



Management of damping off disease in tomato (*Solanum lycopersicum*) using potential biocontrol agent *Pseudomonas fluorescens*

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ABSTRACT

Biocontrol is a potential strategy adapted to control the fungal plant pathogens thus bio-prospecting to find novel native siderophore and enzyme producing *Pseudomonas* isolate for the management of tomato (*Solanum lycopersicum* L.) damping off disease under the prevailing conditions of Punjab state. In the present study, a total of 15 isolates of *Pseudomonas fluorescens* isolated from rhizospheric soils of tomato growing areas of Punjab and evaluated by dual culture plate confrontation assay, of which, two isolates (Pf10 and Pf6) showed maximum antagonistic activity against damping off pathogen (*Rhizoctonia solani*) with a zone of inhibition 14.30 mm and 11.53 mm respectively. These *Pseudomonas* isolates (Pf10 and Pf6) were also analyzed for their siderophore production and enzymatic activity. They were found to effectively produce siderophore and cell wall degrading enzymes such as chitinase and β -1,3 glucanase with higher antagonistic activity against *Rhizoctonia solani*. The present study was carried out at the Plant Pathology Research Farm, Punjab Agricultural University, Ludhiana, Punjab during 2017 and 2018 to confirm the bio-efficacy of these *Pseudomonas* isolates. Amongst the isolates, talc-based bioformulation of Pf10 isolate when applied as the seed+soil treatment had exerted strong inhibition against *Rhizoctonia solani* in tomato. Therefore, based on the findings, it was concluded that *Pseudomonas fluorescens* played a significant role as a potential biocontrol agent against damping off disease in tomato.

Keywords: Chitinase, Damping off, Fluorescent *Pseudomonas*, Glucanase enzyme, Siderophore, Tomato

The Plant growth promoting rhizobacteria (PGPR) are a diversified group of bacteria. They are found naturally in the rhizosphere or rhizoplane or in association with the roots of plants which improves the quality of the plant (Sunar *et al.* 2015). Among them, *Pseudomonas* spp. has been used extensively for plant growth promotion and disease control (Asha *et al.* 2011). *Pseudomonas* spp. are gram negative, rod shaped, non-pathogenic saprophytes that colonize soil, water and plant surface environments. The mechanisms by which *Pseudomonas* protects plants against pathogens are competition, antibiosis, siderophore production, induced systemic resistance, production of lytic and detoxifying enzymes (Haas and Defago 2005).

The siderophores produce yellow green pigments pyoverdins or pseudobactins, which limits the iron availability to pathogens for growth (Schwyn and Neilands 1987). The induced systemic resistance has been activated by encoding defense enzymes chitinase and β -1,3-glucanase activity of bacteria. It is a potential mechanism for

biological control of plant pathogenic fungi (Nielsen *et al.* 1998, Garbeva *et al.* 2004). *Pythium aphanidermatum*, an oomycete fungus is an important soil-borne plant pathogen that causes huge losses in agricultural production. Young tissues and plants are infected much more severely by this pathogen. It causes damping off disease in several plants including tomatoes (*Solanum lycopersicum* L.) and affects the plant both in a pre and post-emergence stage in nursery beds. Pre-emergence damping off is observed when the seed is infected prior to germination. This can result in poor or no germination and is observable as browning or rotting of the seed (Nair *et al.* 2022).

The recognition of plant growth-promoting rhizobacteria is useful for stimulating plant growth and increasing crop yields has evolved over the past few years (Saharan *et al.* 2011). Simultaneously, efforts are being made to minimize the harmful effects of chemical fertilizers in agriculture and move towards ecological methods. The present study is aimed to isolate potential fluorescent *Pseudomonas* from the rhizosphere of tomato growing regions in Punjab and test their bio-efficacy of plant growth promoting factors and their interactions with plants, which are commercially exploited for the management of tomato damping off and this also helps to meet the agricultural sustainability.

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MATERIALS AND METHODS

The present study was carried out at the Plant Pathology Research Farm, Punjab Agricultural University, Ludhiana, Punjab during 2017 and 2018 to confirm the bio-efficacy of *Pseudomonas* isolates.

