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Bioefficacy of *Pseudomonas fluorescens* against Burrowing Nematode *Radopholus Similis* in Banana

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Abstract : The native strains of *Pseudomonas fluorescens* were isolated from the rhizosphere of healthy banana and tested for their efficacy to manage *Radopholus similis* on banana. Among the 40 isolates of *P. fluorescens* tested, isolates PfB 13, PfB 19, PfB 24, PfB 32, PfB 35 and PfB 39 increased germination and the vigour index of rice under roll towel and pot culture conditions. *In vitro,* significant mortality of *R. similis* adult female were observed in the culture filtrate of PfB 13 at 100% concentration. In glasshouse conditions, significantly reduced nematode population and lesion index were observed in plants treated with the isolate PfB13. The plant growth parameters also significantly improved in isolate PfB 13 treated plants when compared to control plants.

Key words: Biological control, Musa spp., Rhizobacterium

Introduction

The plant parasitic nematodes *viz., Radopholus similis, Pratylenchus coffeae, Meloidogyne incognita* and *Helicotylenchus multicinctus* cause serious damage to banana crop. Among them, the burrowing nematode, *R. similis* is the most important pest causing economic losses in India (Krishnappa and Reddy, 1993) and widely distributed in South India (Rajendran *et al.,* 1979). The first report of *R. similis* infestation in India was from banana in the Palakad district of Kerala state (Nair *et al.,* 1966) and about 30-60 per cent reduction in fruit yield (Blake, 1972). In recent years, Plant Growth Promoting Rhizobacteria (PGPR) viz., *Pseudomonas fluorescens* native isolates are reported to be effective against root knot nematode *M. incognita* in banana (Jonathan *et al.,* 2006). The talc formulation of *P. fluorescens* (Pf 1) significantly reduced the infestation of *H. multicinctus* in banana and increased the plant growth and yield (Jonathan *et al.,* 2004). Therefore, an investigation was undertaken for the management of burrowing nematode *R. similis* infesting banana using the rhizobacterium *P. fluorescens.*

Materials and Methods

Soil samples were collected from the rhizosphere of healthy banana plants to isolate native strains of *P. fluorescens* by a serial dilution agar plate technique (Aneja, 2002). One ml each of 10^{-5} and 10^{-6} dilution was pipetted into sterile Petri dishes. King's B medium (King *et al.,* 1954) was cooled to 30 $\pm$ 1°C, poured into the Petri dishes, rotated and incubated at room temperature (28 $\pm$ 1°C) for 24h. The colonies with raised surfaces showing fluorescent colour were individually purified and subcultured.

Suspensions of the *P. fluorescens* isolates were tested for their plant growth promotion activity on rice (IR 20) under *in vitro* conditions by the standard roll-towel method (ISTA, 1993)

Table I. Efficacy of *P. fluorescens* isolates on seed germination and seedling vigour of rice, *Oryza sativa*, using the roll towel method.

