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Evaluation of *Pseudomonas fluorescens* Isolates for their Bio-control Potential against Soil Borne Disease of Tomato

Stanzin Dorjey, Vishal Gupta*, V.K. Razdan and Richa Sharma

Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha - 180 009, India.

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Two major fungal pathogens, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* were isolated from infected root, collar and stem regions of tomato plants. A total of 25 rhizobacterial isolates showing Gram –ve reaction were isolated from the rhizosphere of tomato fields. Six selected isolates, based on their biocontrol activity against the test pathogens showed growth at 4ºC but there was no growth at 41ºC. The maximum growth of these isolates was at pH 7.0 and they isolates showed positive reactions for levan formation, phosphate solubilization, gelatin liquefaction, oxidase test and catalase test, that confirmed the identity of the isolates as *Pseudomonas fluorescens*. Under *in vitro* conditions, carbendazim showed 100 per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *lycopersici* and *R. solani* at all the tested concentrations. Carbendazim also showed maximum plant vigour (1501.54 %), followed by isolate I-23 (1453.77 %).

Keywords: Soil borne disease, tomato, bio-control, management.

Tomato (*Solanum lycopersicum* L.) is one of the most popular commercial vegetable crop grown throughout India and occupies an area of 8.65 lakh hactare, with annual production of 16.862 lakh MT[1]. There are several fungi, bacteria, viruses, nematodes and abiotic factors which adversely affect the productivity of tomato[2]. Out of different biotic stresses, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* are the two most important soil borne pathogens, responsible for causing wilting or root rot in tomato crop. Management of soil-borne diseases has predominantly been based on chemical measures. However, overly dependence on chemical pesticides has not proved very effective, moreover, and their continuous use has proved hazardous by not only polluting soil and environment, but has also led to residual toxicity in soil and ground water. Fluorescent pseudomonads representing group of plant growth promoting rhizobacteria are known to promote plant growth and suppress plant pathogens by multiple mechanisms. *Pseudomonas fluorescens* is considered as putative biocontrol agent against various plant diseases including root diseases[3] because of its production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones[4]. In order to develop an integrated disease management strategy based on eco-friendly measures, the present study was undertaken to explore the beneficial traits of *P. fluorescens* as biocontrol and plant growth promotion in tomato crop.

* To whom all correspondence should be addressed.
E-mail: vishal94gupta@rediffmail.com

Table 1. Cultural characteristics of rhizobacterial isolates

Isolate	Colony characteristics			Microscopic characteristics		
	Colour	Shape	Nature	Cell shape	Gram reaction	Fluorescens
I-1	Light green	Round	Non-spreading	Long rods	Gram –ve	++
I-2	Green	Irregular	Spreading	Long rods	Gram –ve	++
I-3	Green	Irregular	Spreading	Short rods	Gram –ve	+++
I-4	Light green	Round	Non-spreading	Long rods	Gram –ve	+++
I-5	Light green	Round	Non-spreading	Short rods	Gram –ve	++
I-6	Green	Irregular	Spreading	Short rods	Gram –ve	++
I-7	Green	Irregular	Spreading	Long rods	Gram –ve	++
I-8	Light green	Round	Non-spreading	Long rods	Gram –ve	++
I-9	Green	Irregular	Spreading	Short rods	Gram –ve	+++
I-10	Light green	Round	Non-spreading	Long rods	Gram –ve	++
I-11	Light green	Round	Non-spreading	Short rods	Gram –ve	++
I-12	Light green	Round	Non-spreading	Short rods	Gram –ve	++
I-13	Light green	Round	Non-spreading	Long rods	Gram –ve	+++
I-14	Green	Irregular	Spreading	Long rods	Gram –ve	+++
I-15	Light green	Round	Non-spreading	Short rods	Gram –ve	++
I-16	Light green	Round	Non-spreading	Long rods	Gram –ve	++
I-17	Green	Irregular	Spreading	Long rods	Gram –ve	++
I-18	Light green	Round	Non-spreading	Long rods	Gram –ve	+++
I-19	Green	Irregular	Spreading	Short rods	Gram –ve	+++
I-20	Light green	Round	Non-spreading	Long rods	Gram –ve	++
I-21	Green	Irregular	Spreading	Long rods	Gram –ve	++
I-22	Light green	Round	Non-spreading	Short rods	Gram –ve	++
I-23	Light green	Irregular	Spreading	Short rods	Gram –ve	+++
I-24	Light green	Round	Non-spreading	Long rods	Gram –ve	+++
I-25	Light green	Irregular	Spreading	Short rods	Gram –ve	+++

