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Viability of *Pseudomonas fluorescens* in Liquid Formulation and its Effect on Plant Growth Promotion and Inhibition of Root Knot Nematode, *Meloidogyne incognita*

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Viability of the Pfbv 22 isolate of *Pseudomonas fluorescens* in liquid formulation amended with different chemicals was studied. Among amendments tested, the liquid formulation of *P. fluorescens,* Pfbv 22 isolate amended with glycerol (10mM) maintained maximum viability nearly for a year. In the plant growth promotion study conducted with different aged isolates of Pfbv 22 on tomato, the five day old culture recorded a maximum vigour index (3179.56). Results of the *in vitro* study carried out with different aged cultures of Pfbv 22 on eggs and juveniles of *M. incognita* revealed maximum suppression in egg hatching and minimum juvenile morality in the same five day old culture when compared with other aged cultures.

Keywords: Liquid formulation, Plant growth promotion, *Pseudomonas fluorescens*, Root knot nematode,

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Globally plant parasitic nematodes are important antagonists to agricultural and horticultural crops which cause economic loss of about 100 billion dollars every year (Sasser and Frackman, 1987). Apart from direct damage, nematodes also predispose the plants to secondary invasion by fungal and bacterial pathogens. In recent years, plant growth promoting rhizobacteria (PGPR), fluorescent pseudomonds have been reported to be effective in boosting plant growth and vigor and also deleterious to plant pathogens including nematodes (Singh *et al.,* 1990). The liquid formulated *P. fluorescens* was found to be more effective than talc formulation in maintaining the viability of *Pseudomonas* cells (Manikandan *et al*., 2010).

With regard to liquid formulation, not much work has been carried out with *P. fluorescens.* Hence, the present study has been carried out to observe the viability of *P. fluorescens*, Pfbv 22 in liquid formulation amended with different chemical amendments and to observe its plant growth promoting ability and antagonistic effect on root knot nematode, *M. incognita*.

Materials and Methods

The *P. fluorescens* isolate, Pfbv 22 already proved to be effective against *M. incognita* in various high value crops by earlier workers was obtained from the Centre for Plant Protection Studies, TNAU, Coimbatore, India and used for the present study.

Viability studies of P. fluorescens with different chemicals as amendments

The viability of *P. fluorescens* isolate, Pfbv 22 was

tested for a period of 360 days at different time of interval as mentioned in Table 1 by adding different nutrients as amendments *viz*., glycerol (10 mM), trehalose (5 mM), sorbitol (5 mM), glycine (10 mM), mannitol (10 mM), starch (2 %), liquid paraffin (2 %), polyvinlypyrollidone (2%) and gum acacia (2 %) per litre of nutrient broth (NB).

One ml of the log phase culture of Pfbv 22 was inoculated into NB amended with chemicals and incubated at room temperature (25 $\pm$1$_{o}$C). The culture in the broth was examined for viable cell population at different intervals upto its decline phase. Serial dilution was made by transferring one ml of the inoculum into 9 ml sterile water blanks to get 10^{-1} dilution. Similarly dilutions were made upto 10^{-9} and 10 µl was pipetted out from the dilutions and plated in Nutrient media. Plates were incubated for 24 h at room temperature and individual colonies were counted through drop plate technique (Somasegaran and Hoben, 1994). Four replications were maintained for each dilution.

The *P. fluorescens* liquid formulation amended with glycerol (10 mM) maintaining maximum viability of cells was advanced for further study on its effect on plant growth promotion in tomato and *in vitro* efficacy of root knot nematode.

Effect of different days old culture of Pfbv 22 on plant growth promotion

Plant growth promotion ability of aqueous culture of Pfbv 22 was assessed for a period of 360 days at its different storage periods by standard roll towel and pot culture method. In role towel method, tomato (cv. Co 3) seeds (25 / replication) were bacterized with different days old culture of Pfbv 22 and placed

over presoaked germination paper. Seeds were held in position by placing another strip of presoaked germination paper and then gently pressed. Polythene sheets along with seeds were rolled and incubated in growth chamber for 15 days. This treatment was compared with control and replicated four times. Shoot and root length of individual seedlings were measured after 15 days and vigour index was calculated using the following formula, Vigour index = (Mean shoot length + Mean root length) x Germination (%) (ISTA, 1993)

Effect of different day old culture of Pfbv 22 on plant growth of tomato under pot culture condition

In pot culture studies, growth promotion ability was carried out for a period of 360 days in which surface sterilized tomato seeds were steeped in suspension of different aged culture of Pfbv 22 overnight and the treated seeds were sown in pots containing 500 g of sterilized pot mixture and thinned to one plant per pot. Four replications were maintained for each treatment. Germination percentage, shoot and root length were measured 25 days after germination and vigour index was calculated using the above mentioned formula.