P. fluoresescens Isolates	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index
PfB 1	32.40	8.58^{gh}	11.36^{kl}	646.05
PfB 2	92.48	9.23^{fg}	13.11^{hi}	2066.00
PfB 3	77.99	6.17 $^{q-t}$	18.11^{c}	1893.59
PfB 4	45.60	6.08^{r-t}	7.56^{s}	621.98
PfB 5	48.60	7.42^{j-l}	6.30^{t}	666.79
PfB 6	61.41	8.62^{gh}	9.99^{no}	1142.84
PfB 7	45.56	7.21^{k-n}	7.98^{s}	692.05
PfB 8	52.46	7.36^{j-m}	10.59^{l-n}	941.65
PfB 9	69.55	7.56$^{i -k}$	15.95^{ef}	1635.12
PfB 10	44.65	6.65^{m-r}	7.56^{s}	634.47
PfB 11	68.78	6.47^{o-s}	13.45^{h}	1370.09
PfB 12	45.45	5.46^{tu}	7.86^{s}	605.39
PfB 13	99.91	22.56^{a}	23.89^{a}	4640.82
PfB 14	68.68	6.51^{n-r}	8.36^{q-s}	1021.27
PfB 15	60.04	8.2^{1-i}	11.18^{kl}	1164.17
PfB 16	39.00	3.45^{w}	8.32^{q-s}	459.03
PfB 17	40.07	3.17^{w}	7.85^{s}	441.57
PfB 18	53.47	5.78st	8.05^{rs}	739.49
PfB 19	91.00	12.18^{e}	15.53^{ef}	2521.61
PfB 20	66.60	6.71^{l-r}	8.87^{p-r}	1037.62
PfB 21	67.60	6.39^{o-s}	9.66^{op}	1084.98
PfB 22	96.00	9.75^{f}	10.1^{1-n}	1906.56
PfB 23	80.41	6.30^{p-s}	10.63^{l-n}	1361.34
PfB 24	96.00	13.00^{d}	14.43^{g}	2633.28
PfB 25	45.65	4.55^{v}	7.72^{s}	560.12
PfB 26	25.67	3.55^{w}	5.22^{u}	225.12
PfB 27	60.24	6.64^{m-r}	7.99^{rs}	881.31
PfB 28	45.65	6.83^{l-q}	10.25^{mn}	779.70
PfB 29	67.62	7.02^{k-p}	10.0^{1-o}	1151.56
PfB 30	75.15	8.01^{h-j}	10.99^{k-m}	1427.85
PfB 31	56.01	4.93^{uv}	8.96^{pq}	777.97
PfB 32	93.00	9.59^{f}	8.40^{q-s}	1673.07
PfB 33	76.62	7.11^{k-o}	5.54^{tu}	969.24
PfB 34	65.64	7.30^{j-m}	5.52^{tu}	841.50
PfB 35	99.01	13.14^{de}	15.13^{fg}	2799.01
PfB 36	95.43	11.56^{e}	10.95^{k-m}	2148.12
PfB 37	60.41	6.00^{r-t}	3.23^{v}	557.58
PfB 38	89.00	12.12^{e}	12.50^{ij}	2191.18
PfB 39	97.00	13.57^{d}	16.34^{d-j}	2901.27
PfB 40	93.48	11.77^{e}	11.63^{k}	2187.43
Pf 1	98.00	17.40^{c}	16.99^{d}	3370.22
Control	59.51	3.60^{w}	9.64^{op}	787.91
CD (0.05)		0.72	0.88	

Column figures followed by different letters are significantly different from each other at 5% level.

Table 2. Efficacy of *P. fluorescens* isolates on seed germination and seedling vigour of rice, *Oryza sativa*, using the pot culture method.

P. fluoresescens Isolates	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index
PfB 1	31.99	7.48 kl	10.98gj	590.53
PfB 2	94.44	8.28 ij	17.15c	2401.60
PfB 3	75.94l	7.13m-o	17.10 c	1840.02
PfB 4	46.64l	6.99m-p	7.26qr	664.62
PfB 5	48.50	7.42 kl	6.44rs	672.21
PfB 6	67.44	9.64 h	8.78lm-o	1242.24
PfB 7	55.57	8.24 ij	8.48m-o	929.13
PfB 8	62.42l	6.99m-p	11.51g	1154.77
PfB 9	49.51	8.55 i	17.92 c	1310.53
PfB 10	45.62	6.55 n-p	8.56k-o	689.31
PfB 11	66.48	5.47 st	9.45l	991.88
PfB 12	44.46	6.45 op	8.01 no	642.89
PfB 13	99.85	23.45 a	24.76a	4813.76
PfB 14	67.88	5.58 r-t	4.36t	674.72
PfB 15	68.04l	7.21mn	14.18e	1455.37
PfB 16	35.77	4.56 uv	7.39q	427.45
PfB 17	39.05	4.15 vw	6.87q-s	430.33
PfB 18	54.44	6.28 pr	9.02lm	832.93
PfB 19	93.12	13.19 e	12.57fo	2398.77
PfB 20	56.67	5.99 rs	7.98 n	791.67
PfB 21	62.60	6.59 m-p	10.63ij	1077.97
PfB 22	88.01	8.65 i	11.11g	1739.07
PfB 23	72.31l	7.30m	9.62kl	1223.48
PfB 24	95.00	12.00 f	13.41e-r	2413.95
PfB 25	44.15	5.05 tu	6.70s	518.76
PfB 26	24.12	6.04 p-s	6.03s	291.12
PfB 27	50.22	5.99 p-s	8.85lmn	745.26
PfB 28	44.05	6.33 p	11.25g	774.39
PfB 29	67.65	8.05 i-k	11.01g	1289.40
PfB 30	65.15l	7.01m-o	9.98jk	1106.89
PfB 31	59.01	3.90 vw	7.96o	699.85
PfB 32	93.12	9.69 h	7.98 no	1645.43
PfB 33	77.62l	7.15m-o	6.02s	1022.25
PfB 34	64.64	7.57 jk	6.12s	884.92
PfB 35	98.09	14.19 d	16.20dl	2980.95
PfB 36	93.40	10.56 g	9.05m	1831.57
PfB 37	70.42	7.00 l-o	4.29t	795.04
PfB 38	87.23	12.35 f	11.58g	2087.41
PfB 39	97.01	13.47 de	15.34d	2794.85
PfB 40	93.48	11.77 f	11.63f	2187.43
Pf 1	98.45	18.01 c	17.11c	3457.56
Control	60.50	3.59	10.11i-k	828.85
CD (0.05)		0.73	0.88	

