatum* along with pressmud in suppressing spiral nematode infestation on banana. The control is cost-effective and environment-friendly when compared to the chemicals used for the management of nematodes on banana.

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