Isolation and Characterization of native *Pseudomonas* isolates from mid and high hills of Uttarakhand and their evaluation against *Fusarium oxysporum* f. sp. *lycopersici*

Commented [h1]: Fluorescent *Pseudomonas*

Commented [h2]: Soil borne pathogens of tomato

ABSTRACT

Fluorescent *Pseudomonas* spp. are widely recognized for their potential as biocontrol agents against soil-borne phytopathogens However, information on the diversity and efficacy of native fluorescent *Pseudomonas* isolates from different agro-ecological regions of Uttarakhand remains limited. The present investigation was therefore carried out during 2025-26 in the Biocontrol Laboratory, Department of Plant Pathology, College of Hill Agriculture, Campus-Ranichauri, Tehri Garhwal, V.C.S.G. Uttarakhand University of Horticulture and Forestry, Uttarakhand, to isolate, characterize and evaluate fluorescent *Pseudomonas* spp. for their antagonistic potential against major soil-borne pathogens. Seventy rhizospheric soil samples were collected from seven districts (Tehri, Nainital, Pauri, Bageshwar, Uttarkashi, Almora and Chamoli) covering altitudes from 1370 m to 3200 m above mean sea level. Twenty-three fluorescent *Pseudomonas* isolates were obtained using the serial dilution technique on King's B medium. All isolates tested positive for Catalase, KOH and Ammonification tests, while twelve showed positive reaction towards Starch hydrolysis. Antagonistic activity against major soil-borne pathogens was assessed through dual culture assay. Isolates PB1, PB4 and PS40 showed more than 75% inhibition of *Fusarium oxysporum* f.sp. *lycopersici*, while PU2 exhibited the highest inhibition against *Rhizoctonia solani* (94.44%) and PU1 was found most effective against *Sclerotium rolfsii* (86.67%) over control. Screening for biocontrol traits revealed that thirteen isolates produced hydrogen cyanide (HCN), while ten isolates showed siderophore production and phosphate solubilization. Promising putative candidates were further evaluated under glasshouse conditions against tomato wilt caused by *F. oxysporum* f.sp. *lycopersici*. Among the treatments, T5 (*Fusarium* inoculated soil +Seeds bioprimed with PB1) recorded the highest seed germination (93.33%), lowest disease incidence (10.00%) and the maximum disease reduction (90.00%) over T2 (Negative control). The study concludes that isolate PB1 is a potent biocontrol agent with significant potential for managing tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*.

Keywords*:* Biocontrol; *Pseudomonas*; Rhizospheric soil; Antagonistic activity; Tomato wilt; *Fusarium oxysporum* f.sp. *lycopersici*

Introduction

Beneficial microorganisms present in soil play a crucial role in sustainable agriculture by improving soil health, enhancing nutrient availability, and suppressing plant pathogens. Among microorganisms, plant growth-promoting rhizobacteria (PGPR) have gained significant attention for their ability to colonize the rhizosphere and support plant growth through multiple mechanisms **(de Sosa *et al*., 2023)**. PGPR enhance crop growth, yield and stress tolerance by increasing nutrient uptake from the soil and through a variety of mechanisms such as phosphate solubilization, siderophore production, biological nitrogen fixation, phytohormone production, antifungal activity, and induction of systemic resistance **(Abd El-Mageed *et al*., 2022)**. Species of *Pseudomonas* represent an important group of plant growth-promoting rhizobacteria (PGPR), known for their ability to enhance plant growth and confer tolerance to biotic and abiotic stresses through multiple mechanisms.

Pseudomonas spp., a group of ubiquitous, Gram-negative, rod-shaped bacteria belonging to the family Pseudomonadaceae, is widely recognized for its effectiveness in biological control of plant diseases **(Kumaran *et al*., 2010)**. Fluorescent *Pseudomonas* solubilizes phosphate in plants and it also produces various hydrolytic enzymes like amylase, protease, cellulase and chitinase in addition to production of hydrogen cyanide (HCN), siderophore and indole acetic acid **(Khalil *et al*., 2022)**. The genus *Pseudomonas* secretes extracellular enzymes to fend off pathogen invasion. The antifungal compound 2,4-diacetylphloroglucinol is a key factor contributing to the biocontrol potential of *Pseudomonas* spp. **(Delany *et al.,* 2000)**. Besides fending off the pathogens, the genus is also known for enhancing growth and yield of various crops like canola, wheat, rice, maize, soybean, cucumber, brinjal and tomato **(Cattelan *et al.* 1999)** by breaking down phosphorus, potassium into plant available forms **(Wang *et al*., 2020)**. In tomato, *Pseudomonas* has been extensively studied for its ability to manage several soil-borne and foliar pathogens, thereby improving plant health and productivity.

