Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 953-959

Received: 16-09-2020
Accepted: 18-10-2020

Jayanta IN
Dept. of Plant Pathology, Agriculture College, University of Agricultural Sciences, Raichur, Karnataka, India

Mallesh SB
Scientist Agriculture Research Station, Hagari, Ballari, Karnataka, India

Ramesh G
Assistant Professor. Department of Horticulture, Agriculture College, University of Agricultural Sciences, Karnataka, India

Zaheer Ahamed B
Scientist (Plant Pathology), Krishi Vigyan Kendra, Kalaburgi, Karnataka, India

Amaresh YS
Assistant. Prof. Dept. of Plant Pathology, Agriculture College, University of Agricultural Sciences, Raichur, Karnataka, India

Sreedevi SC
Scientist and Head, AICRP, Groundnut, MARS, Raichur, Karnataka, India

Corresponding Author:
Ramesh G
Assistant Professor. Department of Horticulture, Agriculture College, University of Agricultural Sciences, Karnataka, India

Isolation and *in vitro* screening of native fluorescent Pseudomonads against *Fusarium* and root knot wilt disease complex pathogens of tomato

Jayanta IN, Mallesh SB, Ramesh G, Zaheer Ahamed B, Amaresh YS and Sreedevi SC

Abstract
Tomato (*Solanum lycopersicum* Mill.) is the most popular vegetable crop world-wide. Tomato crop is known to be affected by different diseases caused by fungi, bacteria, viruses and nematodes. Among different diseases affecting tomato, root-knot and wilt complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* was observed in severe form. Twenty fluorescent Pseudomonads were isolated from the healthy rhizosphere of tomato growing areas of North Eastern Karnataka by serial dilution method on King's B medium. Dual culture technique under *in vitro* evaluation of fluorescent Pseudomonads against *F. oxysporum* f. sp. *lycopersici* revealed that the per cent inhibition of was maximum (78.52) in FP- 13 followed by FP-4 (74.82%) and FP-6 (73.71%). Least inhibition of 20.74 per cent was observed in FP-19 followed by FP-20 (35.55%). In case of nematode juvenile mortality, isolate FP-3 showed maximum inhibition of 71.05 (%), followed by FP-13 (70.54%), FP-18 (70.05%), FP-1 (69.77%), FP-6 (68.83%), FP-4 (68.33%) and FP-5 (67.83%).The highest decrease in egg hatching was recorded in isolate FP-3 (2.24), followed by FP-1 (2.41), FP-13 (2.57), FP-18 (2.83), FP-14 (5.66), FP-6 (5.83) and FP-2 (6.58). Further, seven isolates *viz.*, FP-1, FP-3, FP-4, FP-5, FP-6, FP-13 and FP-18 were found commonly efficacious against test pathogens *i.e.*, *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* and were used for further studies.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, fluorescent pseudomonads, *Meloidogyne incognita* tomato and wilt

Introduction
Tomato **(***Solanum lycopersicum* Mill.) belonging to the nightshade family *i.e.*, solanaceae, is one of the most important popular vegetable in the world. It is native of Peru in South America and the word tomato comes from the Aztec word Tomatl. This crop spread to North America, primarily by migrating birds and then the crop spread to different countries. Spanish priests introduced the tomato crop to Europe around 1550. In Europe it was known as *Poma amoris*- Amorous apple or love apple. It was also known as *Poma peruviana*, apple of Peru. It was Robert Gibbon Johanson, an ordinary farmer in the U.S.A who first ate tomato on a hot day of August, 1820 to demonstrate its edibility. From then onwards, the tomato spread throughout the world.

Global tomato production is currently around 170.75 million tons and China is the leading country both in area (1 million ha) and production (52.72 million tons). India ranks second with production of 21.24 million tons followed by USA, Turkey and Egypt. In India top five tomato producing states are Madhya Pradesh, Karnataka, Andhra Pradesh, Telangana and Gujarat. Karnataka has an area of 63.73 thousand ha and annual production of about 2138.13 thousand tons with an average productivity of 33.55 tons/ha (Anon., 2017) [2].

Among the fungal diseases, *Fusarium* wilt is the most important disease and is a limiting factor in tomato production (Sokhi *et al.*, 1991) [17]. *Fusarium oxysporum* f. sp. *lycopersici* is a soil borne pathogen that is highly specific to tomato and is worldwide in distribution (Walker, 1969) [19]. Among the nematode diseases in tomato, root knot nematode (*Meloidogyne* species), sting nematode (*Belonolaimus longicaudatus*), stubby-root nematode (*Paratrichodorus* and *Trichodorus* spp.) and lesion nematode (*Pratylenchus* sp.) are important. Of these, root knot nematode (*M. incognita*) is the most dominant species accounting for 64 per cent of total

population which is widely prevalent, inflicting serious loss to tomato fruit yield (Sasser, 1980) [15].

Material and methods

Isolation of fluorescent Pseudomonads

Soil samples were collected from the healthy rhizosphere of tomato, growing areas from North Eastern Karnataka during the survey. After scraping, the top 2-3 cm soil with the kurpie upto a depth of 20-40 cm, at a distance of 25-50 cm from the base of the plant the soil was collected from 3-4 places randomly. About 250 g soil and 10 g of roots were collected in polythene bags with proper label. From these rhizospheric soil samples, fluorescent Pseudomonads were isolated in laboratory conditions by using serial dilution technique on King's 'B' and it was characterized.