++ =Good fluorescens; +++ =very good fluorescens

MATERIALS AND METHODS

Isolation and identification of causal organisms associated with diseased plant samples

Isolation of fungal pathogens was done from infected plant parts such as root and/or stem/ collor region of tomato plants[5]. Sterilized bits were asceptically transferred onto petriplates having pre-poured PDA + streptomycin sulphate and then incubated at 25+2^{0}C in BOD incubator. The petriplates were regularly observed for any fungal growth. The fungal growths obtained from diseased plant parts were purified using hyphal tip method[6]. Pure culture of each fungus thus obtained was used for further studies. Morphological examinations and cultural characteristics of the isolated fungi were recorded for their identification[7,8]. Pure cultures thus obtained were mass multiplied on Potato Dextrose (PD) broth for further studies.

Isolation and identification of rhizobacterial isolates

Rhizobacterial isolates were collected from the rhizosphere of tomato crop grown at University Research Farm, by serial dilution agar plating method[9]. Aliquots from 10^{-7} dilution was placed in the centre of pre-poured petriplate containing King's B medium. The inoculated plates were then incubated in inverted position for 24 hours in BOD incubator at 25+2^{0}C. Single colonies were purified by streak plate method[10]. Pure cultures were mass multiplied on nutrient broth medium and examined for their Gram reaction, colony morphology, fluorescence and cell shape[11]. Further, all the isolates were screened for bio-control potential against *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* by dual culture method[12]. Specific biochemical tests such as gelatin liquefaction, production of levan, catalase test,

oxidase test, arginine hydrolysis, indole production, phosphorous solubilization and growth at 4 and 41^{0}C were conducted for identification of *P. fluorescens*[13].

Effect of hydrogen ion concentration (pH)

To studying the effect of pH, the King's B agar medium was amendment with varying hydrogen ion concentrations (pH) ranging from 3 to 14. The neutral pH of the King's B agar medium was adjusted to acidic pH values by addition of 0.1N HCl and the alkaline pH by adding 0.1N NaOH before adding agar-agar and autoclaving. Sterilized petriplates were poured with 15-20 ml medium having different pH values and allowed to solidify. Then the plates were spotted with 10 ¼l of overnight cultures of the test organisms and incubated for 48 h at 28+2^{0}C. The observations on the ability of the isolates to grow at different hydrogen ion concentrations were recorded.

Table 2. *In vitro* evaluation of rhizobacterial isolates against pathogens found associated with infected tomato plants

Isolate	*Fusarium oxysporum* f. sp. *lycopersici*		*Rhizoctonia solani*	
I-1	-	-	-	-
I-2	-	-	-	-
I-3	-	-	-	-
I-4	-	-	-	-
I-5	-	-	-	-
I-6	-	-	-	-
I-7	+	+	+	+
I-8	-	-	-	-
I-9	-	-	-	-
I-10	-	-	-	-
I-11	-	-	-	-
I-12	-	-	-	-
I-13	-	-	-	-
I-14	-	-	-	-
I-15	+	+	+	+
I-16	-	-	-	-
I-17	-	-	-	-
I-18	+	+	+	+
I-19	-	-	-	-
I-20	-	-	-	-
I-21	-	-	-	-
I-22	-	-	-	-
I-23	+	+	+	+
I-24	+	+	+	+