Collection and isolation of *Pseudomonas*: In 2017–2018 and 2018–2019, 15 isolates of *Pseudomonas* spp. were collected from rhizospheric soils of various geographical locations in Punjab district and crop. Isolation of fluorescent pseudomonads was done on King's B media, following the serial dilution technique. Bergey's Manual of Systematic Bacteriology was followed to characterize the *Pseudomonas* isolates (Sneath 1986) as a standard protocol. The pure cultures were maintained on KB slants at 4°C in refrigerator and used whenever required. The pathogen *Rhizoctonia solani* was isolated and grown on PDA slants from naturally infected sick tomato soils.

Confrontation assay: The *Pseudomonas* isolates were evaluated against *R. solani* by a dual-culture confrontation assay using King's B medium (Dennis and Webster 1971). The inhibition zone in diameter (mm) was measured after 7 days of incubation. Petri plates inoculated with fungal pathogen discs (*R. solani*) alone served as control.

Molecular identification of Pseudomonas spp. using 16S rDNA primers

DNA isolation and PCR amplification: The genomic DNA was extracted from a 72-hour-old culture following the method of Stafford *et al.* (2005) with minor modifications. Amplification of the 16S rDNA gene was done by using universal primers: forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR amplification was carried out in 30 µl containing 50 ng/µl of template DNA, 2 U/µl of Taq DNA polymerase, 1X buffer, 1.5 mM MgCl₂, and 0.2 mM dNTPs. The PCR conditions consisted of initial denaturation at 94°C for 5 min, then 35 cycles each of 1 min denaturation at 94°C, 1 min annealing at 57°C, 2 min extension at 72°C, followed by holding the reaction at 72°C for 10 min. PCR products were resolved by gel electrophoresis running at 5 V/cm for 3 h on a 0.8% agarose gel prepared in 0.5X TBE buffer. The loaded PCR products were visualised by using the SYNGENE gel documentation system.

Sequencing and analysis of 16S rDNA gene: Desired 1500 bp unique band was eluted and purified from agarose gel by using Nucleospin® Gel and a PCR clean-up kit (Promega, Madison, WI, USA). Purified PCR products were sent for outsourced sequencing to Bioserve Biotechnologies (India) Pvt Ltd. Then, final sequences were searched in BLASTn tool and identified on the basis of sequence similarities with previously available sequences in NCBI's GenBank database.

Siderophore production: The siderophore production was determined by using chrome azurol S (CAS) agar medium (Schwyn and Neilands 1987). *P. fluorescens* isolates were CAS inoculated in plates as a spot inoculation and

incubated at 28±2°C. The formation of a bright zone with a yellowish colour in the dark-blue medium indicated the production of siderophores.

Qualitative estimation of chitinase enzyme activity: The ability of *Pseudomonas fluorescens* isolates to degrade chitin was determined by using the spot bioassay technique on King's B medium containing 1.0% colloidal chitin (w/v) (Viswanathan and Samiyappan 2001). Four spots on Petri plates were inoculated and incubated at 28°C. A zone of clearance was observed and it served as an indicator for chitinolytic activity.

Quantitative estimation of chitinase enzyme activity: The chitinase production in *P. fluorescens* strains was assessed in chitin peptone medium as method described by (Lim *et al.* 1991). The amount of reducing sugar produced was determined by measuring the optical density at 575 nm. The amount of enzyme produced per 1 µmole of reducing sugars per minute is defined as one unit of chitinase activity.

Quantitative estimation of β-1,3-glucanase enzyme activity: β-1,3-glucanase activity was performed according to the method described (Lim *et al.* 1991) and analyzed in terms of production of reducing sugar formed during assay using a spectrophotometer at 540 nm. The β-1,3-glucanase enzyme activity was expressed as a unit of glucose per ml of culture filtrate.

In vivo assessment of *Pseudomonas* spp. against *R. solani*: The biocontrol efficacy of *Pseudomonas* isolates against *R. solani* was evaluated during the 2017 and 2018 at the Plant Pathology Research Farm, PAU, Ludhiana. The experiment was conducted in completely randomized design with three replications. The talc based formulation was prepared by 600 ml of inoculated King's B broth per kg of talc powder. The *Pseudomonas* bio-formulation was applied as (a) seed dressings (10 and 15 g per kg of seed); (b) soil treatment (10 and 15 g per kg of FYM, then mixed in soil), and (c) combination (seed dressing + soil treatment) for field trials. A chemical fungicide Carbendazim 50 % W.P. (1.5 g/kg seed) was used as a standard check for comparison. An untreated control treatment was also maintained. Seed germination (%), per cent disease incidence (%), per cent disease inhibition (%), and plant growth parameters (root length, shoot length, fresh weight, dry weight, and yield) were recorded.