These isolates were maintained in laboratory for further studies. Characterization of the different isolates of fluorescent Pseudomonads were carried out according to the Laboratory Guide for identification of Plant Pathogenic Bacteria published by the American Phytopathological Society (Schaad, 1992) [16]. For each test, 24 to 48 h. old cultures were used. The cultures of fluorescent Pseudomonads were used for studying the biocontrol efficacy. The cultures were sub cultured on NA slants and allowed to grow at 25 ˚C for 10-15 days and such slants were preserved in refrigerator at 4 ˚C and further sub cultured once in 30 days.

In vitro screening of fluorescent Pseudomonads against *F. oxysporum* f. sp. *lycopersici*

Fluorescent Pseudomonads from healthy rhizosphere and rhizoplane of major tomato growing areas of North Eastern Karnataka were tested for their inhibitory activity against mycelial growth of *Fusarium* by following the dual culture technique (Dennis and Webster, 1971) [3]. Mycelial discs of 5 mm diameter of seven days-old culture of *Fusarium* were placed in the middle of the Petri plate containing 20 ml PDA medium. Twenty four hour old culture of each fluorescent Pseudomonads were streaked parallel on either side of the fungal disc (30 mm away from the disc). The plates were incubated at room temperature (28 ± 2 ˚C) for 8-10 days, until the plate was covered completely by the fungus in control. The plates with only fungal disc without bacterial streaks were served as control. Each treatment was replicated three times. After incubation, *i.e.* when control plate reached 90 mm diameter, the radial growth of pathogen were measured. Per cent inhibition over control was calculated by using the formula of Vincent (1947) [18] as follows;

$$\text{Per cent inhibition} = \frac{(C- T)}{C} \times 100$$

I = Per cent inhibition of mycelium

C = Growth of mycelium in control, T = Growth of mycelium in treatment

In vitro screening of fluorescent Pseudomonads against *M. incognita*

Preparation of cell free extract

A single colony of each rhizobacterial strains were cultured in screw-capped test tubes containing 10 ml of sterilized nutrient broth, incubated at 28 ˚C with mechanical shaker at 150 rpm for 48 h. The cultures were subsequently passed through sterilized Whatman filter papers no.1 and 42 and then it was concentrated by centrifugation at 10,000 rpm for 10 min. The supernatant was collected and finally passed through Millipore filter of 0.22 µm. This was designated as undiluted standard cell free filtrate of cent per cent (100%) concentration. The cell free extract was further diluted to 75, 50 and 25 per cent respectively and these dilutions were use to study their effect on nematodes (Niknam and Dhawan, 2001) [12]. *In vitro* evaluation of PGPR strains against root-knot nematode were carried by enumeration of egg hatching and juvenile mortality.

In vitro egg hatching test

Egg masses were collected from culture plants and treated with NaCl (1%) to dissolve the egg matrix and to separate the individual eggs. A known number of eggs (50) were carefully transfer to each vials, containing 5 ml of cell free culture filtrate of fluorescent Pseudomonads of 100, 75, 50 and 25 per cent concentrations. Inoculated vials were incubated at room temperature (28 ± 2 ˚C) for 36 h. Two controls were maintained by transferring 50 eggs to a vial containing sterilized nutrient broth and water. After 48 h, the number of juveniles hatched were counted under stereo binocular microscope and per cent inhibition of egg hatching in different dilutions in each vials were calculated.

In vitro mortality test of juveniles

Freshly hatched, 50 active juveniles were counted in a counting dish using a stereo binocular microscope and carefully transferred to individual vials containing 5 ml of each of the bacterial cell free filtrates of different concentrations (100, 75, 50 and 25 per cent). Each treatment was replicated three times and arranged in completely randomized design and incubated at 28 ± 2 ˚C. Observations were recorded at 12 h, 24 h and 48 h after exposure period and per cent mortality of juveniles were calculated.

Results and Discussion

During the random survey, soil samples were collected from healthy rhizopshere of tomato fields from selected villages of North Eastern Karnataka *viz.*, Kalaburgi, Yadagir, Raichur and Koppal for the isolation of fluorescent Pseudomonads. This was carried out by standard procedure of serial dilution technique on King's B medium (KBM) (King *et al.*, 1954) [8]. After 48 h of incubation, all the isolates were checked for fluorescence under UV light and representative type of colonies were selected and further purified on KBM. Pure isolates were preserved at -80 ˚C after an addition of glycerol to a final concentration of 40 per cent (v/v). A total of 20 fluorescent Pseudomonads were isolated from the healthy rhizospheric soil of tomato and were designated as FP-1, FP-2 and so on upto FP-20 (Table 1).

Plant growth promoting rhizobacteria (PGPR) has an important role in sustainable agriculture. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens and solubilizing nutrients for easy uptake by plants.

Sakthivel *et al.* (1986) [14] isolated several strains of *P. fluorescens* from rhizosphere of crop plants and were

identified as biotype C, whereas Laha and Verma (1998) [9] isolated the six fluorescent *Pseudomonas* spp. from rhizosphere of healthy cotton seedlings using King's B medium or *Pseudomonas* agar for fluorescin (PAF). Yeole and Dube (2000) [20] isolated twelve rhizobacterial fluorescent Pseudomonads from chilli, cotton, groundnut and soybean. Anand *et al.* (2010) [11] also collected several isolates of *P. fluorescens* from the rhizosphere of chilli, sunflower and cotton crops and characterized few of them.