Evaluation of fungicides against the test pathogens

The efficacy of propiconazole (Tilt 25), tebuconazole (Folicur 25), hexaconazole (Malconda 5 EC) and difenoconazole (Score 25) at 10, 25 and 50 ppm concentrations, and carbendazim (Bavistin 50), mancozeb + carbendazim (SAAF), metalaxyl + mancozeb (Ridomil-MZ), triademifon (Bayleton) and carboxin + thiram (Vitavax Power) at 50, 100 and 250 ppm were evaluated *in vitro*, against the test pathogens i.e., *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani*, using poisoned food technique[14]. Petriplates containing PDA amended with the desired concentrations of fungicides were inoculated with 5mm discs of individual pathogen and incubated at 25+2^{0}C. Petriplates without any fungicide served as check. The experiment was conducted under Completely Randomized Design with three replications. The radial growth of mycelium was recorded in each treatment and per cent inhibition over check was calculated[15].

Evaluation of *Pseudomonas fluorescens* isolates for the management of soil borne disease of tomato

Potting soil (soil: FYM at 2:1) was

Table 3. Biochemical characteristic of *Pseudomonas fluorescens* isolates

Biochemical tests	*Pseudomonas fluorescens* isolates					
	I-7	I-15	I-18	I-23	I-24	I-25
Growth at 4^{0}C	+	+	+	+	+	+
Growth at 41^{0}C	-	-	-	-	-	-
Levan formation	+	+	+	+	+	+
Arginine hydrolysis	+	+	+	+	+	+
Indole production	+	+	+	+	+	+
Phosphorous solubilization	+	+	+	+	+	+
GelatinLiquefaction	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+

Table 4. Effect of hydrogen ion concentrations (pH) on growth of *Pseudomonas fluorescens* isolates

Isolate	Growth at different pH levels											
	3	4	5	6	7	8	9	10	11	12	13	14
I-7	-	-	+	++	+++	+++	++	+	+	+	-	-
I-15	-	-	++	++	+++	+++	++	+	+	+	-	-
I-18	-	-	+	++	+++	+++	++	++	+	+	-	-
I-23	-	-	++	+++	+++	+++	+++	++	++	+	-	-
I-24	-	-	+	++	+++	++	++	+	+	+	-	-
I-25	-	-	++	++	+++	+++	++	+	+	+	-	-

- Absent; + Fair; ++ Good; +++ Very good

Table 5. *In vitro* evaluation of fungicides against the test pathogens

Fungicide	Conc. (ppm)	Radial growth (mm)		Per cent inhibition over control	
		Fol	Rs	Fol	Rs
Propiconazole (Tilt)	10	25.36	19.50	71.82	78.33
	25	20.53	15.60	77.18	83.33
	50	14.10	11.63	84.33	87.07
Tebuconazole (Folicur)	10	18.40	8.50	79.56	87.07
	25	11.70	0.00	87.00	100.00
	50	0.00	0.00	100.00	100.00
Hexaconazole (Malconda)	10	26.73	23.43	70.30	73.96
	25	23.73	22.80	73.63	74.67
	50	14.20	16.10	84.22	82.11
Difenoconazole (Score)	10	48.50	63.43	46.11	29.52
	25	36.93	57.10	58.97	36.56
	50	30.83	39.80	65.74	55.78
Carbendazim (Bavistin)	50	0.00	0.00	100.00	100.00
	100	0.00	0.00	100.00	100.00
	250	0.00	0.00	100.00	100.00
Triademifon (Bayleton)	50	59.63	68.06	33.74	24.38
	100	47.96	57.86	46.71	35.71
	250	36.13	42.70	59.86	52.56
Mancozeb + Carbendazim (SAAF)	50	19.16	13.76	78.71	84.71
	100	14.13	23.60	84.30	73.78
	250	8.87	0.00	90.14	100.00
Mancozeb + Metalaxyl (Ridomil-MZ)	50	22.03	25.93	75.52	71.18
	100	17.90	21.10	80.11	76.56
	250	12.13	11.60	86.52	87.11
Carboxin+thiram (Vitavax power)	50	61.80	22.80	31.33	74.67
	100	52.03	14.00	42.18	84.44
	250	41.83	9.36	53.52	89.60
Control		90.00	90.00	0.00	0.00
CD (P=0.05)		2.542	3.394		
$S.E_m(+)$		0.894	1.194		