In vitro *assay of different aged cultures of Pfbv 22 on eggs and juveniles of M. incognita*

In vitro assay was carried out with the culture filtrate of different aged culture of Pfbv 22. About 5 ml of cell free culture filtrate of different aged and concentrations (5, 15 and 25 %) of Pfbv 22 were taken in 50 mm Petri dish and five egg masses of *M. incognita* collected from pure culture of *M. incognita* maintained on tomato were placed in each dish and incubated at room temperature (28±1°C). Egg masses placed in Nutrient broth served as standard check and distilled water served as control. All the treatments were replicated four times in CRD. Observations were made on number of hatched juveniles at 24, 48 and 72 h of exposure.

For the effect of culture filtrates of Pfbv 22 on juveniles of *M. incognita,* five ml of cell free culture filtrate of different aged and concentrations (5, 15 and 25 %) of Pfbv 22 were taken in 50 mm Petri dish and about 100 second stage juveniles of *M. incognita* collected from pure culture of *M. incognita* were inoculated in each dish and incubated at room temperature (28±1°C). The juveniles placed in nutrient broth served as standard check and distilled water as control. Four replications were maintained for each treatment. Observations were made on the number of dead juveniles after 24, 48 and 72 h of exposure period and per cent mortality was calculated. All the experimental data were statistically analyzed as per the method given by Gomez and Gomez (1984).

Results and Discussion

Effect of different chemicals as amendments on the viability of Pfbv 22

Among the different chemicals used as amendments with the isolate of Pfbv 22, the addition of glycerol (10 mM) in nutrient broth was effective in maintaining the propagules of Pfbv 22 nearly for a year when compared with other chemicals. The maximum propagules interms of cfu was registered in 5 days old culture of Pfbv 22 amended with glycerol (95.75 x 10_9 cfu/ml). The cfu of the isolate tend to decrease with other aged cultures. The NB without addition of any chemical as amendment maintained the propagule load only upto 60 days and it is least (1.20 x 10_2 cfu/ml) among different combination (Table 1).

Enhanced survival of *Pseudomonas* cells in liquid medium might be due to the addition of amendments to the medium. The increase in cell/ propagules viability with the addition of glycerol was already proved by Chavan and Kadam (2009) in *Verticillium lecani* and Poonguzhali (2002) with phosphobacteria.

Table 1. Propagules of *P. fluorescens* (Pfbv 22) in nutrient broth supplemented with chemicals as amendments

Days	Propagules (cfu/ml)									
	Nutrient amendments									
	Glycerol	Trehalose	Sorbitol	Glycine	Mannitol	Starch	Poly Vinyl Pyrollidone (PVP)	Gum acacia	Liquid paraffin	Nutrient broth
0	50.25×10^9	50.00×10^9	50.00×10^9	50.00×10^9	50.00×10^9	50.75×10^9	50.25×10^9	50.00×10^9	50.00×10^9	50.00×10^9
2	74.00×10^9	72.50×10^9	67.75×10^9	53.50×10^9	51.00×10^9	61.25×10^9	60.00×10^9	56.00×10^9	50.25×10^9	50.25×10^9
5	95.25×10^9	84.50×10^9	81.00×10^9	60.75×10^9	58.75×10^9	73.75×10^9	71.00×10^9	62.00×10^9	57.50×10^9	5.00×10^9
15	78.00×10^9	72.25×10^9	65.75×10^9	7.40×10^8	4.57×10^8	60.50×10^9	55.00×10^9	10.75×10^9	2.68×10^8	2.33×10^8
30	43.00×10^9	39.00×10^9	32.25×10^9	6.15×10^6	4.73×10^5	28.50×10^9	23.25×10^8	7.12×10^8	3.20×10^5	3.10×10^5
45	8.80×10^8	6.25×10^8	5.00×10^8	3.08×10^5	2.20×10^4	5.78×10^8	7.60×10^7	5.62×10^6	1.08×10^3	1.00×10^3
60	2.30×10^8	8.80×10^7	4.10×10^7	2.03×10^3	1.60×10^3	2.50×10^8	2.40×10^6	1.45×10^5	1.15×10^2	1.20×10^2
90	8.30×10^7	3.40×10^7	2.10×10^7	2.05×10^2	1.00×10^2	2.20×10^7	3.70×10^5	2.83×10^4	-	-
120	3.80×10^7	5.40×10^6	5.60×10^6	-	-	1.68×10^6	2.48×10^2	2.15×10^2	-	-
150	1.80×10^7	1.60×10^6	1.10×10^6	-	-	3.40×10^5	-	-	-	-
180	5.00×10^6	4.40×10^5	3.90×10^5	-	-	2.05×10^5	-	-	-	-
210	2.60×10^5	5.23×10^4	1.70×10^4	-	-	1.43×10^3	-	-	-	-
240	3.80×10^3	2.20×10^2	2.50×10^2	-	-	2.33×10^2	-	-	-	-
270	3.30×10^3	-	-	-	-	-	-	-	-	-
300	3.20×10^3	-	-	-	-	-	-	-	-	-
330	3.00×10^3	-	-	-	-	-	-	-	-	-
360	2.60×10^2	-	-	-	-	-	-	-	-	-
CD (0.05)	2.8827	1.9821	1.3575	1.5820	1.3260	2.3707	2.0731	1.5630	0.8867	0.2071