Rajalaxmi and Naik (2013) [13] also isolated 101 isolates of fluorescent Pseudomonads from rhizospheric and endophytic region of different crops. A total of 36 isolates of fluorescent Pseudomonads were isolated from the healthy rhizospheric soil of tomato, grown in different areas of North Eastern Karnataka (Mahesh, 2016) [10].

In vitro screening of fluorescent Pseudomonads against _F. oxysporum_ f. sp. _lycopersici_

Fluorescent Pseudomonads from healthy rhizosphere and rhizoplane of major tomato growing areas of NE Karnataka were tested for their inhibitory activity against mycelial growth of *Fusarium* by following the dual culture technique.

Twenty fluorescent Pseudomonads isolates were screened by dual culture method against *Fusarium* sp. All the isolates significantly inhibited the growth of the fungus.

The per cent inhibition varied from 20.74 to 78.52. Maximum per cent inhibition of 78.52 per cent was observed in FP- 13 followed by FP-4 (74.82%) and FP-6 (73.71%). Least inhibition of 20.74 per cent was observed in FP-19 followed by FP-20 (35.55%) (Table 2).

There was a significant difference observed between the isolates in mycelial inhibition (Fig. 1). The difference in inhibition was noticed mainly because they were isolated from the different regions of soil. Added to this the inherent potentiality, such as growth in the culture media, production of antibiotics, volatile compounds, siderophore *etc.*, might have played an important role in inhibition of mycelia growth *in vitro.*

Similar findings have been reported by Mallesh (2008) [11] who screened 50 isolates of fluorescent Pseudomonads against the *Fusarium* spp. of which seven isolates where found highly efficacious in inhibiting the mycelial growth of the pathogen. The present results were in accordance with, Kannahi and Malathi (2013) [7], where they screened *Bacillus* spp. and *Pseudomonas* spp. against *F. oxysporum* f. sp. *lycopersici. Bacillus* spp. (36.7%) was found to have more antagonistic activity against test pathogens. The *Pseudomonas* spp. showed 35.5 per cent of growth inhibition against test pathogen.

Effect of culture filtrates of fluorescent Pseudomonads on egg hatching of *M. incognita*

Hatching of *M. incognita* juveniles increased with decrease in concentration of fluorescent Pseudomonads. However, hatching was significantly reduced in all concentrations of fluorescent Pseudomonads.

The interaction between treatment and concentration was significant which indicated that an increase in concentration tended to modify the effect of other in a significant manner.

The culture filtrates of fluorescent Pseudomonads at 100, 75, 50 and 25 per cent significantly inhibited the hatching of eggs. The greatest decrease in egg hatching was recorded in isolate FP-3 (2.24) followed by FP-1 (2.41) and FP-13 (2.57). Maximum number of juveniles were hatched in control (50.00) followed by FP- 20 (11.74) (Table 3).

Effect of culture filtrates of fluorescent Pseudomonads on juvenile mortality of *M. incognita*

Cell free culture filtrates of twenty fluorescent Pseudomonads were tested *in vitro* for their nematicidal action on *M. incognita*. Data indicated that various fluorescent Pseudomonads isolates and their different concentrations were highly deleterious to the nematode (Table 4). In general, juvenile mortality increased with increase in exposure period and increase in concentration of fluorescent Pseudomonads. No nematode mortality was recorded in control (distilled water). A maximum nematode juvenile mortality was observed in FP-3 (71.05%), followed by FP-13 (70.54%) and FP-18 (70.05%). The lowest juvenile mortality was recorded in FP-11 (62.16%) followed by FP-20 (63.17%) and FP-7 (63.77%). In the present study, *in vitro* bioassay with cell free culture filtrates of twenty fluorescent Pseudomonads at different concentrations revealed an increased juvenile mortality and egg hatching inhibition with increase in exposure period as well as concentration. *Meloidogyne incognita* juveniles and eggs were highly vulnerable to the native fluorescent Pseudomonads. Among the twenty fluorescent Pseudomonads tested, ten isolates showed significantly higher ovicidal and larvicidal action on *M. incognita* juveniles and eggs respectively (Fig. 2 and Fig.3). Reduction in mobility and viability of juveniles and eggs of *M. incognita* are induced by secondary metabolites such as 2, 4-diacetylphlorglucinol (DAPG), pyrolnitrin, tropolone, pyocyanin, phenazines and lytic enzymes which are produced in culture filtrates of *P. fluorescens* (Elsherif and Grossmann, 1996 and Dunne *et al.*, 1998) [5, 4]. Similar toxic property of *P. fluorescens* culture filterates was also reported on the juvenile mortality of *M. incognita* and *Heterodera cajani* (Gokte and Swarup, 1988) [6]. The juvenile mortality and egg hatching inhibition of *M. incognita* observed in the present study might be due to antibiosis. Mallesh (2008) [11] tested 50 PGPR strains against egg hatching and juvenile mortality of root knot nematode. Among the fifty PGPR strains tested, seventeen strains showed significantly higher larvicidal and ovicidal action on *M. incognita* juveniles and eggs respectively.