Fol = *Fusarium oxysporum* f.sp. *lycopersici*; Rs = *Rhizoctonia solani*

Table 6. Effect of seed treatment and talc based formulation of *Pseudomonas fluorescens* isolates for the management of soil borne disease of tomato

Isolate	Vigour index (%)	Disease incidence %	
		Fusarium oxysporum f.sp. *lycopersici*	*Rhizoctonia solani*
I-7	1212.62	12.50	29.16
I-15	1120.54	16.67	27.08
I-18	1253.82	27.08	10.41
I-23	1453.77	14.58	16.67
I-24	1299.70	25.00	18.75
I-25	1317.27	20.83	27.08
I-7+ Talc	1193.06	22.91	29.16
I-15+ Talc	1265.95	18.75	33.33
I-18+ Talc	1220.90	22.91	25.00
I-23 + Talc	1386.93	18.75	18.75
I-24 + Talc	1157.31	25.00	25.00
I-25 + Talc	1291.43	29.17	29.17
Talc alone	458.20	64.58	64.58
Carbendazim	1501.54	8.33	14.58
Control	453.70	72.91	62.50
$S.E_{m}(+)$	41.97	2.21	2.90
CD(P=0.05)	121.81	6.41	8.43

autoclave-sterilized for 1 h on two consecutive days and was placed in pots. Seeds of tomato (Heem Sohna), treated individually with the selected isolates of *Pseudomonas fluorescens* (I-07, I-15, I-18, I-23, I-24 and I-25) were grown on nutrient agar medium for 48 hours and harvested with sterile distilled water (1×10^8 cfu/ml). The bacteria coated seeds were spread on sterile filter paper, dried over night and sown in the pots. Thirty-day-old tomato seedlings pre treated with isolates of *P. fluorescens* as seed treatment were transplanted (4 seedlings pot^{-1}) in earthen pots filled with sterilized potting soil. Ten days after transplanting, soil application with 10 ml of bacterial suspension (1×10^8 cfu ml^{-1}) of the isolates (I-07, I-15, I-18, I-23, I-24 and I-25) was done and one day after soil application 50 ml of conidial suspension of *Fusarium oxysporum* f. sp. *lycopersici* (1000 microconidia ml^{-1}) and *Rhizoctonia solani* (8×10^5 cfu/g) was inoculated per pot. Further talc based bioformulation were also prepared by inoculation a loop full of *P. fluorescens* isolates into the nutrient broth[16]. Ten days after transplanting, soil application with talc based formulation (1g/pot) was done and one day after soil application, 50 ml of conidial suspension of *F. oxysporum* f. sp. *lycopersici* (1000 microconidia ml^{-1}) and *R. solani* (8×10^5 cfu/g) was inoculated per pot. Carbendazim as seed treatment at 2g kg^{-1}seed and after transplanting 2g pot^{-1}as soil application was included as check, whereas, untreated seeds served as control. Observations regarding disease incidence and Vigour Index were recorded.

RESULTS AND DISCUSSION

Identification of plant pathogens

Two major fungal pathogens *viz.*, *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* were found associated with the infected roots of tomato plants based on their cultural and morphological characteristic[17, 18, 19].