Column figures followed by different letters are significantly different from each other at 5% level.

and in pots containing 1 kg of sterilized soil. An untreated control was also maintained. The germination percentage of rice seeds was recorded and the vigour index of the resulting seedlings was calculated using the formula Vigour index = germination (%) x seedling length (shoot length + root length (Baki and Anderson, 1973).

P. fluorescens isolates were selected based on their growth promotion activity and their antagonestic effect on *R. similis* were then assessed *in vitro.* These isolates are maintained at the Department of Nematology, Tamil Nadu Agricutlural Unversity, Coimbatore, India. The effect of the culture filtrates of the isolates were tested for their efficacy on mortality of *R. similis* at different concentrations (100, 75, 50 and 25%). To study the nematicidal effect of *P. fluorescens* isolates, one ml each of the bacterial cell free filtrates of different concentrations (100, 75, 50 and 25%) were poured into separate Syracuse dish. *R. similis* adult females are introduced into each dish @ 100 nematodes in 0.1 ml of sterile water and incubated at 27 $\pm$ 1°C. Each treatment was replicated thrice. The inactive nematodes from each dilution were transferred separately into sterile distilled water and kept overnight to check whether mortality was permanent or temporary. Observations were recorded on the mortality of nematodes after 24, 48 and 72 h of exposure period and per cent mortality was calculated. A sterile blank and King's B broth were also maintained as check.

P. fluorescens isolates were formulated in purified talc powder (sterilized at 105°C for 12h) with calcium carbonate 15 g (to adjust the pH to neutral) and carboxy methyl cellulose (CMC) 10 g (adhesive), the method described by Vidhyasekaran and Muthamilan (1995). At the time of application, the populations of bacteria in the talc formulations were maintained as 2.5-3 x 10^8 cfu/g.

The talc-based formulations of promising *P. fluorescens* isolates were tested against *R. similis* infesting banana under glass house conditions. The experiment was arranged at the Department of Nematology, Coimbatore, India during December, 2005 to February, 2006. Tissue culture banana plantlets cv. Nendran obtained from Spic Agro Biotech, Coimbatore, India were planted in pots filled with 10 kg of a steam-sterilized pot mixture (Red soil : Sand : Farmyard manure; 2 :1 : 1) in the glasshouse. At the time of planting, 10 g of each of the *P. fluorescens* isolates in talc formulation were applied to the soil in each pot and mixed thoroughly. A biocontrol product, Pf 1, already developed by the Department of Plant Pathology,Tamil Nadu Agricultural University, Coimbatore, India and the chemical, carbofuran 3G, were also included as treatments as furnished in Table 5 as standared check. Untreated banana plants were maintained for comparison. A completely randomized design was adopted with three replications for each treatment. Five days after planting, *R. similis* were inoculated in the root zone at 10000 /pot. Regular watering was done with tap water passed through a 325-mesh sieve. The experiment was repeated during March to June 2006, to confirm the biocontrol potential of the *P. fluorescens* isolates. Observations on plant height, pseudostem girth, number of leaves per plant, total leaf area, root weight, shoot weight, root lesion index and number of nematodes / 5g root were recorded on 90 days after the treatment and all the data were statistically analysed (Gomez and Gomez, 1984).