Solanum lycopersicum (tomato) is an important solanaceous crop worldwide, ranking just after potato and sweet potato in terms of cultivated area, while standing as the most extensively processed vegetable crop **(Tamburino *et al.*, 2020)**. The crop suffers from many fungal diseases such as Early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Septoria leaf spot (*Septoria lycopersici*), Leaf Mold (*Passalora fulva*), Buckeye rot (*Phytophthora parasitica*), Anthracnose (*Colletotrichum coccoides)* and *Fusarium* wilt (*Fusarium oxysporum*

f.sp. *lycopersici*) **(Ketelaar and Kumar, 2002)**. Among all these diseases, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersic*i is the most devastating fungal disease of tomato which is known to cause yield loss from 10 to 100% **(Haq and Ijaz, 2020)**. In India the disease causes yield loss up to 47.94% **(Choudhary *et al*., 2023)**. The management of this disease remains to be a challenging task due to its soil-borne nature. The conventional management of *Fusarium* wilt relies on the use of synthetic fungicides, which are often expensive, hazardous to the environment and pose significant risks to human health **(Jaiswal *et al*., 2016)**. Therefore, making use of eco-friendly control methods for management of *Fusarium* wilt of tomato has become a necessity. In this context, seed biopriming has emerged as an effective and widely recognized approach for improving plant health and biotic stress tolerance **(Haruna *et al.,* 2024)**.

Seed biopriming, defined as the coating of seeds with beneficial microorganisms **(Mathre *et al.,* 1999),** is an effective approach to enhance seed germination, seedling vigour and tolerance to biotic and abiotic stresses **(Harman and Taylor, 1988)**. It also improves plant resistance against pathogens and overall plant performance under stressed conditions **(Rawat *et al.,* 2016)**. In this context, the use of bacterial biocontrol agents such as *Pseudomonas* spp. has gained importance for disease management and growth promotion. Building on this potential, the present study was undertaken to manage *Fusarium* wilt of tomato through seed biopriming in the mid and high hills of Uttarakhand using effective *Pseudomonas* isolates.

Material and Methods

The study was carried out in the Biological Control Laboratory of the Department of Plant Pathology, College of Hill Agriculture, Campus- Ranichauri, Tehri Garhwal, V.C.S.G., Uttarakhand University of Horticulture and Forestry, Uttarakhand.

Source of Materials (Soil, Pathogen, Plant material) and Isolation of fluorescent *Pseudomonas*

Soil Samples

Survey and collection of rhizospheric soil samples was done from seven districts (*viz*., Tehri, Nainital, Pauri, Bageshwar, Uttarkashi, Almora and Chamoli) of Uttarakhand from various crops. Seventy rhizospheric soil samples were collected covering altitudes from 1370 m to 3200 m above mean sea level. The soil attached to secondary roots was gently removed using

a brush, thoroughly mixed and about 10 g of composite soil was collected per field in properly labelled sample bags for further analysis.

Fungal Plant Pathogens

Fungal plant pathogens *viz*., *Fusarium oxysporum* f.sp. *lycopersici, Rhizoctonia solani* and *Sclerotium rolfsii.* were obtained from the microbial repository of the Department of Plant Pathology, College of Hill Agriculture, Campus- Ranichauri, Tehri Garhwal, V.C.S.G., Uttarakhand University of Horticulture and Forestry, Uttarakhand.

Seed Material

The seed material for the present investigation, comprised of the local tomato variety "S-21" (*Solanum lycopersicum*) was obtained from the local market of Ranichauri, Tehri Garhwal.

Isolation of fluorescent *Pseudomonas*

For the selective isolation of fluorescent *Pseudomonas* isolates, 'serial dilution' technique was used on Nutrient Agar Medium (NAM) plates followed by incubation at 28± 2 °C for 48 hr. After incubation, plates were examined under UV light and colonies giving yellow-green fluorescence were isolated on KB media. Mother cultures were then maintained at 4 °C for further study.