Identification and characterization of rhizboacterial isolates

Twenty five rhizobacterial isolates were obtained from the healthy tomato plants, (Table 1). Out of which, 14 were long rods and 11 were short rods. Majority of the isolates formed light green pigmented colonies (I-1, I-4, I-5, I-8, I-10, I-11, I-12, I-13, I-15, I-16, I-18, I-20, I-22, I-23, I-24 and I-25)

whereas, the others formed green pigmented colonies (I-2, I-3, I-6, I-7, I-9, I-14, I-17, I-19 and I-21). Such a variation in the colony colour may be attributed to the production of different pigments/ metabolites[20]. The isolates formed round to irregular shaped colonies, while the round shaped colonies were non-spreading, (I-1, I-4, I-5, I-8, I-10, I-11, I-12, I-13, I-15, I-16, I-18, I-20, I-22 and I-24) the irregular shaped colonies were of the spreading nature, (I-2, I-3, I-6, I-7, I-9, I-14, I-17, I-19, I-21, I-23 and I-25). All the 25 isolates exhibited fluorescence on King's B agar under the UV light, however, there was variation among the isolates with respect to the intensity of fluorescence. The fluorescence under UV light is one of the key characters and the direct detection of fluorescence around the colonies is helpful for the identification of fluorescent pseudomonads[21]. Further, out of 25 isolates of fluorescent pseudomonads, I-07, I-15, I-18, I-23, I-24 and I-25 showed inhibition of pathogens *viz*., *Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani* (Table 2)

Identification of *Pseudomonas fluorescens*

The six putative rhizobacterial isolates having bio-control potentials were identified based on biochemical test *viz*., levan formation, arginine hydroysis, indole production, phosphorous solubilization, gelatin liquefaction, oxidase test and catalase test (Table 3) which confirmed the identification of the selected isolates (I-7, I-15, I-18, I-23, I-24 and I-25) to be *P. fluorescens* [22]. All the isolates showed positive growth at 4^0C, whereas, there was no growth at 41^0C. Earlier workers have also reported the similar results[23]. For physiological characterization, the effect of pH on growth was also studied and all the selected isolates showed maximum growth at pH 7.0, whereas, no isolate could grow at extreme acidic pH of 3.0 and 4.0 or alkaline pH of 13.0 and 14.0 (Table 4). All the isolates were able to grow in the pH range of 5.0 to 12.0. The selected isolates showed very good to good growth at pH 6.0 to 9.0.[24] isolated and characterized 216 fluorescent pseudomonads with high phosphate solubilizing ability from alkaline and Ca-rich soils with low phosphate availability. These reports indicate the ability of the fluorescent pseudomonads to sustain higher pH and NaCl concentration in the soil.

Evaluation of fungicides against the test pathogens

While evaluating the fungicides at different concentrations against *F. oxysporum* f. sp. *lycopersici* and *R. solani*, it was observed that all the treatments significantly reduced the mycelial growth of the test pathogens (Table 6). However, carbendazim, at all the tested concentrations and tebuconazole at 50 ppm, were responsible for complete inhibition of *F. oxysporum* f. sp. *lycopersici*, whereas, carbendazim (50, 100 and 250 ppm), tebuconazole (25 and 50 ppm) and mancozeb + metalaxyl (250 ppm) resulted in complete inhibition of *R. solani*. Our results are in accordance with[25] who tested Captan, Emison-6, Foltaf, JKstein, Kavach, Shield-75 and Vitavax against *R. solani*, the causal agent of damping off in eggplant under *in vitro* conditions and concluded that JKstein and Vitavax were the most effective fungicides, whereas, Sheld-75 was least effective in checking the linear growth of the fungus. Carbendazim and benomyl were the most effective *in vitro* fungitoxicants against *Fusarium* spp. compared to captafol, thiram, thiophenate methyl and captan[26]. The efficacy of carbendazim and carboxin against *R. solani* under *in vitro* conditions has also been advocated by other researchers[27, 28].

Evaluation of *Pseudomonas fluorescens* isolates for the management of soil borne disease of tomato

The selected *P. fluorescens* isolates (I-7, I-15, I-18, I-23, I-24 and I-25) and carbendazim were tested in pot culture against *F. oxysporum* f. sp. *lycopersici* and *R. solani*. The fungicide carbendazim showed maximum plant vigour (1501.54%) followed by isolate I-23 with 1453.77 per cent vigour index (Table 7). Similarly[29] have also reported that *P. fluorescens* increased plant vigour and consistently reduced the disease incidence under green house conditions and the disease protection was comparable with fungicide carbendazim.

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