FIELD EVALUATION OF TALC BASED BIOFORMULATIONS OF BIOCONTROL AGENTS FOR THE MANAGEMENT OF *RADOPHOLUS SIMILIS* AND *HELICOTYLENCHUS DIHYSTERA* IN BANANA

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Summary. Two field experiments were conducted in banana cvs Neipoovan and Nendran at Onampalayam and Pannimadai villages of Coimbatore district, Tamil Nadu, India, respectively, to assess the bioefficacy of two promising native isolates of the bacteria *Pseudomonas fluorescens* (Pfbv22) and *Bacillus subtilis* (Bbv57) against burrowing nematode *Radopholus similis* and spiral nematode *Helicotylenchus dihystera*. The biocontrol agents were compared with the standard chemical carbofuran. Combined application of *P. fluorescens* and *B. subtilis* following paring of corms and coating (pralinage) each at 5 g/corm and soil application at 1.25 kg/ha significantly reduced the nematode population infesting banana. The treatment also significantly enhanced the plant height, pseudostem girth, number of leaves, total leaf area and fruit yield.

Keywords: *Bacillus subtilis*, burrowing nematode, biological control, *Musa* spp., *Pseudomonas fluorescens*, spiral nematode.

Banana (*Musa* spp.) is an important commercial fruit crop grown worldwide both in tropical and subtropical areas and ranks first in terms of production in India. Studies carried out in major banana growing areas of Tamil Nadu State have shown frequent associations of root knot nematode, burrowing nematode and spiral nematode causing yield loss of 30-60% (Jonathan, 1994). In recent years, native isolates of the plant growth promoting rhizobacterium *Pseudomonas fluorescens* Migula have been reported to be effective in suppressing the populations of spiral nematode *Helicotylenchus multicinctus* (Cobb) Golden (Jonathan *et al*., 2004), root knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. (Jonathan *et al*., 2006) and burrowing nematode *Radopholus similis* (Cobb) Thorne (Senthilkumar *et al*., 2008) in banana. Application of fluorescent pseudomonads prior to invasion is thought to protect the crop from the pathogens by strengthening the cell wall structure and causing biochemical and physiological changes in the plant system (Chen *et al*., 2000). *Pseudomonas fluorescens* was also reported to be effective against *M. incognita* in many crops *viz*., tomato and brinjal (Anita and Rajendran, 2002), chickpea (Khan *et al*., 2001), turmeric (Srinivasan *et al*., 2001) and medicinal coleus (*Coleus forskohlii*) (Senthamarai *et al*., 2008; Seenivasan and Devrajan, 2008). Therefore, an investigation was carried out to test the efficacy of two promising native isolates *viz*., *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) individually and in combination in the management of the burrowing nematode *R. similis* and the spiral nematode *H. dihystera* (Cobb) Sher in banana.

MATERIALS AND METHODS

Two field experiments were conducted during 2007-2008 in banana cvs Neipoovan (*Musa* AB) and Nendran (French Plantain, *Musa* AAB) at Onampalayam and Pannimadai villages of Coimbatore district, respectively, to study the efficacy of biocontrol agents in suppressing *R. similis* and spiral nematode *H. dihystera*. The experiments were laid out in a randomized block design with five treatments replicated five times.

Sixty-five native strains of plant-growth-promoting rhizobacteria were isolated from the rhizosphere of healthy banana plants at different localities of Tamil Nadu State. Among these, two isolates, *P. fluorescens* (Pfbv22) and *Bacillus subtilis* (Bbv57), were found promising. The two selected isolates were formulated in purified talc powder (sterilized at 105 °C for 12 h) with calcium carbonate 15 g (to adjust the pH to neutral) and carboxy methyl cellulose (CMC) 10 g (adhesive), following the method described by Vidhyasekaran and Muthamilan (1995). The population loads of the talc formulations were 2.5-3 $\times 10^8$ CFU per gram and their bio-efficacies were compared with that of a granular formulation of the nematicide carbofuran (3% a.i.). Untreated banana plants were maintained for comparison.

