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Viability studies of *Pseudomonas fluorescens*, Pf 1 in liquid formulation, its effect on plant growth and on root knot nematode, *Meloidogyne incognita*

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ABSTRACT:

Viability of *Pseudomonas fluorescens,* Pf 1 in liquid formulation amended with different chemicals was studied for a period of one year. Among the amendments tested, NB with 10 mM glycerol showed maximum viability of Pf 1 cells (360 days). Similarly, in stickers, NB with starch (2%) showed the maximum viability of Pf 1 cells (240 days). Plant growth promotion studies carried out in tomato cv Co3 under roll towel and pot culture method revealed that fifth day old culture of Pf 1 showed enhanced plant growth when compared with other subsequent day old cultures. Under *in vitro* studies against *Meloidogyne incognita*, the same fifth day old culture at its 25 per cent concentration recorded the least egg hatching and maximum juvenile mortality. However, the efficiency of different days old cultures remained effective over control for a period of one year when stored as liquid formulation along with chemical amendments.

Key words: Liquid formulation, *P. fluorescens,* Plant growth, Root knot nematode, Viability

Root knot nematode, *Meloidogyne* spp are important sedentary endoparasites causing damage to a wide range of crops worldwide (Sikora and Fernandez 2005). Use of chemical nematicides to control the nematodes was a short term solution. This was because nematode population was found to increase several months after nematicides application or new races of nematodes developed due to the selection pressure caused by use of resistant varieties.

An awareness of recent problems in the use of nematicides is the impetus behind the strong movement in determining the potential of biocontrol management of plant parasitic nematodes. Among the biocontrol agents, *Pseudomonas fluorescens* plays a major role in promoting plant growth and reducing nematode incidence. Moreover liquid formulation of biocontrol agents was proved to be more stable than their talc formulation (Manikandan *et al.* 2010). Hence, the present investigation was undertaken to study the viability of *P. fluorescens*, Pf 1 in liquid formulation and its influence on plant growth promotion and root knot nematode, *Meloidogyne incognita*.

MATERIALS AND METHODS

Pseudomonas fluorescens, Pf 1 used in the present study was obtained from Centre for Plant Protection Studies, Tamil Nadu Agricultural University Coimbatore, India. The efficacy of culture against plant pathogens and nematodes in a wide range of crops was already proved by our previous workers (Saravanakumar *et al*. 2009, Manikandan *et al*. 2010).

Different chemical amendments, *viz.* glycerol (10 mM), trehalose (5 mM), sorbitol (5 mM), glycine (10 mM), mannitol (10 mM) and the stickers, viz. starch (2%), polyvinylpyrollidone (PVP) (2%), gum acacia (2%) and liquid paraffin (2%) were separately added to 1 litre of Nutrient broth (NB) and King's B (KB) broth (Tamilvendan and Thangaraju 2006). One ml of log phase culture of Pf 1 was inoculated into each of chemically amended NB and KB broths. Pf 1 culture grown in NB and KB without any amendation served as control. The flasks were incubated at room temperature (28±2°C) for a period of one year and broth cultures were analyzed for viable cell population at monthly intervals upto its lag phase.

Sterilized Nutrient Agar (NA) and King's B (KB) medium was poured into sterile Petri plates separately at 10 ml/plate. The plates were incubated at room temperature for 48 h. Petri plates were radially marked on outside bottom into eight equal sectors (Fig 1). Four sectors were used for replications of one dilution and four for another, allowing two dilutions per plate. Serial dilution was prepared upto 10^{-9}th dilution. From dilutions of 10^{-2} to 10^{-9}, 10 µl was pipetted out from each and placed on the respective quadrant in the Petri plate. Plates were incubated undisturbed at room temperature (28±2°C) for 24 h and individual colonies were counted through drop plate method (Somasegaran and Hoben 1994).