Table 1: Native fluorescent Pseudomonads from tomato rhizosphere from different parts of North Eastern Karnataka

Sl. No.	Districts	Isolates	Taluk	Village	Isolate Designation
1	Kalaburgi	5	Kalaburgi	Ganajalakhed	FP-1
				Tajsultanpur	FP-2
			Chittapur	Nalwar	FP-3
			Aland	Kadaganchi	FP-4
				Mogha K	FP-5
2	Yadagir	5	Yadagir	Tumkur	FP-6
				Hattikuni	FP-7
			Shahapur	Hayyal	FP-8

				Chamnal	FP-9
			Shorapur	Hunsagi	FP-10
3	Raichur	5	Raichur	Chandrabanda	FP-11
			Manvi	Kavital	FP-12
				Pamanakallur	FP-13
			Lingasugur	Echanal	FP-14
				Bupura	FP-15
4	Koppal	5	Koppal	Ginigera	FP-16
			Yelaburga	Nelajeri	FP-17
				Narasapur	FP-18
			Kustagi	Menedal	FP-19
				Hulihyder	FP-20
Total isolates					20

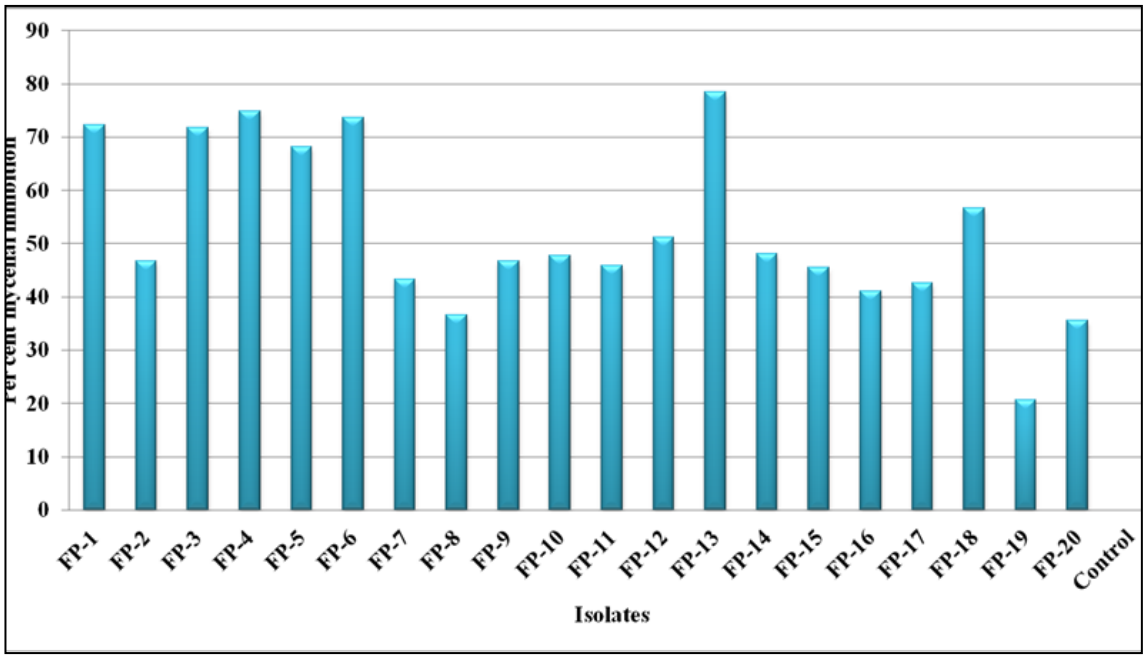

Fig 1: *In vitro* screening of fluorescent Pseudomonads against *F. oxysporum* f. sp. *lycopersici*

Table 2: *In vitro* screening of fluorescent Pseudomonads against *F. oxysporum* f. sp. *lycopersici* by dual culture method

Sl. No.	Isolates	Radial mycelial growth (mm)	Per cent mycelial inhibition
1	FP-1	25.00	72.22* (58.20)**
2	FP-2	48.00	46.66 (43.09)
3	FP-3	25.33	71.85 (57.96)
4	FP-4	22.66	74.82 (59.89)
5	FP-5	28.66	68.15 (55.65)
6	FP-6	23.66	73.71 (59.16)
7	FP-7	51.00	43.33 (41.17)
8	FP-8	57.00	36.66 (37.27)
9	FP-9	48.00	46.66 (43.09)
10	FP-10	47.00	47.77 (43.73)
11	FP-11	48.66	45.93 (42.67)
12	FP-12	44.00	51.11 (45.64)
13	FP-13	19.33	78.52 (62.39)
14	FP-14	46.66	48.15 (43.94)
15	FP-15	49.00	45.55 (42.45)
16	FP-16	53.00	41.11 (39.88)
17	FP-17	51.66	42.60 (40.75)
18	FP-18	39.00	56.66 (48.83)
19	FP-19	71.33	20.74 (27.10)
20	FP-20	58.00	35.55 (36.61)
21	Control	90.00	0.00
S. Em..±			0.57
C.D. at 1%			2.19

Table 3: *In vitro* screening of fluorescent Pseudomonads on egg hatching of *M. incognita*