Commented [h3]: KB media

Commented [h4]: Nutrient Agar Medium

***In vitro* characterization of isolated *Pseudomonas* isolates**

Morphological and Biochemical characterization of bacterial isolates

The *Pseudomonas* isolates form characteristic colonies on the King's B media, which was taken as a tool for preliminary identification. Cultural characterization of isolates was observed by different characteristics of colonies such as shape, nature, and colour pigmentation of the colony **(Srinivasa *et al*., 2015)**. Biochemical characterization of bacterial isolates was performed using various test like Gram's staining Catalase test, KOH test, Starch hydrolysis and Ammonification test **(Bagul *et al*., 2023)**.

***In vitro* evaluation for biocontrol and other traits of fluorescent *Pseudomonas* isolates (Antagonistic activity, HCN production, Phosphate solubilization and Siderophore production)**

Evaluation for Antagonistic Activity

In vitro antagonistic activity of *Pseudomonas* spp. against *R. solani, S. rolfsii* and *F. oxyspo*rum f.sp. *lycopersici* was studied using dual culture technique **(Jishma *et al*., 2021)**. A three to four day old culture of bacterial isolate was streaked at one side of petri dish (1 cm away from the

edge) and 5 mm mycelial plug from three to four day old cultures of pathogens were placed at the opposite side of the petri dish perpendicular to the bacterial streak on PDA medium. Petri dishes were then incubated at 28± 2 °C for 7 days. Petri dishes inoculated with sole fungal discs were served as a control. Each treatment was replicated five times. Observations on the width of mycelial growth of tested pathogens were recorded and the percent inhibition of pathogen growth was calculated using the following formula **(Kamaruzzaman *et al*., 2021)**.

The percent fungal growth inhibition was estimated by $I = \frac{C-T}{C} \times 100$,

Where:

I = percent mycelial growth inhibition of the test pathogen.

C = mycelial growth of the pathogen in the control plate

T = mycelial growth of the pathogen in the test plate.

Evaluation for HCN production, Phosphate solubilization and Siderophore production

The isolates were evaluated for plant growth-promoting traits, namely hydrogen cyanide (HCN) production, siderophore production, and phosphate solubilization. HCN production was determined by the modified method **(Miller and Higgins, 1970)** where a colour change from white to yellow-orange, brown, or brick red indicated a positive reaction. Siderophore production was assessed using the Chrome Azurol S (CAS) agar plate assay **(Schwyn and Neilands, 1987)** after incubation at 28 ± 2 °C for 72 hours, and the appearance of an orange halo zone confirmed the production. Phosphate solubilization was tested on Pikovskaya's agar medium **(Linu *et al*., 2019)** incubated at 28 ± 2 °C for 48 hours, where the formation of clear halo zone around bacterial colonies indicated tricalcium phosphate solubilization.

Developing bioformulations of promising *Pseudomonas* isolates exhibiting broad action properties

Putative candidates (PS1, PS2, PS10, PB1, and PB4) of fluorescent *Pseudomonas* spp. were selected for preparing talc-based bioformulation based on their performance in the above studied traits. Sterilized King's broth was inoculated with the selected isolates separately and incubated at 28 ± 2°C for 48–72 hrs on an incubator shaker at 150 rpm. **(Sendhilvel *et al*., 2007)**. After 72 hrs the inoculated broth was mixed with pre-sterilized raw talc (1:2 v/w) having 1.0 % carboxymethyl cellulose (CMC). The talc-based bioformulation was dried and the final CFU value was maintained at 10^{-7} g^{-1}. Talc-based bioformulation with calculated CFU value

(Gupta and Dohroo, 2014) was packed in pre-autoclaved bags and stored at -20 °C till further use.

Evaluation of putative candidates of *Pseudomonas* isolates for wilt disease (caused by *F. oxysporum* f.sp. *lycopersici*) supression in tomato under glass house conditions

***Fusarium* inoculum preparation**

A pure culture of *F. oxysporum* f. sp. *lycopersici* was prepared on sterilized barnyard millet grains and incubated at 28 °C for 12 days **(Saber *et al*., 2015)**. Colonized grains were air-dried and grained to obtain a fine powder, which was passed through 80 mesh sieve to obtain a pure spore powder.