Maximum viable cells of Pf 1 were observed in nutrient

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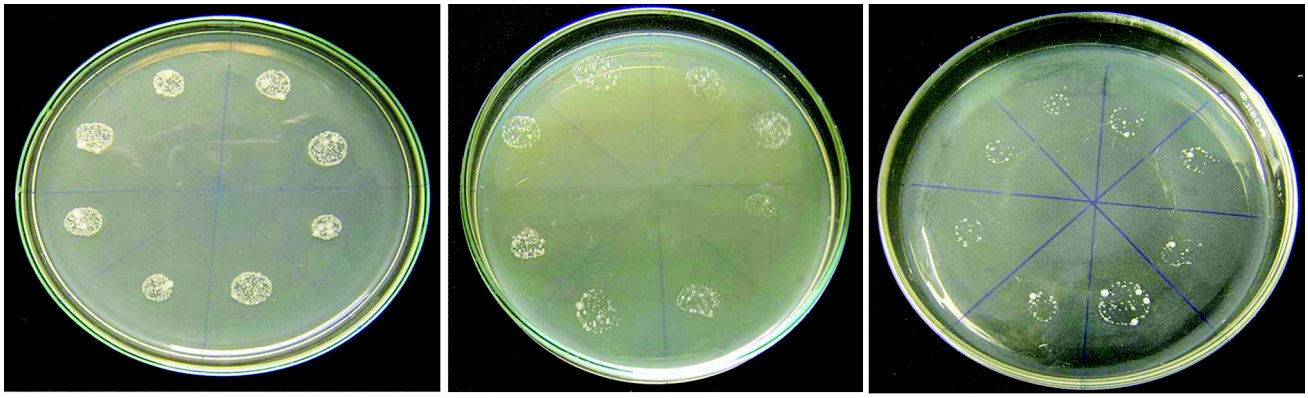

Fig 1 Viability of *P. fluorescens,* Pf 1 in nutrient broth amended with glycerol

broth amended with glycerol (10 mM). This formulation was used to study plant growth promotion and *in vitro* antagonistic effect on *M. incognita*.

Plant growth promotion activity of different days old (0 day to 360 days) culture of Pf 1 grown in nutrient broth amended with glycerol (10mM) was assessed based on the seedling vigour index by the standard roll towel method (ISTA 1993). Twenty five seeds of tomato cv Co 3 were bacterized with different days old culture of *P. fluorescens* (Table 3) and kept over the pre-soaked germination paper. The seeds were held in position by placing another pre-soaked germination paper strip and gently pressed. Polythene sheet along with seeds were then rolled and incubated in growth chamber for 15 days. Three replications were maintained for each treatment. Vigour indices of the seedlings were calculated by recording the root length, shoot length and germination per cent of the individual seedlings by formula:

$$\text{Vigour index} = \text{Per cent germination} \times \text{seedling length (shoot length + root length)}$$

Surface sterilized tomato seeds of cv Co 3 were steeped in suspension of different days old culture of Pf 1 for overnight (Table 3). Treated seeds were sown in pots containing 500 g of sterilized pot mixture (sand: red soil: FYM (1:2:1). An untreated control was also maintained. Germination percentage, shoot length and root length were measured 25 days after seed germination in pot and the vigour index was calculated.

Table 1 Population of *P. fluorescens* (Pf 1) in nutrient broth amended with different chemicals and stickers