Sl. No.	Isolates	Concentration								Mean (J_2 hatched)
		100%		75%		50%		25%		
		No. of J_2 hatched	%IOC*	No. of J_2 hatched	%IOC*	No. of J_2 hatched	%IOC*	No. of J_2 hatched	%IOC*	
1	FP-1	0.00	100.00 (90.00)**	1.33	97.33 (80.60)	3.66	92.67 (74.30)	4.66	90.67 (72.22)	2.41
2	FP-2	4.00	92.00 (73.58)	5.66	88.67 (70.34)	7.33	83.33 (65.91)	9.33	81.33 (64.40)	6.58
3	FP-3	0.00	100.00 (90.00)	1.66	96.67 (79.49)	3.33	93.33 (75.04)	4.00	92.00 (73.58)	2.24
4	FP-4	4.33	91.33 (72.88)	6.66	86.67 (68.59)	8.33	83.33 (65.91)	8.66	82.67 (65.40)	6.99
5	FP-5	5.33	89.33 (70.94)	7.33	85.33 (67.48)	8.66	82.67 (65.40)	9.00	82.00 (64.90)	7.58
6	FP-6	2.00	96.00 (78.47)	4.33	91.33 (72.88)	8.00	84.00 (66.43)	8.33	83.33 (65.91)	5.83
7	FP-7	6.33	87.33 (69.15)	7.66	84.67 (66.95)	9.33	81.33 (64.40)	9.66	80.67 (63.92)	8.24
8	FP-8	5.66	88.68 (70.34)	8.00	84.00 (66.43)	9.66	80.67 (63.92)	10.33	79.33 (62.96)	8.41
9	FP-9	6.00	88.00 69.74)	8.66	82.67 (65.40)	10.33	79.33 (62.96)	12.33	75.33 (60.22)	9.33
10	FP-10	5.33	89.33 (70.94)	7.33	84.00 (66.43)	10.00	80.00 (63.44)	12.00	76.00 (60.67)	8.66
11	FP-11	6.66	86.68 (68.59)	9.66	80.67 (63.92)	11.66	76.67 (61.12)	13.66	72.67 (58.49)	10.41
12	FP-12	6.00	88.00 (69.74)	9.33	81.33 (64.40)	12.33	75.33 (60.22)	12.66	74.67 (59.79)	10.08
13	FP-13	1.66	96.67 (79.49)	1.66	96.67 (79.49)	3.33	93.33 (75.04)	3.66	91.33 (72.88)	2.57
14	FP-14	7.33	85.33 (67.48)	9.00	82.00 (64.90)	12.00	76.00(60.67)	13.33	73.33 (58.91)	10.41
15	FP-15	4.33	91.33 (72.88)	7.33	85.33 (67.48)	9.33	81.33 (64.40)	10.33	79.33 (62.96)	7.83
16	FP-16	5.33	89.33 (70.94)	8.33	83.33 (65.91)	10.33	79.33 (62.96)	12.33	74.67 (59.79)	9.08
17	FP-17	6.33	87.33 (69.15)	8.66	82.67 (65.40)	10.66	78.67 (62.50)	13.66	74.00 (59.35)	9.82
18	FP-18	1.33	97.33 (80.60)	2.00	96.00 (78.47)	3.66	92.67 (74.300)	4.33	91.33 (72.88)	2.83
19	FP-19	7.00	86.00 (68.03)	10.33	79.33 (62.96)	12.66	74.68 (59.79)	14.33	71.33 (57.63)	11.08
20	FP-20	7.66	84.68 (66.95)	11.33	77.33 (61.57)	13.33	73.33 (58.91)	14.66	70.67 (57.21)	11.74
21	Distilled water	50	0.00 (0.00)	50	0.00 (0.00)	50	0.00 (0.00)	50	0.00 (0.00)	50.00
	Mean	6.79	86.13 (68.14)	8.86	82.06 (64.95)	10.25	78.19 (62.16)	11.96	76.03 (60.69)	
	S. Em. ±	0.78		0.74		0.84		0.90		
	CD@1%	3.01		2.85		3.22		3.46		

* Mean of three replications
** Figures in the parentheses are arc sine transformed values
IOC- Inhibition over control