Statistical analysis: The data was analysed by one-way ANOVA (analysis of variance), and mean comparison was done by Duncan's multiple range test (DMRT) in SPSS Statistics 26.0 software (IBM Corporation, NY, USA). All statistical analyses were carried out at the P=0.05 level of significance.

RESULTS AND DISCUSSION

Confrontation assay: The dual-culture confrontation assay was performed with 15 isolates of *Pseudomonas*. The results in Table 1 show the variation in the antagonistic activity of *P. fluorescens* isolates against *R. solani*. Among them, isolate Pf10 inhibited the maximum mycelial growth (65.93%) with a 14.30 mm inhibition zone against

Table 1 Siderophore, quantitative and qualitative chitinase activity, glucanase activity and inhibitory effect of *Pseudomonas* isolates against *R. solani*

<i>Pseudomonas</i> isolates	Siderophore coloured zone (mm)	Quantitative clear zone (mm)*	Chitinase activity (unit/ml)*	β -1,3-glucanase (unit/ml)*	Confrontation assay (% inhibition)		
					<i>R. solani</i> radial growth (mm)*	Inhibition zone (mm)*	Inhibition (%)
Pf1	25.33 ^c ±0.58	8.00 ^{de} ±1.00	0.75 ^h ±0.17	1.14 ^{ab} ±0.01	40.00 ^{bcd} ±0.10	10.63 ^{cdef} ±0.04	55.56
Pf2	28.67 ^b ±1.53	10.67 ^{cd} ±1.53	4.89 ^d ±0.29	1.05 ^{ab} ±0.01	39.00 ^{bcd} ±0.10	10.13 ^{def} ±0.03	56.67
Pf3	18.33 ^{gh} ±5.77	9.33 ^{cde} ±1.53	1.13 ^{gh} ±0.12	0.68 ^{ab} ±0.01	40.33 ^{bcd} ±0.06	5.43 ⁱ ±0.04	55.19
Pf4	16.63 ^{gh} ±2.52	8.67 ^{de} ±0.58	0.72 ^h ±0.21	0.64 ^{ab} ±0.01	39.33 ^{bcd} ±0.45	9.43 ^{efg} ±0.04	56.30
Pf5	23.67 ^{cd} ±1.53	11.67 ^{bc} ±0.58	1.04 ^{gh} ±0.05	0.55 ^b ±0.01	41.50 ^{abc} ±0.25	9.03 ^{fg} ±0.02	53.89
Pf6	32.69 ^a ±1.15	15.00 ^a ±1.00	14.03 ^a ±0.37	1.50 ^a ±0.14	32.33 ^e ±0.15	11.53 ^{bcd} ±0.05	64.07
Pf7	19.00 ^{efg} ±1.00	8.00 ^{de} ±1.00	1.84 ^{fgh} ±0.15	0.52 ^b ±0.01	42.67 ^{ab} ±0.31	10.83 ^{cde} ±0.10	52.59
Pf8	31.67 ^{ab} ±1.53	13.67 ^{ab} ±1.53	12.23 ^b ±0.27	1.39 ^{ab} ±0.05	36.67 ^d ±0.15	12.67 ^b ±0.06	59.26
Pf9	21.00 ^{def} ±1.00	8.33 ^{de} ±1.53	2.03 ^{efg} ±0.04	0.62 ^{ab} ±0.03	40.67 ^{bc} ±0.21	10.53 ^{def} ±0.05	54.81
Pf10	33.67 ^a ±1.53	15.33 ^a ±1.53	14.20 ^a ±0.40	1.52 ^a ±0.02	30.67 ^e ±0.06	14.30 ^a ±0.03	65.93
Pf11	28.77 ^b ±1.53	9.00 ^{de} ±2.00	3.03 ^e ±0.09	0.84 ^{ab} ±0.01	44.67 ^a ±0.15	9.33 ^{efg} ±0.10	50.37
Pf12	19.00 ^{efg} ±1.00	6.67 ^e ±1.53	9.77 ^c ±0.28	0.48 ^b ±0.01	38.33 ^{cd} ±0.31	12.20 ^{bc} ±0.03	57.41
Pf13	15.33 ^h ±1.53	14.65 ^a ±1.53	10.14 ^c ±0.24	0.75 ^{ab} ±0.01	41.67 ^{abc} ±0.31	9.83 ^{efg} ±0.02	53.70
Pf14	21.97 ^{de} ±1.53	7.00 ^e ±1.00	2.10 ^{efg} ±0.16	0.77 ^{ab} ±0.01	40.33 ^{bcd} ±0.25	8.37 ^{gh} ±0.04	55.19
Pf15	17.85 ^{fgh} ±1.53	8.67 ^{de} ±0.58	2.59 ^{ef} ±0.10	0.54 ^b ±0.01	42.67 ^{ab} ±0.31	7.20 ^h ±0.02	52.59
Control	-	-	-	-	90.00	-	-
CD (P=0.05)	3.41	2.30	0.33	0.21	3.95	0.72	-