Days	Population (cfu/ml)									
	Chemical (nutrient) amendments					Stickers				Nutrient broth
	Glycerol (10 mM)	Trehalose (5 mM)	Sorbitol (5 mM)	Glycine (10 mM)	Mannitol (10 mM)	Starch (2%)	PVP (2%)	Gum acacia (2%)	Liquid paraffin (2%)	
0	50.25×10^9	50.00×10^9	50.00×10^9	50.00×10^9	50.00×10^9	50.50×10^9	50.25×10^9	50.00×10^9	50.00×10^9	50.00×10^9
2	73.00×10^9	71.25×10^9	67.25×10^9	52.50×10^9	50.25×10^9	60.00×10^9	58.25×10^9	55.00×10^9	50.00×10^9	51.00×10^9
5	93.75×10^9	83.25×10^9	80.50×10^9	58.50×10^9	57.25×10^9	72.50×10^9	69.75×10^9	60.25×10^9	55.50×10^9	4.25×10^9
15	77.00×10^9	71.00×10^9	64.00×10^9	7.15×10^8	4.37×10^8	59.00×10^9	53.50×10^9	10.25×10^9	2.43×10^8	2.08×10^8
30	41.75×10^9	36.75×10^9	31.25×10^9	5.905×10^6	4.58×10^5	27.25×10^9	20.00×10^8	6.62×10^8	3.02×10^5	2.85×10^5
45	7.90×10^8	5.75×10^8	5.00×10^8	2.95×10^5	1.98×10^4	5.73×10^8	4.93×10^7	5.17×10^6	1.00×10^3	8.50×10^2
60	1.80×10^8	8.50×10^7	4.10×10^7	1.93×10^3	1.45×10^3	2.40×10^8	2.02×10^6	1.40×10^5	1.25×10^2	1.03×10^2
90	7.50×10^7	3.10×10^7	2.00×10^7	1.88×10^2	1.00×10^2	1.70×10^7	3.28×10^5	2.35×10^4		
120	3.40×10^7	4.70×10^6	5.50×10^6			1.60×10^6	2.18×10^2	1.88×10^2		
150	1.50×10^7	1.50×10^6	1.00×10^6			2.93×10^5				
180	5.00×10^6	3.70×10^5	3.10×10^5			1.85×10^5				
210	2.20×10^5	4.23×10^4	1.45×10^3			1.20×10^3				
240	5.23×10^4	2.20×10^2	2.33×10^2			1.15×10^2				
270	3.40×10^3									
300	2.83×10^2									
360	1.15×10^2									
CD (P=0.05)	1.8597	2.7076	2.3237	2.8492	1.4645	2.9360	2.1010	1.5030	1.8166	0.7073

Table 2 Population of *P. fluorescens* (Pf 1) in King's B broth amended with different chemicals and stickers

Days	Population (cfu/ml)									
	Chemical (nutrient) amendments					Stickers				Broth alone
	Glycerol (10 mM)	Trehalose (5 mM)	Sorbitol (5 mM)	Glycine (10 mM)	Mannitol (10 mM)	Starch (2%)	PVP (2%)	Gum acacia (2%)	Liquid paraffin (2%)	
0	49.25×10^9	48.75×10^9	49.50×10^9	49.50×10^9	49.50×10^9	50.00×10^9	48.75×10^9	50.00×10^9	49.75×10^9	47.75×10^9
2	62.00×10^9	47.50×10^9	44.75×10^9	34.25×10^9	30.50×10^9	43.75×10^9	38.00×10^9	35.55×10^9	24.75×10^9	1.97×10^8
7	2.60×10^8	2.27×10^8	1.52×10^8	1.02×10^8	1.02×10^8	1.45×10^8	1.45×10^8	1.12×10^8	1.02×10^8	7.00×10^6
21	2.20×10^6	1.80×10^6	1.30×10^6	1.00×10^6	9.60×10^6	1.40×10^6	1.20×10^6	1.20×10^6	9.90×10^6	1.15×10^4
30	2.80×10^2	2.35×10^2	2.30×10^2	2.10×10^2	2.03×10^2	2.63×10^2	2.43×10^2	2.23×10^2	2.02×10^2	1.12×10^2
CD (P=0.05)	1.9360	2.4536	1.5444	2.1226	0.9532	1.0111	4.2049	4.8449	2.5666	1.4949

Table 3 Influence of different days old culture of *P. fluorescens* (Pf 1) on the growth of tomato cv. Co3