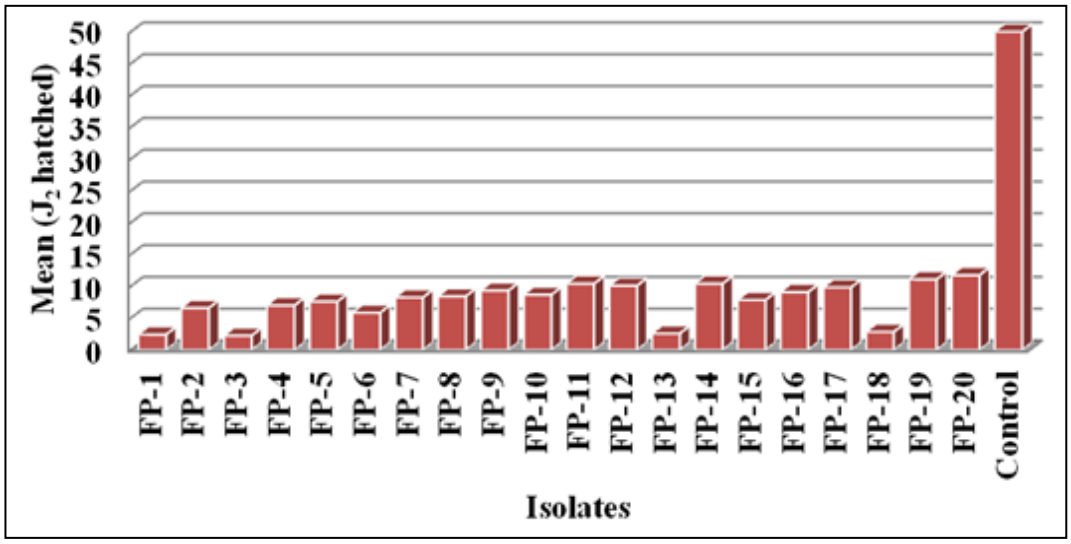

Fig 2: Efficacy of fluorescent Pseudomonads on egg hatching of *M. incognita*

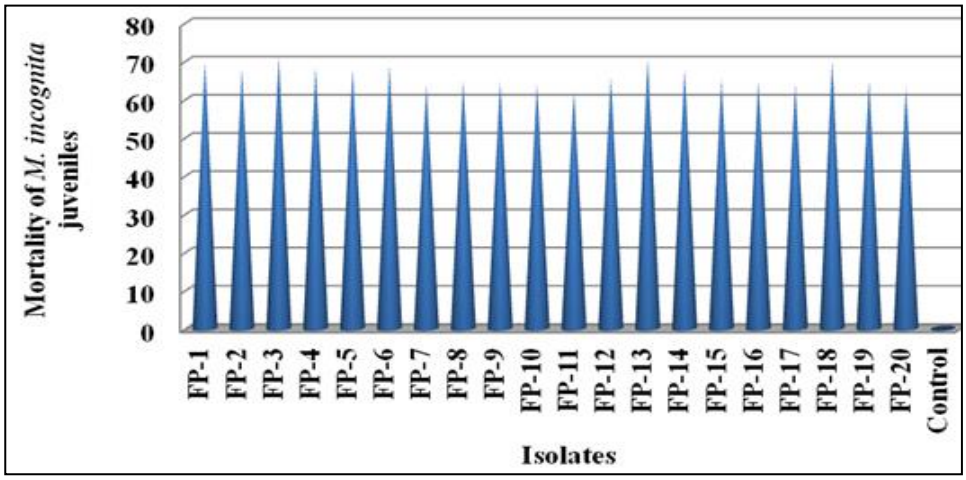

Fig 3: Efficacy of fluorescent Pseudomonads on juvenile mortality of *M. incognita*

Table 4: *In vitro* screening of fluorescent Pseudomonads on juvenile mortality of *M. incognita*