Results and Discussion

Forty native isolates of *P. fluorescens* were obtained from healthy banana rhizospheres.

Table 3. Effect of of *P. fluorescens* culture filtrates on mortality of *R. similis*

S.No.	Mortality of *R. similis*											
	C1			C2			C3			C4		
	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3
PfB 13	56.86 (48.87)	72.88 (58.31)	81.77 (64.71)	50.43 (45.12)	57.42 (49.27)	72.69 (58.51)	44.35 (41.75)	52.38 (46.35)	67.69 (55.36)	31.39 (34.07)	37.39 (37.67)	47.69 (43.67)
PfB 19	39.44 (38.66)	54.69 (47.69)	61.87 (51.37)	26.07 (30.67)	29.69 (32.99)	42.38 (40.60)	14.77 (21.99)	19.71 (26.33)	24.09 (29.34)	9.69 (18.09)	13.69 (21.69)	17.78 (24.85)
PfB 24	46.65 (42.72)	61.87 (51.37)	69.87 (56.17)	31.76 (33.81)	39.09 (38.61)	53.98 (46.74)	22.98 (28.19)	28.99 (31.96)	34.67 (35.67)	10.77 (18.70)	15.88 (22.77)	18.98 (25.35)
PfB 32	42.65 (40.41)	58.37 (47.89)	64.88 (53.14)	29.76 (32.38)	35.65 (36.20)	47.69 (43.67)	17.12 (24.33)	24.55 (29.34)	31.56 (33.84)	10.77 (18.39)	14.97 (22.20)	18.98 (25.12)
PfB 35	51.77 (45.58)	65.98 (53.71)	73.69 (59.14)	36.77 (37.08)	44.87 (41.53)	59.87 (52.54)	25.38 (30.25)	29.87 (33.43)	35.11 (36.45)	13.76 (21.48)	16.98 (24.06)	22.22 (27.95)
PfB 39	35.39 (36.48)	51.34 (45.59)	54.71 (47.70)	23.89 (28.64)	27.69 (31.74)	38.88 (38.24)	12.65 (20.27)	16.77 (23.56)	20.87 (26.56)	9.78 (17.67)	12.38 (20.45)	16.77 (23.51)
Pf1	51.69 (47.31)	67.45 (56.39)	76.98 (59.58)	40.87 (39.21)	48.88 (44.11)	63.88 (52.97)	28.87 (32.10)	33.61 (35.43)	41.33 (39.81)	16.87 (23.83)	21.78 (27.61)	27.54 (31.57)
Control check (KB broth)	5.87 (13.31)	9.44 (17.45)	13.65 (21.43)	2.65 (8.76)	4.78 (12.47)	7.78 (15.35)	1.39 (6.55)	1.69 (7.34)	3.38 (10.51)	0.00 (0.13)	0.00 (0.13)	0.69 (3.88)
Control (Dist. H_2o)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)	0.00 (0.13)

Figures in parentheses are sine transformed values.

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C1-100 percent concentration; C2 – 75 percent concentration; C3 – 50 percent concentration; C4 – 25 percent concentration H1-24 hours; H2 – 48 hours; H3 – 72 hours.

	CD (p = 0.01)
Treatment (T)	1.37
Concentration(C)	0.41
Hour (H)	0.35
T x C	2.76
C x H	0.72
T x H	2.37
T x C x H	4.77

Table 4. Effect of talc formulations of *P. fluorescens* isolates on growth of banana cv. Nendran infested with *R. similis**