Treatments/ Days	Roll towel method				Pot culture method			
	Germination percentage	Shoot length (cm)	Root length (cm)	Vigour index	Germination percentage	Shoot length (cm)	Root length (cm)	Vigour index
5	97.33^{a}	12.20^{a}	19.5^{a}	3 085.47	97.33^{a}	12.07^{a}	20.3^{a}	3 153.60
15	96.33^{ab}	12.00^{a}	19.3^{a}	3 018.44	96.67^{ab}	11.93^{ab}	20.0^{ab}	3 086.89
30	96.00^{ab}	11.80^{ab}	19.2^{ab}	2 979.20	96.00^{bc}	11.83^{ab}	19.7^{abc}	3 024.00
60	95.33^{bc}	11.47^{bc}	19.0^{abc}	2 904.49	95.33^{cd}	11.73^{ab}	19.3^{a-d}	2 961.69
90	95.00^{bcd}	11.40^{bc}	18.7^{bcd}	2 859.50	94.67^{de}	11.67^{b}	19.0^{b-e}	2 903.11
120	94.33^{cde}	11.30^{c}	18.4^{cd}	2 804.84	94.00^{ef}	11.27^{c}	18.7^{c-f}	2 813.73
180	94.00^{c-f}	11.23^{cd}	18.2^{de}	2 763.60	93.33^{fg}	11.23^{c}	18.3^{d-g}	2 759.556
240	93.67^{d-g}	11.13^{cd}	17.7^{ef}	2 697.60	92.67^{gh}	11.20^{c}	18.0^{e-h}	2 705.87
270	93.33^{efg}	11.00^{cd}	17.5^{fg}	2 660.00	92.33^{gh}	11.07^{c}	17.7^{fgh}	2 653.04
300	93.00^{efg}	10.86^{d}	17.3^{fg}	2 615.78	92.00^{h}	10.93^{cd}	17.3^{gh}	2 600.53
360	92.67^{fg}	10.46^{e}	17.1^{g}	2 550.50	92.00^{h}	10.67^{d}	17.0^{h}	2 545.33
Control	92.33^{g}	10.03^{f}	17.0^{g}	2 496.08	91.67^{h}	10.10^{e}	17.0^{h}	2 487.22

Values are mean of three replications, Means followed by a common letter within a column are not significantly different (P = 0.05) by DMRT (Duncan's Multiple Rate Test)

For the preparation of cell free filtrate, a single bacterial colony was incubated at 28° C on a shaker at 100 rpm for 2-3 days in Nutrient Broth (NB). The culture was subsequently passed through sterilized Whatman filter paper No. 1 and 42 respectively concentrated by centrifugation at 6000 rpm for 10 min. and supernatant was collected and finally passed through a Millipore filter of 0.22 µm which was finally designated as undiluted standard filtrate of cent per cent concentration (Niknam and Dhawan 2002).

Egg masses used in the present study was collected from the pure culture of *M. incognita* from tomato roots which was maintained in the glass house of Department of Nematology, Tamil Nadu Agricultural University, India. Juveniles hatched out from the egg masses were used for the mortality studies.

About five ml cell free filtrate of different days old (0-360 days) Pf 1 was taken at different concentrations (5, 15 and 25%) in a 50 mm Petri dish (Table 4) and one egg mass of *M. incognita* were placed in each dish and incubated at 28± 1°C. Egg mass placed in nutrient broth without bacteria served as standard check along with distilled water as control. The experiment was replicated four times. Observation on number of hatched juveniles was made after 24, 48 and 72 h of exposure.

About five ml of cell free filtrate of different days old (0 -360 days) Pf 1 was taken at different concentrations, viz. 5, 15 and 25 per cent and poured into separate Petri dishes. Infective juveniles (second stage juveniles (J_2)) of *M. incognita* were introduced into Petri dishes at 100 juveniles per dish and the dishes were incubated at 28± 1° C. Juveniles placed in nutrient broth without bacteria served as standard check along with distilled water as control. Each treatment was replicated four times. Observations were recorded on the mortality of juveniles after 24, 48 and 72 h of exposure period and per cent mortality was calculated. Inactive nematodes were transferred separately from each dilution into sterile distilled water and kept overnight to check whether mortality was permanent or temporary.