*Mean of three replications; Pf1 to Pf15, *Pseudomonas fluorescens*; CD, Critical difference. Superscript values indicate Duncan's grouping means with the same letter are not significantly different values after $\pm$ indicate standard deviation.

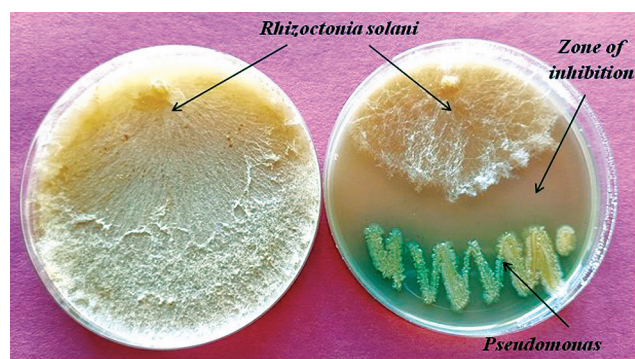


Fig 1 Dual culture of *Pseudomonas* isolate (Pf10) against *R. solani* on PDA (right) and a control plate inoculated with *R. solani* alone (left).

Rhizoctonia solani (Fig 1). It is followed by the isolate Pf6, which inhibits mycelial growth by 64.07%.

Molecular identification of 16s rDNA from *Pseudomonas* isolates: The molecular identification of the *Pseudomonas* isolates was done by using the universal primers 27F and 1492R. PCR amplification of DNAs extracted from *Pseudomonas* isolates showed the amplified PCR products with band size of 1500 bp (Fig 2), which confirmed the genus of the bacteria. The 1500 bp amplicons were eluted and purified from agarose gel and sequenced. The sequence resembled more than 95% with *Pseudomonas fluorescens* (U44827) in blast analysis.

Estimation of siderophore production from *Pseudomonas*

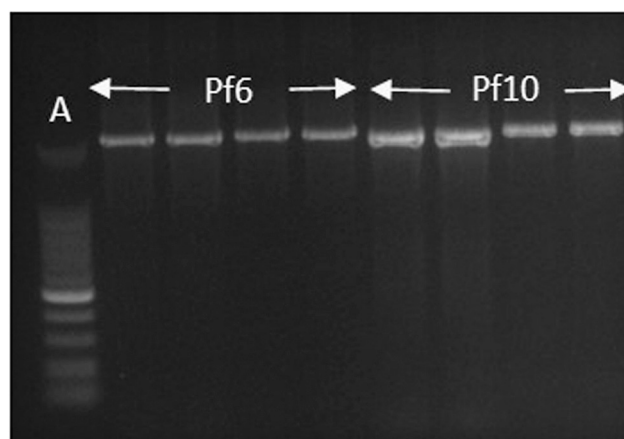


Fig 2 DNA profile generated using Universal primers with 2 isolates of *Pseudomonas* (Pf6 and Pf10) [A, 100 bp ladder].

isolates: All *Pseudomonas* isolates exhibited siderophore production on CAS media. On the Chrome Azural S (CAS) plates, all 15 *Pseudomonas* isolates produced yellow colour halo zones, indicating the production of siderophores. The coloured zone of *Pseudomonas* isolates varies in diameter from 15.33 to 33.67 mm. Pf 10 produced the most siderophores, measuring 33.67 mm in diameter, followed by Pf6 (32.69 mm), whereas Pf 13 isolate showed the least amount of siderophore with a diameter of 15.33 mm (Table 1).