Effect of different aged culture of Pfbv 22 on plant growth in tomato

The results of roll towel method revealed that five days old culture of Pfbv 22 recorded the highest vigour index of 3180.68 with seed germination of 97.67 per cent (Table 2). Although the plant growth promoting ability of Pfbv 22 tend to decrease in the subsequent day old cultures, it was higher than control which recorded a vigour index of 2600.81 with 93.67 per cent germination.In pot culture studies, five days old culture of Pfbv 22 performed better than other aged cultures in improving the plant growth parameters of tomato. It recorded a vigour index of 3179.56 with 97.33 per cent germination

Table 2. Influence of different aged culture of *P. fluorescens* (Pfbv 22) on growth of tomato cv. Co3

Age of culture (Days)	Roll towel method				Pot culture method			
	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
5	97.67$_{a}$	12.80$_{a}$	19.8$_{a}$	3180.68	97.33$_{a}$	12.27$_{a}$	20.4$_{a}$	3179.56
15	97.33$_{ab}$	12.70$_{ab}$	19.7$_{a}$	3153.60	96.67$_{ab}$	12.00$_{ab}$	20.0$_{ab}$	3093.33
30	97.00$_{abc}$	12.50$_{ab}$	19.5$_{a}$	3107.23	96.33$_{bc}$	11.70$_{bc}$	19.7$_{bc}$	3024.87
60	96.33$_{a-d}$	12.43$_{abc}$	19.3$_{ab}$	3060.19	95.67$_{cd}$	11.60$_{bcd}$	19.5$_{c}$	2975.23
90	96.00 $_{b-e}$	12.33$_{abc}$	19.2$_{abc}$	3030.40	95.33$_{d}$	11.57$_{cd}$	19.3$_{cd}$	2945.80
120	95.67$_{c-f}$	12.23$_{bcd}$	18.9$_{bcd}$	2975.23	95.00$_{de}$	11.50$_{cde}$	18.9$_{de}$	2891.17
180	95.33$_{d-g}$	12.17$_{bcd}$	18.7$_{cd}$	2945.8	94.33$_{ef}$	11.43$_{cde}$	18.5$_{ef}$	2823.71
240	94.67$_{e-h}$	11.93$_{cde}$	18.6$_{de}$	2887.33	94.00$_{f}$	11.13$_{ef}$	18.2$_{fg}$	2754.20
270	94.33$_{fgh}$	11.77$_{de}$	18.2$_{ef}$	2823.71	93.67$_{fg}$	11.20$_{def}$	17.9$_{gh}$	2725.70
300	94.00$_{gh}$	11.47$_{ef}$	18.0$_{f}$	2773.00	93.00$_{gh}$	11.00$_{f}$	17.6$_{hi}$	2656.70
360	93.67$_{h}$	11.10$_{f}$	17.7$_{fg}$	2700.72	92.67$_{h}$	10.60$_{g}$	17.4$_{hi}$	2597.76
Control	93.67$_{h}$	10.40$_{g}$	17.4$_{g}$	2600.81	92.33$_{h}$	10.00$_{h}$	17.3$_{i}$	2517.62

(Table 2). The growth promoting ability of the culture tends to decrease with increase in number of days of storage. Production of phytohormones by *P. fluorescens* is attributed as core of the mechanism for the improvement in plant growth of tomato. The present study is in confirmatory with the report of Jonathan *et al*. (2005) in betelvine and Senthil Kumar *et al*. (2008) in banana in which the authors demonstrated the improvement in plant growth due to the *P. fluorescens*.

In vitro *efficacy of different aged cultures of P. fluorescens on* M. *incognita*

The study revealed a negative relation between concentration of aqueous formulation and number of eggs hatched. Significant reduction in egg hatching was observed in the culture filtrate of different days old Pfbv 22 with the least being observed in five day old culture filtrate (6.33) at 25 per cent concentration after 72 h exposure period (Table 3).