The data of one year was pooled and the critical

Table 4 Effect of different days old *P. fluorescens* (Pf 1) culture filtrate on the hatching of *M. incognita* eggs

Days	No. of hatched juveniles/egg mass								
	5%			15%			25%		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
5	33.67 (35.47)	67.33 (55.14)	98.00 (81.87)	20.67 (27.04)	36.00 (36.87)	36.67 (37.27)	3.67 (11.04)	5.33 (13.35)	6.67 (14.96)
30	34.33 (35.87)	67.67 (55.35)	98.33 (82.58)	21.33 (27.51)	36.33 (37.07)	37.67 (37.86)	4.67 (12.48)	6.33 (14.58)	7.67 (16.07)
120	38.33 (38.25)	56.00 (48.45)	88.00 (69.73)	24.00 (29.33)	37.33 (37.66)	39.00 (38.65)	5.67 (13.77)	6.67 (14.96)	8.00 (16.43)
170	40.00 (39.23)	59.00 (50.18)	89.00 (70.63)	27.33 (31.52)	38.00 (38.06)	39.67 (39.04)	6.00 (14.18)	7.33 (15.71)	8.33 (16.78)
210	41.33 (40.01)	61.67 (51.75)	90.33 (71.89)	28.00 (31.95)	38.67 (38.45)	40.33 (39.43)	6.33 (14.58)	8.00 (16.43)	9.67 (18.11)
360	43.33 (41.17)	65.33 (53.93)	91.67 (73.22)	30.33 (33.42)	39.00 (38.65)	40.67 (39.62)	8.00 (16.43)	8.67 (17.12)	10.00 (18.43)
Nutrient broth	72.67 (51.94)	102.33 (75.76)	148.33 (83.93)	65.67 (45.47)	87.33 (65.14)	126.00 (81.87)	53.67 (35.47)	82.33 (55.14)	118.00 (85.78)
Distilled water	78.67 (53.66)	154.33 (65.56)	198.00 (74.66)	78.67 (53.66)	154.33 (65.56)	198.00 (74.66)	78.67 (53.66)	154.33 (65.56)	198.00 (74.66)
Mean	47.79 (41.95)	79.21 (57.02)	112.71 (76.06)	37.00 (34.99)	58.37 (44.68)	69.75 (48.55)	20.84 (21.45)	34.87 (26.61)	45.79 (32.65)
		CD (P=0.05)							
Treatment(T)		(11.07)							
Concentration (C)		(6.77)							
Hour(H)		(6.77)							
T×C		(19.17)							
C×H		(11.74)							
T×H		(19.17)							
T×C×H		(33.21)							

Mean of 4 replications, figures in parentheses are square root transformed values. No. of hatched juveniles per 5 egg masses

differences (CD) was calculated at P=0.05 to test for significant differences between treatments (T) (Pansey and Sukhatme 1978). The data were square root and arcsine transformed before analysis. The treatment means were compared by Duncan's multiple range test (DMRT) (Gomez and Gomez 1984)

RESULTS AND DISCUSSION

Effect of chemical amendments on the viability of Pf 1

Among the different amendments tested in nutrient broth, glycerol (10 mM) maintained the maximum population of Pf 1 throughout the period of observation (one year). Among different amendments tested, maximum population of Pf 1 was maintained in NB amended glycerol (10 mM) for a year. Population load was calculated as 1.15 $\times 10^2$ cfu/ml at 360th day (Table 1). Initial population of 10^9 was maintained up to 30 days and thereafter the population decreased gradually. In NB amended with different stickers, NB amended starch (2%) recorded maximum viable cells of Pf 1. It recorded the Pf 1 population for a period of 240 days with 1.15 $\times 10^2$ cfu/ml at 240th day (Table 1). On the contrary, NB without any amendation showed population load of 1.03 $\times 10^2$ cfu/ml at 60th day of incubation itself.