Corms of uniform size, weighing approximately 1.5 and 1.25 kg, respectively, for Neipoovan and Nendran were selected for the field experiments. The outer surfaces of the corms were peeled to a depth of 1 cm (paring) before dipping the corms in clay slurry mixed in a proportion of 1: 5 (clay: water) and talc-based formulations of *P. fluorescens* (Pfbv22) alone at 10 g/corm (T1), *B. subtilis* (Bbv57) alone at 10 g/corm (T2), combinations of *P. fluorescens* and *B. subtilis*, each at the rate of 5 g/corm (T3) and carbofuran granules at 33 g/corm (T4) were sprinkled over the corms (pralinage). Un-

treated corms (T5) were maintained as control. The corms were planted at a spacing of 2.1 × 2.1 m (plot size of 40 m^2 with 8 plants/plot) for banana cv. Neipoovan and 2 × 2 m (plot size of 35 m^2 with 8 plants/plot) for cv. Nendran. Pre-treatment soil samples of 250 cm^3 from the respective plots were taken with an auger prior to planting to a depth of 15 cm (5 samples per plot). The soil samples were mixed thoroughly and representative sub-samples of 250 cm^3 were used for the estimation of initial nematode population.

The crop was irrigated once in 10 days and weeding was done regularly. 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 separate doses, at 90, 150 and 210 days after planting. Three small holes were made to a depth of 15 cm around each plant and spot application of *P. fluorescens* (Pfbv22) alone at 2.5 kg/ha *viz.,* 10 g/plant (T1), *B. subtilis* (Bbv57) alone at 2.5 kg/ha *viz.*, 10 g/plant (T2), combinations of *P. fluorescens* and *B. subtilis* each at the rate of 1.25 kg/ha *viz*., 5 g each/plant (T3) and carbofuran granules at 1 kg a.i/ha *viz*., 4 g/plant (T4) were given at the third and fifth month after planting, around the sucker.

Plant height, pseudostem girth, number of leaves and total leaf area were recorded at 180 days after planting. Bunch weight was recorded at the time of harvest (300 days after planting). To assess population densities of major plant parasitic nematodes infesting banana, post-treatment soil and root samples were collected at 90, 180, 270 and 300 (harvest) days after planting, to a depth of 15 cm from five points in each plot, and mixed thoroughly to get representative sub-samples of 250 cm^3 and 5 g, respectively. Soil samples were processed by Cobb's sieving and decanting method (Cobb, 1918) and Modified Baermann funnel technique (Schindler, 1961). Root samples were washed thoroughly, cut to a length of 1 cm and nematodes were extracted by a mistifier technique at 25 ± 5 °C for 12 hours.

Data were statistically analyzed and standard error and critical differences determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

A combined application of plant growth promoting rhizobacteria *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) at 5 g/corm and soil application (SA) at 1.25 kg/ha at the third and fifth month after planting significantly increased plant height, pseudostem girth, number of leaves, total leaf area and bunch yield (Tables I and II). The microbial mixture also resulted in significant reductions in the infestations of *R. similis* and *H. dihystera* and resulted in heavier bunches. The two biocontrol agents were significantly more effective than the standard chemical carbofuran. *Pseudomonas fluorescens* (Pfbv22) alone as paring and pralinage at 10 g/corm and SA at 2.5 kg/ha was the next best treatment in reducing the infestation of burrowing and spiral nematodes (Tables III to VI). *Bacillus subtilis* (Bbv57) at 10 g/corm and SA at 2.5 kg/ha and the chemical treatment carbofuran were equally effective in decreasing nematode infestation and enhancing plant growth and yield. The results obtained from site I (Onampalayam) are very similar to those from site II (Pannimadai).

Increase in plant growth and reduction in nematode populations by plant growth promoting rhizobacteria may be due to induced systemic resistance or multiple defence mechanisms (Wei *et al*., 1996). *Pseudomonas fluorescens* is capable of surviving in and colonizing the rhizosphere of all field crops and is reported to promote plant growth by secreting auxins, gibberellins and cytokinins (Vidhyasekaran, 1998). The suppression of phytonematodes by *P. fluorescens* may be attributed to several mechanisms, such as induced systemic resistance, production of antibiotics and siderophores, competition for nutrients and alteration of specific root exudates such as polysaccharides and amino acids, which modify nematode behaviour (Oostendorp and Sikora, 1990; Aatlen *et al*., 1998). *Pseudomonas fluorescens* and *B. subtilis* were reported to induce systemic resistance in banana against lesion nematodes (Shanthi and Rajendran, 2006).