Qualitative assay of chitinase: All *Pseudomonas*

Table 2 Effect of *Pseudomonas* treatments on germination, damping off disease incidence and on growth parameters (Root length, shoot length, fresh weight, dry weight and yield) of tomato (Pooled data of 2017–18 and 2018–19) under field conditions

Treatment	Germination (%)	Damping off disease incidence %	Damping off disease reduction (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Yield (q/acre)
	Mean	Mean		Mean	Mean	Mean	Mean	Mean
Pf6 Seed (10 g)	68.33 ^g ± 7.89	31.67 ^b ± 5.39	39.67	9.22 ^{cd} ± 1.22	13.50 ^e ± 1.34	1.85 ^{efg} ± 0.10	1.52 ^{ab} ± 0.16	197.86 ^{def} ± 0.12
Pf6 Seed (15 g)	74.17 ^{defg} ± 5.39	25.83 ^{bcd} ± 3.94	50.80	9.75 ^{cd} ± 1.25	14.75 ^e ± 2.39	2.01 ^{efg} ± 0.14	1.72 ^a ± 0.14	203.87 ^{cd} ± 0.05
Pf6 Soil (10 g)	71.67 ^{fg} ± 4.33	28.33 ^{bc} ± 4.33	46.03	9.25 ^{cd} ± 0.75	15.92 ^{de} ± 1.45	2.43 ^{def} ± 0.14	1.60 ^a ± 0.09	194.50 ^f ± 0.11
Pf6 Soil (15 g)	78.33 ^{bcd} ± 5.26	21.67 ^{defg} ± 5.26	58.72	10.08 ^{cd} ± 0.88	18.67 ^d ± 1.76	2.70 ^{cde} ± 0.21	1.75 ^a ± 0.07	205.46 ^c ± 0.16
Pf6 Seed+Soil (10 g)	80.00 ^{bcd} ± 2.50	20.00 ^{efg} ± 2.50	61.90	10.50 ^c ± 1.34	23.33 ^c ± 1.76	2.77 ^{cde} ± 0.17	1.82 ^a ± 0.02	196.56 ^{ef} ± 0.10
Pf6 Seed +Soil (15 g)	82.50 ^b ± 2.89	17.50 ^g ± 4.33	66.66	14.50 ^b ± 1.00	27.33 ^b ± 1.76	3.16 ^{bcd} ± 0.07	1.88 ^a ± 0.07	207.24 ^{bc} ± 0.13
Pf10 Seed (10 g)	71.67 ^{fg} ± 5.77	28.33 ^{bc} ± 5.77	46.03	13.25 ^b ± 1.82	13.58 ^e ± 1.51	2.68 ^{cde} ± 0.17	1.38 ^{ab} ± 0.08	203.32 ^{cde} ± 0.07
Pf10 Seed (15 g)	75.83 ^{cdef} ± 2.89	24.17 ^{cdef} ± 5.77	53.96	13.55 ^b ± 1.19	14.50 ^e ± 1.19	2.75 ^{cde} ± 0.17	1.57 ^a ± 0.04	204.18 ^{cd} ± 0.34
Pf10 Soil (10 g)	72.50 ^{efg} ± 2.89	27.50 ^{bcd} ± 2.89	47.61	13.47 ^b ± 0.95	16.50 ^{de} ± 2.66	3.11 ^{bcd} ± 0.08	1.63 ^a ± 0.09	201.46 ^{cde} ± 0.11
Pf10 Soil (15 g)	79.17 ^{bcd} ± 3.94	20.83 ^{efg} ± 3.94	60.32	13.83 ^b ± 1.58	22.50 ^c ± 2.65	3.69 ^{abc} ± 0.14	1.73 ^a ± 0.07	203.18 ^{cde} ± 0.17
Pf10 Seed+Soil (10 g)	80.83 ^{bc} ± 6.44	19.17 ^{fg} ± 6.44	63.48	15.42 ^b ± 1.42	23.92 ^c ± 1.59	4.08 ^{ab} ± 0.05	1.83 ^a ± 0.07	206.85 ^{bc} ± 0.12
Pf10 Seed+Soil (15 g)	94.17 ^a ± 3.94	5.83 ^h ± 1.44	88.89	23.67 ^a ± 1.26	34.49 ^a ± 1.16	4.53 ^a ± 0.11	2.22 ^a ± 0.07	221.86 ^a ± 0.30
Carbendazim 50% WP	92.50 ^a ± 2.89	7.50 ^h ± 2.89	85.71	9.25 ^{cd} ± 0.81	9.00 ^f ± 1.00	1.48 ^{fg} ± 0.05	0.74 ^{bc} ± 0.12	213.03 ^b ± 0.14
Control	47.50 ^h ± 4.33	52.50 ^a ± 4.33	-	7.20 ^d ± 1.09	8.33 ^f ± 0.87	1.10 ^g ± 0.07	0.42 ^c ± 0.05	81.80 ^g ± 0.13
Max	94.17	52.50	88.89	23.67	34.49	4.53	2.22	221.86
Min	47.50	5.83	39.67	7.20	8.33	1.10	0.42	81.80
CD (P=0.05)	9.38	9.42	-	2.3	3.2	0.31	0.18	12.51