Addition of amendments in King's B broth did not bring any significant changes in the survival of Pf 1. The population load was maintained only up to 30 days with all the amendments tested in KB broth (Table 2).

Enhanced survival of *Pseudomonas* cells in liquid medium may be due to the addition of amendments and stickers to the medium. Poonguzhali (2002) reported the higher growth rate of phosphobacteria in the medium amended with glycerol. Similar result was obtained by Chavan and Kadam (2009) where they found that the addition of glycerol (2%) increased the viability and virulence of *Verticillium lecani*. *P. fluorescens* showed complete viability when plated onto the medium supplemented with 10 mM glycerol (Manikandan *et al*. 2010). Moreover, the polymeric additives such as starch had the property of colloidal stabilization. The improvement of survival is analogous to the protective colloid effect where bacteria represent one colloid and the suspension the other (Deaker *et al.* 2004).

Effect of different days old Pf 1 on the growth of tomato cv Co 3

In roll towel method, Pf 1 cultures induced plant growth

promotion in tomato cv Co 3 significantly over untreated control. Among different days old culture, fifth day old culture of Pf 1 recorded the maximum vigour index (3085.47) (Table 3).

Similar result was revealed under pot culture condition in which fifth day culture performed better than the subsequent day old cultures with the vigour index of 3153.60. Activity of the culture was decreased as age of the culture increased (Table 3).

The mechanism by which plant growth is improved include production of phytohormones, enhanced availability of nutrients, production of antibiotics, reduction of ethylene level, induced systemic resistance and competition for space and nutrition (Holland 1997). Bacterized grapevines enhanced fresh weight of shoots and roots and accelerated growth of vine with more lignin deposits (Barka *et al.* 2002). Maximum increase in growth of banana was observed in the plants treated with native *P. fluorescens* isolate, PfB 13 (Senthilkumar *et al.* 2008). Similar study conducted by Devapriyanga *et al.* (2012) and Jonathan *et al*. (2012) with native isolates of *P. fluorescens* from black pepper and observed enhanced germination and seedling vigour due to treatment with *P. fluorescens.*

Effect of different days old Pf 1 on M. incognita *eggs and juveniles*

The study revealed a negative relationship between the concentration of aqueous formulation and the number of eggs hatched. Significant reduction in egg hatching was observed with different days old culture of Pf 1 with the least being observed with fifth day old culture of Pf 1 (6.67 J_2/egg mass) at 25 per cent concentration after 72 h of exposure period (Table 4). Similar trend was also observed in 15 and 5 per cent concentrations. Maximum egg hatching was recorded in distilled water (198 J_2/egg mass) followed by 25% concentration of NB (118 J_2/ egg mass) at 72 h of exposure period.

In mortality study, gradual increase in juvenile mortality was observed with increase in concentration of culture filtrate. Significant difference in mortality rate was observed with different days old Pf 1 culture at all its exposure period (Table 5). Highest juvenile mortality of 88.67 per cent was recorded in the culture filtrate of fifth day old culture of Pf 1 at its 25 per cent concentration after 72 h exposure period. Similar trend was observed in 15 and 5 per cent concentrations of culture filtrates. Lowest juvenile mortality was recorded in distilled water (1.67%) followed by nutrient broth (8.67%).

Table 5 Effect of different days old *P. fluorescens* (Pf 1) culture filtrate on per cent mortality of *M. incognita* juveniles