Pot preparation

Soil sample was prepared by adding mixture of Soil: Sand: Farmyard manure (3:1:1), then transferred (5 kg/pot) into sterilized plastic pots (30 cm diameter). Infestation was done by thoroughly mixing 10 g of prepared fungal inoculum per pot up to 5 cm depth and incubating under warm, humid conditions for eight days, except for T1(Positive control) as described by **(Saber *et al*., 2015)**.

Seed biopriming and seed sowing

Seeds were bioprimed with talc-based bioformulations of five putative candidates of fluorescent *Pseudomonas* isolates separately while T1 (Positive control) and T2 (Negative control) received non bioprimed seeds **(Table 1)**. Ten seeds per pot were sown.

Percent Disease Incidence (PDI) was calculated first after 30 days of sowing and was next calculated 60 days after sowing (when all plants in T2 (Negative control) wilted) by using the formula **(Shiva *et al*., 2020)**.

$$\text{PDI \%} = \frac{\text{No. of plants wilted}}{\text{Total number of plants examined}} \text{ X } 100$$

Percent Disease Reduction (PDR) over Negative control was recorded according to **Alwathnani *et al.*, (2012)**.

$$\text{PDR \%} = \frac{\text{DIC -DIT}}{\text{DIC}} \text{ X } 100$$

Where:

DIC = Disease incidence in Negative control

DIT = Disease incidence in treatment

Statistical Analysis

The replicated data of each treatment obtained in laboratory observations were statistically analysed using a Complete Randomised Design (CRD), using MS-Excel and OPSTAT. The mean value of the data was subjected to analysis of variance, as accepted by **(Gomez and Gomez, 1984)**.

Results and Discussion

Isolation of *Pseudomonas* isolates from rhizospheric soil samples

Out of 70 soil samples tested (**Table 2**), 23 soil samples harboured *Pseudomonas* isolates. The number of *Pseudomonas* isolates isolated from soil samples varied across seven different districts. Maximum number of *Pseudomonas* isolates (six) were isolated from Tehri district followed by Nainital and Pauri district (four isolates each). Similar approach for the isolation of *Pseudomonas* from rhizospheric soils has been documented where the respective researchers successfully employed serial dilution technique, highlighting its reliability and widespread applicability in microbial studies (**Suman *et al*., 2018; Joshi *et al*., 2018; Kipgen *et al*., 2021)**.

***In vitro* characterization of fluorescent *Pseudomonas* isolates**

Morphological and Biochemical Characterization

A total of 23 fluorescent *Pseudomonas* isolates were morphologically characterized on King's B medium and exhibited considerable variation in colony traits. All isolates produced characteristic fluorescence under UV light **(Fig. 1)**. Six bacterial isolates (PS1, PS32, PS40, PS43, PS45 and PU2) showed spreading type growth and remaining 17 showed non- spreading type growth **(Fig. 2a)**. Colony colour ranged from cream white to white, pale yellow and white pink **(Fig. 2a and Fig. 2b)**. These observations are in agreement with the findings of **Soesanto *et al.*, (2010)**.

Further, biochemical characterization revealed that all isolates were Gram negative, rod shaped and tested positive for Catalase and KOH tests, indicating typical *Pseudomonas* characteristics **(Table 4 and Fig. 3)**. In Starch hydrolysis test, 12 isolates exhibited positive results, and the rest 11 showed negative results. All the isolates showed positive results for Ammonification test **(Table 4)**. These findings are consistent with earlier reports by **Behrendt *et al.*, (2003)** and

(Hugh and Leifson, 1953) confirming the identity and functional diversity of fluorescent *Pseudomonas* isolates.