These rhizobacteria induce profuse root development and reduce populations of *M. incognita* in banana and tomato (Jonathan *et al*., 2000). In the present investigation, the combined application of bioagents was found to be superior to single applications in suppressing nematode infestation and promoting plant growth. Similar studies made by Panneerselvam *et al*. (2008) revealed the superior effect of microbial combinations against root lesion nematode *Pratylenchus coffeae* Sher *et* Allen in coffee plants. Thus the present study indicates the potential of a combination of *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) in suppressing the burrowing and spiral nematodes infesting banana.

The bacterial biocontrol agents can be prepared on a large scale in talc-based formulation for commercial use under Indian conditions for the banana crop. Regarding the economics of the combined treatment, the cost/benefit ratios for cv. Neipoovan and cv. Nendran were found to be 1:2.9 and 1:3.2 respectively (Tables I and II).

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Table I. Bioefficacy of talc-based formulations of *Pseudomonas fluorescens* and *Bacillus subtilis* on growth components, yield (8 bunches/plot of 40 m^2) and cost/benefit ratio, of banana cv. Neipoovan, in a field infested with *Radopholus similis* and *Helicotylenchus dihystera* at Onampalayam.

Treatment	180 Days after planting				Bunch weight/plant (kg)	C : B ratio[1]
	Plant height (cm)	Pseudostem girth (cm)	Number of leaves/plant	Total leaf area (m^2)		
P. fluorescens (Pfbv22) paring and pralinage at 10 g/corm + SA at 2.5 kg/ha	190.6	64.2	14.8	1.02	16.33	1 : 2.7
B. subtilis (Bbv 57) at 10 g/corm + SA at 2.5 kg/ha	178.2	58.8	14.2	0.95	14.22	1 : 2.1
Combination of *P.fluorescens* (Pfbv 22) + *B. subtilis* (Bbv 57) paring and pralinage at 5 g/corm + SA at 1.25 kg/ha each	203.6	70.6	15.2	1.17	18.29	1 : 3.2
Carbofuran paring and pralinage at 33 g/corm + SA at 1 kg a.i/ha	175.8	56.4	13.2	0.88	14.02	1 : 1.8
Control	163.4	51.6	12.4	0.76	11.95	-
SE	2.129	1.433	0.380	0.029	0.179	-
CD at 5%	4.514	3.038	0.805	0.060	0.380	-

SA = Soil application.
[1]C : B ratio = Cost : benefit ratio.

Table II. Bioefficacy of talc-based formulations of *P. fluorescens* and *B. subtilis* on growth components, yield (8 bunches/plot of 40 m^2) and cost/benefit ratio of banana cv. Nendran, in a field infested with *R. similis* and *H. dihystera* at Pannimadai.

Treatment	180 Days after planting				Bunch weight/plant (kg)	C : B[1] ratio
	Plant height (cm)	Pseudostem girth (cm)	Number of leaves/plant	Total leaf area (m^2)		
P. fluorescens (Pfbv22) paring and pralinage at 10 g/corm + SA at 2.5 kg/ha	182.2	66.2	15.2	0.89	13.55	1 : 2.5
B. subtilis (Bbv 57) at 10 g/corm + SA at 2.5 kg/ha	171.8	57.2	14.8	0.78	11.87	1 : 1.8
Combination of *P. fluorescens* (Pfbv 22) + *B. subtilis* (Bbv 57) paring and pralinage at 5 g/corm + SA at 1.25 kg/ha each	195.6	71.8	15.6	1.01	15.17	1 : 2.9
Carbofuran paring and pralinage at 33 g/corm + SA at 1 kg a.i/ha	168.4	55.6	13.6	0.76	11.67	1 : 1.5
Control	151.2	47.4	12.6	0.59	9.62	-
SE	1.994	0.858	0.293	0.015	0.204	-
CD at 5%	4.227	1.819	0.622	0.032	0.433	-

SA = Soil application.
[1]C : B ratio = Cost : benefit ratio.