Days	Per cent of dead juveniles								
	5%			15%			25%		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
5	5.33 (13.35)	12.67 (20.85)	13.33 (21.42)	19.00 (25.84)	36.67 (37.27)	37.67 (37.86)	49.67 (44.81)	87.67 (69.44)	88.67 (70.33)
30	5.00 (12.92)	11.33 (19.67)	12.67 (20.85)	17.33 (24.60)	34.33 (35.87)	35.67 (36.67)	49.33 (44.62)	85.00 (67.21)	86.67 (68.58)
120	4.67 (12.48)	10.67 (19.06)	11.67 (19.97)	16.33 (23.84)	31.33 (34.04)	32.33 (34.65)	47.33 (43.47)	80.67 (63.92)	82.67 (65.40)
170	4.33 (12.01)	8.33 (16.78)	9.33 (17.79)	14.00 (21.97)	29.33 (32.79)	30.67 (33.63)	44.00 (41.55)	75.33 (60.22)	77.67 (61.80)
210	3.67 (11.04)	7.33 (15.71)	8.33 (16.78)	13.67 (21.70)	26.67 (31.09)	27.67 (31.73)	44.00 (41.55)	71.00 (57.42)	72.67 (58.48)
360	2.67 (9.40)	6.00 (14.18)	7.33 (15.71)	10.33 (18.75)	25.33 (30.22)	26.33 (30.87)	43.00 (40.98)	68.00 (55.55)	70.33 (57.00)
Nutrient broth	0.67 (4.68)	1.67 (7.42)	2.00 (8.13)	1.33 (4.86)	3.67 (4.09)	5.00 (6.13)	4.00 (6.74)	6.00 (12.35)	8.67 (16.01)
Dis water	0.67 (4.68)	1.33 (6.63)	1.67 (7.42)	0.67 (4.68)	1.33 (6.63)	1.67 (7.42)	0.67 (4.68)	1.33 (6.63)	1.67 (7.42)
Mean	3.38 (10.07)	7.42 (15.04)	8.29 (16.01)	11.75 (18.21)	23.90 (26.45)	24.94 (27.29)	35.75 (33.47)	60.23 (48.94)	61.97 (50.41)
		CD (P=0.05)							
Treatment (T)		(5.60)							
Concentration (C)		(3.43)							
Hour(H)		(3.43)							
T×C		(9.70)							
C×H		(5.94)							
T×H		(9.70)							
T×C×H		(16.81)							

Mean of 4 replications, figures in parentheses are arcsine transformed values

The study conducted by Spiegel *et al.* (1991) also indicated the effectiveness of *P. chitinolytica* against *M. incognita* due to its strong chitinolytic and proteolytic activity. Biocidal property of the culture filtrate increased with increase in its exposure period and concentration. The results of Kamra and Dhawan (1999) also confirmed the present findings where they have reported the toxic effect of cell free filtrate of *P. fluorescens* on the eggs and juveniles of *H. avenae* with a significant reduction in egg hatching and larval mortality in S/10 and S/100 dilutions when compared to distilled water. Similar study conducted by Siddiqui (2000), Seenivasan and Lakshmanan (2001) revealed an antagonistic effect of *P. fluorescens* on nematode eggs and juveniles.

The report of Cannayane and Rajendran (2001) was also in accordance with the present findings where they have confirmed the antagonistic effect of *P. fluorescens* culture filtrate on *M. incognita* juveniles collected from bhendi crops. Combined application of native strains of *P. fluorescens* (Pf 123) and *Bacillus subtilis* (Bs 214) isolated from black pepper at its 100 per cent concentration were found to be effective against *M. incognita* 72 h after exposure of the culture filtrate to nematode (Devapriyanga *et al*. 2012).

REFERENCES

Kamra A and Dhawan S C. 1999. Effect of *Pseudomonas fluorescens* culture filtrate on the emergence and mortality of juveniles of *Heterodera avenae*. *(In) Proceedings of Rational Approaches in Nematode Management for Sustainable Agriculture.* Gujarat Agricultural University, Anand, India, pp 116–8.

Barka E A, Gognies S, Nowak J, Audran J C and Belarbi A. 2002. Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. *Biological Control* **24**: 135–42.

Cannayane I and Rajendran G. 2001. Management of *Meloidogyne incognita* by bacterial and fungal culture filtrates on bhendi, *Abelmoschus esculentus* L. *Current Nematology* **12**: 85-89.