Table 3. Effect of different aged culture of *P. fluorescens*, Pfbv 22 on per cent egg hatching of *M. incognita*

Age of culture (Days)	Concentration*								
	5 %			15 %			25 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
5	35.00 (36.27)	55.67 (48.25)	87.33 (69.15)	19.67 (26.33)	35.67 (36.67)	36.33 (37.07)	3.00 (9.97)	4.67 (12.48)	6.33 (14.58)
30	37.67 (37.86)	58.67 (49.99)	88.33 (70.03)	20.67 (27.04)	35.67 (36.67)	36.67 (37.27)	4.33 (12.01)	5.67 (13.77)	6.67 (14.96)
120	38.33 (38.25)	61.00 (51.35)	89.33 (70.94)	23.00 (28.66)	36.67 (37.27)	37.67 (37.86)	5.00 (12.92)	6.00 (14.18)	7.00 (15.34)
170	39.67 (39.04)	64.67 (53.53)	90.67 (72.21)	26.67 (31.09)	37.67 (37.86)	38.67 (38.45)	5.33 (13.35)	6.67 (14.96)	7.33 (15.71)
210	42.00 (40.40)	67.33 (55.14)	91.67 (73.22)	27.00 (31.31)	38.00 (38.06)	39.00 (38.65)	5.67 (13.77)	7.67 (16.07)	9.33 (17.79)
360	44.00 (41.55)	71.00 (57.42)	92.33 (73.93)	30.00 (33.21)	38.67 (38.45)	40.00 (39.23)	7.33 (15.71)	8.00 (16.43)	9.67 (18.11)
Nutrient broth	34.00 (35.67)	67.67 (55.35)	98.00 (81.87)	34.00 (35.67)	67.67 (55.35)	98.00 (81.87)	34.00 (35.67)	67.67 (55.35)	98.00 (81.87)
Distilled water	34.67 (36.07)	68.00 (55.55)	98.33 (82.58)	34.67 (36.07)	68.00 (55.55)	98.33 (82.58)	34.67 (36.07)	68.00 (55.55)	98.33 (82.58)

Figures in parentheses are square root transformed values

* Mean of 4 replications

	CD (p=0.05)		
Treatment(T)	(11.21)	CXH	(11.89)
Concentration (C)	(6.86)	TXH	(19.42)
Hour(H)	(6.86)	TXCXH	(33.64)
TXC	(19.42)		

The mortality rate of the juveniles increased with increase in concentration of the culture filtrate. The highest juvenile mortality of 89.33 per cent was recorded with five days old culture filtrate of Pfbv 22

Table 4. Effect of different aged culture of *P. fluorescens,* Pfbv 22 on juveniles of *M. incognita*

Age of culture (Days)	Concentration*								
	5 %			15 %			25 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
5	5.67	13.33	14.00	19.33	37.33	38.67	51.33	88.33	89.33
30	5.33	12.00	13.00	18.00	35.67	37.00	50.67	86.33	87.67
120	5.00	11.00	12.00	16.67	32.33	32.67	47.67	81.67	83.33
170	4.67	8.67	10.00	14.67	30.00	31.33	45.67	77.00	78.33
210	4.00	7.67	8.67	14.33	27.33	28.00	44.33	72.33	74.00
360	3.00	6.67	8.33	11.33	26.33	27.00	43.33	69.33	71.67
Nutrient broth	0.67	1.67	2.00	0.67	2.00	2.00	1.00	2.00	2.00
Distilled water	0.67	1.33	1.67	0.67	1.33	1.67	0.67	1.33	1.67

Figures in parentheses are arcsine transformed values * Mean of 4 replications

	CD (p=0.05)
Treatment(T)	(5.83)
Concentration (C)	(3.57)
Hour(H)	(3.57)
TXC	(10.11)
CXH	(6.19)
TXH	(10.11)
TXCXH	(17.51)

at 25 per cent concentration after 72 h exposure period (Table 4). The 360 days old culture of Pfbv 22 recorded the egg hatching of 9.67 and juvenile mortality of 71.67 at 25 per cent concentration in 72 h exposure period which is noted to be higher than control. Maximum egg hatching (98.33) and minimum juvenile mortality (1.67) was recorded in control. The results of the present study is in agreement with the findings of Seenivasan and Lakshmanan (2001) and Siddiqui (2000) who observed that the culture filtrate of *P. fluorescens* showed antagonistic effect on root knot nematode eggs and juveniles. The reports of the toxic effect of cell free filtrate of *P. fluorescens* against eggs and juveniles of *Heterodera avenae* made by Anju Kamra and Dhawan (1999) were also supportive to the present findings.

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