*Mean of three replications; Pf6 and Pf10, *Pseudomonas fluorescens*; CD, Critical difference. Values are significant at 5% level as per Duncan grouping means with the same letter are not significantly different. Values after ± indicate standard deviation.

isolates were subjected on the chitin agar plates to check their qualitative chitinase enzyme activity. Data presented in Table 1 reveals a clear zone for all 15 *Pseudomonas* isolates, ranging from 6.67–15.33 mm. The diameter of the cleared halo zone indicated chitinolytic activity. The isolate Pf10 formed the largest clear zone, measuring 15.33 mm, followed by Pf6 (15.00 mm) and the minimum clear zone was formed by isolate Pf12 (6.67 mm).

Quantitative assay of chitinase and β -1,3-glucanase: The data revealed the efficacy of *Pseudomonas* isolates for the production of chitinase and β -1,3-glucanase (Table 1). The enzyme activity varied from 0.72 to 14.20 units/ml for chitinase and from 0.48–1.52 units/ml for β -1,3-glucanase. The maximum chitinase and β -1,3-glucanase enzyme

activities were expressed by the isolate Pf10 with 14.20 units/ml and 1.52 units/ml, respectively. It was followed by the isolate Pf6 with 14.03 units/ml chitinase and 1.50 units/ml β -1,3-glucanase enzyme activity.

Bio-efficacy of *Pseudomonas* isolates against damping off and its growth promotion in tomato: As per the *in vitro* analysis, the *Pseudomonas* isolates Pf10 and Pf6 showed strong enzymatic and siderophore activity. These two isolates have been chosen for additional field trials to assess bio-efficacy and plant growth promotion. Among the different *Pseudomonas* treatments, the combined treatment (seed+soil) at 15 g dose of Pf10 and Pf6 showed maximum disease inhibition with 88.89% and 85.71%, respectively. In the control treatment, the frequency of disease incidence

was highest at 52.50%.

In the field experiment, the *Pseudomonas* treatments were also tested for their plant growth-attributing characters such as germination, root and shoot length, fresh and dry weight, and yield of tomato plants. There was a significant amount of growth stimulation when compared to the untreated control. There is a variation in the plant growth promotion of different treatments. Pf10 seed+soil treatment at a 15-g dose had the highest germination rate (94.17%), followed by T8 (92.50%). The fluorescent *Pseudomonas* treatment PF10 stimulated the maximum root length (23.67 cm), shoot length (34.49 cm), fresh weight (4.53 g), dry weight (2.22 g), and yield (225.86 q/acre) of the tomato. All the treatments of Pf6 and Pf10 showed significant increase in root length, shoot length, fresh weight, dry weight and yield as compared to the control (Table 2).