Sl. No.	Isolates	Mortality of *Meloidogyne incognita* juveniles																Total mean
		100%				75%				50%				25%				
		12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	
1	FP-1	60.67* (51.17)**	88.67 (70.34)	99.33 (85.31)	82.89 (65.57)	56.67 (48.84)	80.00 (63.44)	84.67 (66.95)	73.78 (59.20)	53.33 (46.91)	63.33 (52.74)	76.67 (61.12)	64.44 (53.40)	45.33 (42.33)	59.33 (50.38)	69.33 (56.38)	57.99 (49.60)	69.77 (56.65)
2	FP-2	58.67 (50)	86.67 (68.59)	93.33 (75.04)	79.55 (63.12)	54.00 (47.3)	77.33 (61.57)	81.33 (64.40)	70.88 (57.35)	50.00 (45)	64.00 (53.14)	78.67 (62.50)	64.22 (53.27)	44.67 (41.95)	56.00 (48.45)	68.00 (55.56)	56.22 (48.58)	67.71 (55.38)
3	FP-3	63.33 (52.74)	84.67 (66.95)	100.00 (90)	82.66 (65.40)	61.33 (51.55)	81.33 (64.40)	86.00 (68.03)	76.22 (60.82)	57.33 (49.22)	65.33 (53.93)	79.33 (62.96)	67.33 (55.14)	46.67 (43.10)	57.33 (49.22)	70.00 (56.79)	58.00 (49.61)	71.05 (57.45)
4	FP-4	63.33 (52.74)	83.33 (65.91)	96.67 (79.49)	81.11 (64.24)	62.00 (51.95)	76.00 (60.67)	79.33 (62.96)	72.44 (58.34)	56.67 (48.84)	63.33 (52.74)	74.67 (59.79)	64.89 (53.67)	43.33 (41.17)	54.67 (47.68)	66.67 (54.74)	54.89 (47.81)	68.33 (55.76)
5	FP-5	61.33 (51.55)	85.33 (67.48)	98.67 (83.38)	81.77 (64.73)	61.33 (51.55)	74.67 (59.79)	78.67 (62.50)	71.55 (57.77)	55.33 (48.06)	62.67 (52.34)	74.67 (59.79)	64.22 (53.27)	42.67 (40.79)	54.00 (47.30)	64.67 (53.54)	53.78 (47.17)	67.83 (55.45)
6	FP-6	64.67 (53.54)	82.00 (64.9)	95.33 (77.52)	80.66 (63.92)	63.33 (52.74)	76.67 (61.12)	79.33 (62.96)	73.11 (58.77)	56.67 (48.84)	64.00 (53.14)	77.33 (61.57)	66.00 (54.34)	44.00 (41.56)	55.33 (48.06)	67.33 (55.14)	55.55 (48.19)	68.83 (56.07)
7	FP-7	58.67 (50)	80.00 (63.44)	94.67 (76.66)	77.78 (61.88)	50.00 (45)	64.67 (53.54)	75.33 (60.22)	63.33 (52.74)	46.67 (43.1)	60.67 (51.17)	75.33 (60.22)	60.89 (51.29)	41.33 (40.01)	53.33 (46.91)	64.67 (54.00)	53.11 (46.79)	63.77 (53.00)
8	FP-8	60.67 (51.17)	82.67 (65.44)	96.67 (79.49)	79.98 (63.43)	50.00 (45)	71.33 (57.63)	80.67 (63.92)	67.33 (55.14)	45.33 (42.33)	61.33 (51.55)	74.00 (59.35)	60.22 (50.90)	40.00 (39.24)	51.33 (45.77)	63.33 (52.74)	51.55 (45.89)	64.77 (53.60)
9	FP-9	58.67 (50)	83.33 (65.91)	97.33 (80.6)	79.77 (63.28)	49.33 (44.62)	70.67 (57.21)	82.67 (65.40)	67.55 (55.28)	44.67 (41.95)	62.00 (51.95)	73.33 (58.91)	60.00 (50.77)	39.33 (38.84)	50.67 (45.39)	61.33 (51.55)	50.44 (45.26)	64.44 (53.40)
10	FP-10	60.67 (51.17)	80.67 (63.92)	94.67 (76.66)	78.67 (62.50)	50.67 (45.39)	71.33 (57.63)	83.33 (65.91)	68.44 (55.83)	47.33 (43.47)	60.67 (51.17)	72.00 (58.06)	60.00 (50.77)	39.33 (38.84)	48.67 (44.24)	58.00 (49.61)	48.66 (44.24)	63.94 (53.10)
11	FP-11	57.33 (49.22)	78.67 (62.5)	93.33 (75.04)	76.44 (60.97)	48.67 (44.24)	68.00 (55.56)	79.33 (62.96)	65.33 (53.93)	45.33 (42.33)	57.33 (49.22)	71.33 (57.63)	57.99 (49.60)	38.67 (38.46)	47.33 (43.47)	60.67 (52.17)	48.89 (44.37)	62.16 (52.04)
12	FP-12	58.67 (50.00)	84.67 (66.95)	95.33 (77.52)	79.55 (63.12)	56.67 (48.84)	73.33 (58.91)	76.67 (61.12)	68.89 (56.10)	51.33 (45.77)	63.33 (52.74)	75.33 (60.22)	63.33 (52.74)	41.33 (40.01)	52.67 (46.54)	62.67 (52.34)	52.22 (46.28)	65.99 (54.33)
13	FP-13	65.33 (53.93)	85.33 (67.48)	100.00 (90)	83.53 (66.06)	62.67 (52.34)	74.67 (59.79)	82.67 (65.40)	73.33 (58.91)	56.67 (48.84)	65.33 (53.93)	79.33 (62.96)	67.11 (55.01)	44.00 (41.56)	61.33 (51.55)	69.33 (56.38)	58.22 (49.74)	70.54 (57.13)
14	FP-14	64.67 (53.54)	88.67 (70.34)	96.00 (78.47)	83.11 (65.74)	60.00 (50.7)	64.67 (53.54)	83.33 (65.91)	69.33 (56.38)	56.00 (48)	63.33 (52.74)	76.67 (61.12)	65.33 (53.93)	42.00 (40.40)	53.33 (46.91)	65.33 (53.93)	53.55 (47.04)	67.83 (55.45)
15	FP-15	62.00 (51.95)	82.00 (64.9)	94.67 (76.66)	79.55 (63.12)	58.00 (49.61)	65.33 (53.93)	78.00 (62.03)	67.11 (55.01)	53.33 (46.91)	61.33 (55.00)	73.33 (58.91)	62.66 (52.34)	41.33 (40.0)	50.67 (39.00)	62.67 (52.34)	51.55 (45.89)	65.21 (53.86)
16	FP-16	61.33 (51.55)	80.00 (63.44)	93.33 (75.04)	78.22 (62.19)	54.00 (47.3)	66.67 (54.74)	79.33 (62.96)	66.66 (54.74)	50.00 (45.00)	63.33 (52.74)	74.67 (59.79)	62.66 (52.34)	39.33 (38.84)	53.33 (46.91)	62.67 (52.34)	51.77 (46.02)	64.82 (53.63)
17	FP-17	59.33 (50.38)	81.33 (64.4)	95.33 (77.52)	78.66 (62.49)	49.33 (44.62)	65.33 (53.93)	80.00 (63.44)	64.88 (53.66)	45.33 (42.33)	62.00 51.95)	75.33 (60.22)	60.88 (51.29)	37.33 (37.67)	50.67 (45.39)	65.33 (53.93)	51.11 (45.64)	63.88 (53.06)
18	FP-18	64.00 (53.14)	86.00 (68.03)	98.67 (83.38)	82.89 (65.57)	60.67 (51.17)	73.33 (58.91)	83.33 (65.91)	72.44 (58.34)	54.00 (47.3)	66.67 (54.74)	79.33 (62.96)	66.66 (54.74)	45.33 (42.33)	60.67 (51.17)	68.67 (55.97)	58.22 (49.74)	70.05 (56.83)
19	FP-19	59.33 (50.38)	81.33 (64.4)	94.67 (76.66)	78.44 (63.34)	55.33 (48.06)	67.33 (55.14)	79.33 (62.96)	67.33 (55.14)	46.67 (43.1)	63.33 (52.74)	72.67 (58.49)	60.89 (51.29)	40.67 (39.63)	55.33 (48.06)	62.67 (52.34)	52.89 (46.66)	64.88 (53.66)
20	FP-20	56.67 (48.84)	80.67 (63.92)	93.33 (75.04)	76.89 (61.27)	51.33 (45.77)	63.33 (52.74)	75.33 (60.22)	63.33 (52.74)	46.09 (42.71)	63.33 (52.74)	71.33 (57.63)	60.25 (50.92)	40.67 (39.63)	54.00 (47.30)	62.00 (51.95)	52.22 (46.28)	63.17 (52.64)
21	Distilled water	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)		
	S. Em. ±	0.81	0.89	0.61		1.07	0.76	0.76		0.73	0.74	0.68		0.74	0.80	0.86		
	CD@1%	3.12	3.42	2.35		4.11	2.90	2.90		2.79	2.85	2.61		2.85	3.07	3.32		