Chavan B P and Kadam R. 2009. Effect of combination of adjuvants on liquid formulations of *Verticillium lecani* (Zimmermann) viegas and their efficacy. *Journal of Biological Control* **23** (1): 73–7.

Deaker R, Roughley R J and Kennedy I R. 2004. Legume seed inoculation technology–a review. *Soil Biology Biochemistry*, **36**: 1 275–88.

Devapriyanga R, Jonathan E I, Sankari Meena K and Kavitha P G. 2012. Bioefficacy of *Pseudomonas* and *Bacillus* isolates against root knot nematode, *Meloidogyne incognita* in black pepper cv Panniyur 1. *Indian journal of Nematology* **42**(1): 57–65.

Gomez K A and Gomez A A. 1984. *Statistical Procedure for Agricultural Research*. John Wiley and Sons, New York.

Holland M A. 1997. Occam's razor applied to hormonology: Are cytokinnins produced by plants. *Plant Physiology* **115**: 865–8.

ISTA. 1993. Proceedings of the International Seed Testing Association. International Rules for Seed Testing. *Seed Science Technology* **21**: 25–30.

Jonathan EI, Raguchander T, Sankari Meena K and Kavitha PG. 2012. Plant growth promoting rhizobacteria in the management of *Radopholus similis* and *Meloidogyne incognita* in black pepper. *The Madras Agricultural Journal* **99**(4-6): 356–8.

Manikandan R, Saravanakumar D, Rajendran L, Raguchander T and Samiyappan R. 2010. Standardisation of liquid formulation of *Pseudomonas fluorescens*, Pf 1 for its efficacy against *Fusarium* wilt of tomato. *Biological control* **54**(2):83–9.

Niknam G R and Dhawan S C. 2002. Systemic resistance induced by *Pseudomonas fluorescens* isolate Pf 1 in tomato against *Rotylenchulus reniformis*. *International Journal of Nematology* **12**(2): 203–8.

Pansey, V G and Sukhatme P V. 1978. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi.

Poonguzhali S. 2002. 'Standardization of media for co-culturing of diazotroph (*Azospirillum*/*Rhizobium*) and phosphobacteria (*B. megaterium).*' M Sc (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, 48 p.

Saravanakumar D, Lavanya N, Muthumeena K, Raguchander T and Samiyappan R. 2009. Fluorescent pseudomonas mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease.*Biocontrol* **54** (2): 273–86.

Seenivasan N and Lakshmanan PL. 2001. Effect of culture filtrates of *Pseudomonas fluorescens* on rice root nematode, *Hirschmanniella gracilis*. *Pestology* **25**: 11–2.

Senthilkumar P, Jonathan EI and Samiyappan R. 2008. Bioefficacy of *Pseudomonas fluorescens* on burrowing nematode, *Radopholus similis* in Banana. *Indian Journal of Nematology* **38(1)**: 46–52.

Siddiqui IA. 2000. Effects of cell free culture filtrates of *Pseudomonas aeruginosa* in control of root rot and root knot disease complex of tomato. *Acta Agrobotanica* **53:** 47–55.

Sikora RA and Fernandez E. 2005. *(in) Plant parasitic nematodes in Subtropical and Tropical Agriculture,* pp 319–92. Luc MR, Sikora A and Bridge J (Eds). CABI Publisher,Wallingford,UK.

Somasegaran P and Hoben H J. 1994. *Handbook for Rhizobia-Methods in Legume-Rhizobium Technology,* p 450. Springer-Verlag, New York, USA.

Spiegel Y, Chon E, Galper S, Sharon E and Chet I. 1991. Evaluation of a newly isolated bacterium, *Pseudomonas chitinolytica* sp. *nov* for controlling the root-knot nematode *Meloidogyne javanica. Biocontrol Science and Technology* **1:** 115–125.

Tamilvendan R and Thangaraju M. 2006. Development and standardization of liquid formulation for *Azospirillum* bioinoculant. *Indian Journal of Microbiology* **46**(4): 379–87.