The antagonistic interactions between the host plant, pathogen, and bacteria are significant for their utilization in plant health management (Thomashow and Weller 1991). The *Pseudomonas* produces both chitinase and β -1,3 glucanases, which digest the hyphae of the fungal cell walls. These enzyme activities could be an effective method for the biological control of plant pathogens.

In this study, 15 isolates of *Pseudomonas* were obtained from rhizospheric soils of tomato-growing regions in Punjab. These were tested for their antagonistic interaction against the pathogen, *R. solani* and for siderophore production, chitinase, and β -1,3-glucanase enzyme activity *in vitro*. Different isolates showed different reactions during investigation by adopting various tests. On the basis of the antagonism, siderophore production, chitinase, and β -1,3-glucanase enzyme activity exhibited by the *Pseudomonas* isolates, one isolate, designated Pf10, which showed positive results in all tests, was selected as a potential isolate for *in vivo* studies.

Similar work was done by Tariq *et al.* (2010), who found that nine strains of antagonistic *Pseudomonas* reduced the fungal growth and caused the lysis of sclerotia in *R. solani* in a dual-culture assay as well as in an extracellular metabolite efficacy test. Choudhary *et al.* (2019) reported maximum inhibition (70%) of *R. solani* by the isolate Pf3 of *P. fluorescens*. *Pseudomonas fluorescens* produces 27 mol of NAG per mg protein per min chitinase activity in *Pseudomonas* isolates, according to Alhasawi and Appana (2017). Suganti *et al.* (2020) reported that 100% and 78% mortality were observed by using *P. fluorescens* MP-13 chitinase against *Helopeltis theivora* in tea. Similarly, Helfish *et al.* (2017) reported *P. fluorescens* producing β -1,3-glucanase (1.76 units/ml) against *R. solani* and *R. oryzae*. Siderophore production of *Pseudomonas* isolates is of great importance and to confirm the production of siderophore of *Pseudomonas* was done in CAS agar plate assay. Similar observations were reported by Singh (2018), who reported that the siderophore production by *Pseudomonas fluorescens* was effective in controlling plant pathogenic fungi and observed a coloured zone of 32.17 mm diameter.

This bacterial isolate was identified as *Pseudomonas*

fluorescens based on its morphology and 16S rDNA gene sequencing. A similar study was done by Georgevia *et al.* (2018) and reported the amplification of the 16S rDNA gene in *Pseudomonas* strains using universal primers 27F and 1492R. The *P. fluorescens* (Pf10) has shown promising traits *in vitro*, and the bio-formulation was prepared for this isolate to check the bio-efficacy of these *in vivo* conditions. The treatments include seed bacterization followed by the direct application of the talc based formulation in soil and the combination of both seed and soil. In comparison to the control, the seed+soil treatment with 15 g of the talc formulation resulted in a significant increase in tomato growth in terms of root length, shoot length, biomass, yield, and inhibition of damping off disease. Our study is in agreement with the previous study made by other researchers, which supports and reflects the same findings. Ahmed and Baker (1987) reported that inoculation of seeds with antagonistic bacteria results in the establishment in the rhizosphere; they protect against root pathogens by producing antibiotics or siderophores, and antagonistic fungi prevented seed decay through the mechanisms of hyperparasitism and the release of certain pathogen-inhibiting enzymes and antibiotics, which are involved in preventing the infection by plant pathogens. Meyer *et al.* (1987) reported that species of *P. fluorescens* produce various compounds that increase the total dry weight of cells, which significantly enhance the yield of vegetable crops. Tanwar *et al.* (2013) also observed that treatment with *T. viride* and *P. fluorescens* increases the uptake of phosphorus, nitrogen, potassium, and other micronutrients, which leads to an increase in plant mass.

It was concluded from the study that siderophore-producing native fluorescent *Pseudomonas* isolated from the rhizospheric soils of tomato-growing regions in Punjab were effective in suppressing disease caused by *Rhizoctonia solani* in tomato crops, and they also promoted plant growth. Thus, the isolate Pf10 can be used as a commercial bioagent for eco-friendly management of tomato disease as a substitute for chemical fungicides.

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