* Mean of three replications

** Figures in the parentheses are arc sine transformed values

Conclusion

A total of twenty isolates of fluorescent Pseudomonads (FP) were isolated from healthy rhizospheric soils, collected during the survey. Among twenty FP isolates screened *in vitro* against the wilt pathogen, eight isolates *viz.*, FP-1, FP-3, FP-4, FP-5, FP-6, FP-12, FP-13 and FP-18 have inhibited the mycelial growth of the test pathogen (*F. oxysporum* f. sp. *lycopersici*) to an extent of more than 50 per cent. Among the twenty fluorescent Pseudomonads tested, ten isolates *viz.*, FP-1, FP-2, FP-3, FP-4, FP-5, FP-6, FP-12, FP-13, FP-14 and FP-18 showed higher larvicidal action and ten isolates *viz.*, FP-1, FP-2, FP-3, FP-4, FP-5, FP-6, FP-7, FP-13, FP-15 and FP-18 showed higher ovicidal action on *M. incognita* juveniles and its eggs, respectively. Commonly efficacious seven isolates *viz.*, FP-1, FP-3, FP-4, FP-5, FP-6, FP-13 and FP-18 were selected, which showed maximum inhibition of *F. oxysporum* f. sp. *lycopersici* and *M. incognita*.

References

1. Anand M, Naik MK, Ramegowda G, Devikarani GS. Bio-control and PGPR of *Pseudomonas fluorescens* isolates. J Mycopathol Res 2010;46:135-139.
2. Anonymous. Horticulture Database, National Horticulture Board, Gurgoan, India 2017, 453.
3. Dennis C, Webster J. Antagonistic properties of species groups of *Trchoderma* in production of non- volatile antibiotics. Trans. Br. Mycol. Soc 1971;57:25-39.
4. Dunne C, Cronin D, Moknne-Loccoz, Gara FO. Biological control of phytopathogens by phloroglucinol and hydrolytic enzyme producing bacterial inoculants. Bull. OILB/SROP 1998;21:19-25.
5. Elsherif M, Grossman P. Role of biotic factors in the control of soil-borne fungi by fluorescent Psuedomonads. Microbiological Res 1996;151:351-357
6. Gokte N, Swarup. On the potential of some bacterial biocides against root-knot and cyst nematodes. Indian J. Nematol 1988;18:152-153.
7. Kannahi M, Malathi P. Antagonistic effect of tomato rhizospheric microbes against some pathogens. J Che. Pharm. Res 2013;5(9):10-14.
8. King EO, Ward MK, Raney DE. Two simple media for demonstration of pyocyanin and fluorescin. J Lab. Clin. Med 1954;44:301-307.
9. Laha GS, Verma JP. Role of fluorescent Pseudomonads in the suppression of root rot and damping-off of cotton. Indian Phytopath 1998;51:275:278.
10. Mahesh. Studies on fluorescent Pseudomonads for the suppression of wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.). M. Sc(Agri.) Thesis, Univ. Agril. Sci., Raichur, Karnataka, India 2016.
11. Mallesh SB. Plant growth promoting rhizobacteria, their characterization and Mechanisms in the suppression of soil borne pathogens of coleus and ashwagandha. Ph.d Thesis, Univ. Agril. Sci., Dharwad., Karnataka, India 2008.
12. Nicknam GR, Dhawan SC. Effect of seed bacterization, soil drench and bare root dip application methods of *Pseudomonas fluorescens* isolate Pf1 on the suppression of *Rotylenchulus reniformis* infecting tomato (Abstract). In: Proceedings of the National Congress on Centenary of Nematology in India: Appraisal and Future Plans held at IARI, New Delhi, India, 5-7, December 2001, p: 144.
13. Rajalaxmi K, Naik MK. Compatibility of *Pseudomonas fluorescens* (PF-4) with fungicides, insecticides and plant products. Bioinfolet 2013;10(2):620-622.
14. Sakthivel N, Silvamani E, Unnamalai N, Ganamanickam SS. Plant-growth promoting rhizobacteria in enhancing plant growth and suppressing plant pathogens. Curr. Sci 1986;55:22–25.
15. Sasser JN. Root-knot nematode: A global menace to crop production. Plant Disease 1980;63:36-41.
16. Schaad NW. Laboratory guide for identification of plant pathogenic bacteria Eds., Americ. Psychopath. Soc. Minneapolis, USA 1992.
17. Sokhi SS, Munish GD, Grewal RK, Thind TS, Sandhu KS. Current fungal disease problems of important vegatables in India, pp. 382- 404. In: Basic Research for crop disease management (Ed. Vidyasekharan, P.). Day Publishing House, New Delhi, 1991, 409.
18. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature 1947;159:850.
19. Walker JC. Plant Pathology. McGraw-Hill Book Co., New York 1969, 819.
20. Yeole RD, Dube HC. Siderophore mediated antibiosis of rhizobacterial fluorescent Pseudomonads against certain soil borne fungal plant pathogens. J Mycol. Pl Pathol 2000;30:335-338.