Table III. Efficacy of talc-based formulations of *P. fluorescens* and *B. subtilis* on biocontrol of *H. dihystera* in banana cv. Neipoovan at Onampalayam.

Treatment	PTP*/ 250 cm^3 soil	Post-treatment nematode population per 250 cm^3 soil or 5 g roots							
		90 DAP**		180 DAP		270 DAP		300 DAP	
		Soil	Root	Soil	Root	Soil	Root	Soil	Root
P. fluorescens (Pfbv22) paring and pralinage at 10 g/corm + SA at 2.5 kg/ha	103.0	80.8 (1.91)	8.7	64.6 (1.81)	12.3	97.0 (1.99)	16.2	116.8 (2.07)	19.3
B. subtilis (Bbv 57) at 10 g/corm + SA at 2.5 kg/ha	112.0	86.4 (1.94)	12.2	72.8 (1.86)	15.5	104.8 (2.02)	19.5	123.6 (2.09)	23.7
Combination *P. fluorescens* (Pfbv 22) + *B. subtilis* (Bbv 57) paring and pralinage at 5 g/corm + SA at 1.25 kg/ha each	106.0	63.0 (1.81)	5.9	45.4 (1.66)	9.5	76.2 (1.88)	12.9	93.4 (1.97)	15.9
Carbofuran paring and pralinage at 33 g/corm + SA at 1 kg a.i/ha	110.6	90.6 (1.96)	12.6	75.4 (1.88)	15.9	105.6 (2.02)	19.9	127.4 (2.11)	24.4
Control	107.0	162.2 (2.20)	17.5	197.8 (2.30)	20.5	230.4 (2.36)	24.9	257.6 (2.41)	29.5
SE		0.011	0.263	0.008	0.191	0.011	0.224	0.008	0.240
CD at 5%		0.023	0.557	0.018	0.404	0.023	0.475	0.018	0.508

SA = Soil application. *PTP = Pre-treatment nematode population. **DAP = days after planting.
Figures in parentheses indicate log transformation.

Table IV. Efficacy of talc-based formulations of *P. fluorescens* and *B. subtilis* on biocontrol of *R. similis* in banana cv. Neipoovan at Onampalayam.

Treatment	PTP*/ 250 cm^3 soil	Post-treatment nematode population per 250 cm^3 soil or 5 g roots							
		90 DAP**		180 DAP		270 DAP		300 DAP	
		Soil	Root	Soil	Root	Soil	Root	Soil	Root
P.fluorescens (Pfbv22) paring and pralinage at 10 g/corm + SA at 2.5 kg/ha	109.2	91.6 (1.96)	6.7	76.4 (1.88)	10.4	88.8 (1.95)	13.9	100.2 (2.00)	17.3
B. subtilis (Bbv 57) at 10g/corm + SA at 2.5 kg/ha	115.4	99.4 (2.00)	10.3	86.2 (1.94)	13.8	99.6 (2.00)	17.5	112.8 (2.05)	21.8
Combination of *P. fluorescens* (Pfbv 22) + *B. subtilis* (Bbv 57) paring and pralinage at 5 g/corm + SA at 1.25 kg/ha each	111.8	75.8 (1.88)	3.9	57.6 (1.76)	7.2	69.8 (1.84)	10.7	78.4 (1.89)	13.6
Carbofuran paring and pralinage at 33 g/corm + SA at 1 kg a.i/ha	115.2	101.6 (2.01)	10.7	89..2 (1.95)	14.2	102.0 (2.01)	17.9	116.4 (2.07)	22.1
Control	119.0	173.0 (2.24)	15.9	210.6 (2.32)	18	237.8 (2.37)	22.5	249.2 (2.40)	27.2
SE		0.012	0.184	0.008	0.194	0.007	0.164	0.008	0.228
CD at 5%		0.026	0.391	0.016	0.410	0.015	0.347	0.017	0.483

SA = Soil application. *PTP = Pre-treatment nematode population. **DAP = days after planting.
Figures in parentheses indicate log transformation.