S.No	Treatments	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Pseudo stem (cm)	No.of leaves
1	PfB 13 (10 g/pot)	47.67	199.33	54.75	147.68	12.64	7.77
2	PfB 19(10 g/pot)	40.13	138.37	36.83	87.35	8.47	4.06
3	PfB 24(10 g/pot)	39.43	152.39	39.97	77.67	9.06	4.08
4	PfB 32(10 g/pot)	31.53	108.65	36.07	58.05	8.48	6.70
5	PfB 35(10 g/pot)	30.67	142.60	37.83	84.63	6.67	5.65
6	PfB 39(10 g/pot)	38.83	138.93	33.76	73.66	8.98	5.07
7	Pf 1(10 g/pot)	39.13	160.98	40.26	63.32	7.26	6.05
8	Carbofuran 2 g/pot	32.83	140.00	30.48	53.63	5.45	7.05
9	Control	21.5	75.33	19.79	43.77	5.16	4.01
	CD (p = 0.05)	6.12	18.65	9.87	9.87	1.33	NS

*Pooled analysis of data gathered from two pot culture experiments

In the roll-towel and pot culture studies, seeds treated with the six isolates, *viz*, PfB 13, PfB 19, PfB 24, PfB 32, PfB 35 and PfB 39 all germinated and also produced plants with greater root and shoot length than plants from seed treated with other isolates, leading to enhanced vigour indices compared to the effects of other bacterial isolates (Table 1 and Table 2). These seven isolated were selected for further studies.

the culture filtrate of PfB 13 caused significant nematode mortality, at 100% concentration after 72 h of exposure *in vitro* conditions (Table 3). Similar toxic property of *P. fluorescens* culture filtrates was also reported on the juveniles of *M. incognita* and *Heterodera cajani* (Gokte and Swarup, 1988). The studies conducted by Krishnaveni (2005) reported to the toxic effect of native *P. fluorescens* isolate Pfb 34 against the spiral nematode *H. multicinctus* which was isolated from banana crop and in accordance with the results of present study. In the present study, the per cent larval mortality increased with an increase in the exposure period and increase in concentration of culture filtrates. Similler findings reported by Zaki (1994) and Khan and Goswami (1999).

In the glasshouse, the growth of all the isolates of *P. fluorescens* treated banana plants showed significant improvement compared to untreated plants. Among the treatments, plants treated with PfB 13 showed significant enhancement of plant growth and reduced the nematode infestations (Table 4 & Table 5).

P. fluorescens is capable of surviving in and colonizing the rhizophere of all field crops and is reported to promote plant growth by secreting auxins, gibberellins and cytokins (Vidhyasekaran, 1988). The suppression of

Table 5. Efficacy of talc formulations of *P. fluorescens* isolates on *R. similis* infestation in banana cv. Nendran*

S.No.	Treatments	Root population (5g)	Per cent decrease over control	Soil population (250 g)	Per cent decrease over control	Root lesion Index (%)	Per cent decrease over control
1	PfB 13(10 g/pot)	78.33(8.01)	53.83	157.33(10.08)	64.03	11.00	76.09
2	PfB 19(10 g/pot)	92.55(9.45)	45.45	201.21(14.11)	54.00	25.00	45.65
3	PfB 24(10 g/pot)	84.43(9.23)	50.24	210.11(14.53)	51.97	22.00	52.17
4	PfB 32(10 g/pot)	99.53(9.98)	41.34	295.32(17.78)	32.49	30.00	34.78
5	PfB 35(10 g/pot)	90.76(9.65)	46.50	208.76(14.54)	52.29	26.00	43.48
6	PfB 39(10 g/pot)	100.87(10.01)	40.55	224.65(15.33)	48.64	37.00	19.57
7	Pf 1(10 g/pot)	88.21(8.89)	48.01	157.42(11.86)	64.01	13.00	71.74
8	Carbofuran 2 g/pot	76.66(6.97)	54.82	155.74(12.59)	64.40	11.75	74.46
9	Control	169.66(13.91)	-	437.43(20.98)	-	46.00	-
	CD (p = 0.05)	1.40	-	1.12	-	0.79	-

Figures in parentheses are $\sqrt{n}$ transformed

*Pooled analysis of data gathered from two pot culture experiments

phytonematodes by the application of *P. fluorescens* has been due to induced systemic resistance, production of antibiotics and siderophores, competition for nutrients, and alteration of specific root exudated such as polysaccharides and amino acids, which modify nematode behaviour (Oostendorp and Sikora, 1990; Aalten *et al.*, 1998).

Thus study indicated that the rhizobacteria *P. fluorescens* can be mass produced and effectively used against banana burrowing nematode as a component in Integrated Pest Management.

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