Table V. Efficacy of talc-based formulations of *P. fluorescens* and *B. subtilis* on biocontrol of *H. dihystera* in banana cv. Nendran at Pannimadai.

Treatment	PTP*/ 250 cm^3 soil	Post-treatment nematode population per 250 cm^3 soil or 5 g roots							
		90 DAP**		180 DAP		270 DAP		300 DAP	
		Soil	Root	Soil	Root	Soil	Root	Soil	Root
P. fluorescens (Pfbv22) paring and pralinage at 10 g/corm + SA at 2.5 kg/ha	104.0	82.2 (1.91)	12.5	65.2 (1.82)	15.3	93.6 (1.97)	19.4	112.2 (2.05)	23.4
B. subtilis (Bbv 57) at 10 g/corm + SA at 2.5kg / ha	103.6	90.6 (1.95)	15.6	73.6 (1.87)	19.1	105.2 (2.02)	22.7	123.4 (2.09)	26.8
Combination of *P. fluorescens* (Pfbv 22) + *B. subtilis* (Bbv 57) paring and pralinage at 5 g/corm + SA at 1.2 5kg /ha each	101.2	70.8 (1.87)	8.8	54.8 (1.74)	12.1	81.8 (1.91)	16.2	100.6 (2.00)	19.0
Carbofuran paring and pralinage at 33 g/corm + SA at 1 kg a.i/ha	105.0	93.4 (1.97)	16.1	76.4 (1.88)	19.5	108.0 (2.03)	23.2	127.6 (2.10)	27.2
Control	106.4	152.2 (2.18)	19.7	185.8 (2.27)	23.6	219.2 (2.34)	27.6	240.2 (2.38)	32.4
SE		0.010	0.263	0.008	0.190	0.014	0.194	0.016	0.183
CD at 5%		0.021	0.557	0.017	0.403	0.030	0.411	0.034	0.338

SA = Soil application. *PTP = Pre-treatment nematode population. **DAP = days after planting.
Figures in parantheses indicate log transformation.

Table VI. Efficacy of talc-based formulations of *P. fluorescens* and *B. subtilis* on biocontrol of *R. similis* in banana cv. Nendran at Pannimadai.

Treatment	PTP*/ 250 cm^3 soil	Post treatment nematode population per 250 cm^3 soil or 5 g roots							
		90 DAP**		180 DAP		270 DAP		300 DAP	
		Soil	Root	Soil	Root	Soil	Root	Soil	Root
P. fluorescens (Pfbv22) paring and pralinage at 10 g/corm + SA at 2.5 kg/ha	88.2	69.0 (1.84)	9.3	55.4 (1.74)	13.5	66.2 (1.82)	16.7	77.2 (1.89)	22.3
B. subtilis (Bbv 57) at 10 g/corm + SA at 2.5 kg/ha	89.6	77.8 (1.89)	13.2	69.8 (1.84)	16.8	81.6 (1.91)	20.2	93.4 (1.97)	25.6
Combination of *P. fluorescens* (Pfbv 22) + *B. subtilis* (Bbv 57) paring and pralinage at 5 g/corm + SA at 1.25 kg/ha each	90.8	60.2 (1.78)	6.8	47.6 (1.68)	10.3	57.4 (1.76)	14.4	68.6 (1.84)	18.9
Carbofuran paring and pralinage at 33 g/corm + SA at 1 kg a.i/ha	88.8	80.6 (1.91)	13.6	73.2 (1.87)	17.2	85.4 (1.93)	20.6	97.0 (1.99)	26.1
Control	87.6	124.0 (2.09)	17.5	150.6 (2.18)	21.4	164.6 (2.22)	24.9	175.4 (2.24)	29.7
SE		0.011	0.147	0.010	0.145	0.013	0.122	0.009	0.157
CD at 5%		0.024	0.312	0.021	0.308	0.026	0.258	0.019	0.333

SA = Soil application. *PTP = Pre-treatment nematode population. **DAP = days after planting.
Figures in parantheses indicate log transformation.

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