In vitro evaluation for biocontrol and other traits of fluorescent *Pseudomonas* isolates (Antagonistic activity, HCN production, Phosphate solubilization and Siderophore production)

All 23 rhizobacterial isolates were evaluated for their antagonistic efficacy against major soil-borne fungal pathogens *F. oxysporum* f. sp. *lycopersici, R. solani* and *S. rolfsii* using the dual culture assay with four-days-old pathogen cultures, which revealed significant variability among fluorescent *Pseudomonas* isolates. Isolates PB1, PB4 and PS40 showed strong inhibition (>75%) against *F. oxysporum* f. sp. *lycopersici* while PU2 exhibited maximum inhibition (94.44%) against *R. solani*. PS40 also recorded high inhibition (86.67%) against *S. rolfsii,* whereas PU1, PA1, PS45, PB4 and PS43 showed moderate inhibition (>50%) indicating their potential as effective biocontrol agents as shown in **Table 3**. These findings are in agreement with **Singh *et al.*, (2019)** who earlier reported that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* exhibited maximum mycelial inhibition against *F. oxysporum* and also suppressed *R. solani*. Further screening of plant growth-promoting traits revealed that 13 isolates showed positive results for hydrogen cyanide (HCN) production, indicated by a colour change from yellow to orange or brick red to brown with increasing intensity from day 4 to day 7 **(Table 4 and Fig. 4)**. In the phosphate solubilisation assay 10 isolates showed clear zone formation on Pikovskaya's agar and also produced siderophores on Chrome Azurol S medium **(Table 4 Fig. 5)**. These results are consistent with earlier findings by **Kipgen *et al.*, (2021)** and **(Kamei and Simon, 2018)** indicating that HCN production, siderophore production and phosphate solubilisation contributes significantly to the antagonistic and plant growth-promoting potential of fluorescent *Pseudomonas* isolates.

Effect of *Pseudomonas* isolates on wilt disease suppression in tomato caused by *F. oxysporum* f. sp. *lycopersici* under glass house conditions

The antagonistic activity of putative *Pseudomonas* isolates against *F. oxysporum* f. sp. *lycopersici* in tomato was evaluated using selected *Pseudomonas* isolates (PS1, PS2, PB1, PS10, and PB4) applied through seed biopriming. The bioprimed treatments performed significantly better than T2 (Negative control) in terms of wilt suppression and seed germination. Germination ranged from 53.33% to 100% with maximum germination (100%) recorded in T1(Positive control), while Percent Disease Incidence ranged from 0% to 100%

with the highest disease incidence in T2 (Negative control). Among treatments, T5 (Fusarium inoculated soil +Seeds bioprimed with PB1) recorded the highest Percent Disease Reduction over Negative control (90%), indicating strong antagonistic efficiency **(Table 5)**. The reduction in wilt may be attributed to the production of secondary metabolites such as siderophores, antibiotics, volatile compounds and lytic enzymes by *Pseudomonas* isolates. Similar findings were reported by **Saraf *et al*., (2014)** who demonstrated that *Pseudomonas* spp. inhibits phytopathogens by producing antimicrobial metabolites and detoxifying enzymes that degrade fungal cell walls and neutralize toxins. **Shiva *et al*., (2020)** observed significant suppression of tomato wilt caused by *F. oxysporum* f.sp. *lycopersici* where *Pseudomonas* isolates reduced disease incidence to 7.8% and achieved 85.6% control in artificially inoculated pots. These results confirm the potential of *Pseudomonas* isolates as effective biocontrol agents for managing *Fusarium* wilt disease through seed biopriming in tomato.

Conclusion

The present study revealed that native *Pseudomonas* isolates, collected from the mid and high hills of Uttarakhand effectively reduced *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* when applied through seed biopriming. Among the five putative candidates of *Pseudomonas* (PS1, PS2, PS10, PB1 and PB4) selected after various biochemical and biocontrol tests, PB1 was identified as the most promising one for wilt disease suppression in tomato. Also, it was found effective in various *in vitro* biochemical tests, including catalase activity, KOH solubility, starch hydrolysis and ammonification test, as well as in biocontrol traits such as phosphate solubilization, siderophore production and hydrogen cyanide (HCN) production, producing the highest HCN among the five putative candidates of *Pseudomonas* isolates. The enhanced effectiveness observed through seed biopriming highlights its potential for early-stage plant protection and improving seedling vigour. Hence, this isolate is of considerable value and may be further exploited for improving plant health and productivity. However, further evaluation under field conditions and across different crops and pathogens is necessary to validate its broader applicability.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Details for assessing the efficacy of putative candidates of fluorescent *Pseudomonas* isolates applied through seed biopriming for the suppression of wilt disease in tomato caused by *F. oxysporum* f. sp. *lycopersici* under glasshouse conditions are presented below:

Symbol	Treatment
T1	Positive control (Untreated control): Non-*Fusarium* inoculated soil + Non bioprimed seeds
T2	Negative control: *Fusarium* inoculated soil+ Non bioprimed seeds
T3	*Fusarium* inoculated soil + Seeds bioprimed with *Pseudomonas* isolate PS1
T4	*Fusarium* inoculated soil + Seeds bioprimed with *Pseudomonas* isolate PS2
T5	*Fusarium* inoculated soil + Seeds bioprimed with *Pseudomonas* isolate PB1
T6	*Fusarium* inoculated soil + Seeds bioprimed with *Pseudomonas* isolate PS10
T7	*Fusarium* inoculated soil + Seeds bioprimed with *Pseudomonas* isolate PB4

Table 2. Details of the rhizospheric soil samples collected from mid and high hills of Uttarakhand

S. No.	Soil sample code	Crop	Location (Village)	District	Elevation (m)	Harboured *Pseudomonas*	*Pseudomonas* isolate code
1	S1	Cabbage	Ranichauri	Tehri	1950	Yes	PS1
2	S2	Brinjal	Ranichauri	Tehri	1950	Yes	PS2
3	S3	Chilli	Ranichauri	Tehri	1950	No	-
4	S4	Capsicum	Ranichauri	Tehri	1950	No	-
5	S5	Potato	Salamkhet	Tehri	2300	No	-
6	S6	Rye	Salamkhet	Tehri	2300	No	-
7	S7	Rajma	Salamkhet	Tehri	2300	No	-
8	S8	Beans	Salamkhet	Tehri	2300	No	-
9	S9	Amaranth	Salamkhet	Tehri	2300	No	-
10	S10	Cabbage	Salamkhet	Tehri	2300	Yes	PS10
11	S11	Rajma	Ruichanu	Tehri	1930	No	-
12	S12	Maize	Ruichanu	Tehri	1930	No	-
13	S13	Barnyard	Ruichanu	Tehri	1930	No	-
14	S14	Finger millet	Ruichanu	Tehri	1930	Yes	PS14
15	S15	Rajma	Kainchu	Tehri	1918	No	-
16	S16	Barnyard	Kainchu	Tehri	1918	No	-
17	S17	Soybean	Kainchu	Tehri	1918	No	-
18	S18	Rajma	Kotdwara	Tehri	1948	No	-
19	S19	Barnyard	Kotdwara	Tehri	1931	No	-
20	S20	Maize	Kotdwara	Tehri	1931	No	-
21	S21	Tomato	Kotdwara	Tehri	1900	Yes	PS21
22	S22	Chilli	Kotdwara	Tehri	1900	No	-
23	S23	Amaranth	Kotdwara	Tehri	1880	No	-
24	S24	Rye	Maun	Tehri	1950	No	-
25	S25	Raddish	Maun	Tehri	1950	No	-
26	S26	Potato	Maun	Tehri	1950	No	-
27	S27	Barnyard	Maun	Tehri	1950	No	-

28	S28	Amaranth	Maun	Tehri	1900	No	-
29	S29	Chilli	Maun	Tehri	1900	Yes	PS29
30	S30	Rajma	Guriyali	Tehri	1868	No	-
31	DG	Pea	Guriyali	Tehri	1868	No	-
32	S32	Chilli	Pangot	Nainital	2250	Yes	PS32
33	S33	Okra	Pangot	Nainital	2250	Yes	PS33
34	S34	Maize	Sigri	Nainital	2200	No	-
35	S35	Turmeric	Sigri	Nainital	2200	Yes	PS35
36	S36	Pumpkin	Sigri	Nainital	2200	No	-
37	S37	Bitter gourd	Sattal	Nainital	1370	Yes	PS37
39	S38	Sponge gourd	Sattal	Nainital	1370	No	-
40	S39	Cucumber	Sattal	Nainital	1370	No	-
41	S40	Barnyard millet	Kaproli	Pauri	2200	Yes	PS40
42	S41	Pumpkin	Kaproli	Pauri	2180	No	-
43	S42	Maize	Kaproli	Pauri	2180	Yes	PS42
44	S43	Rice	Kaproli	Pauri	2150	Yes	PS43
45	S44	Finger millet	Mursheti	Pauri	1770	No	-
46	S45	Amaranth	Mursheti	Pauri	1800	Yes	PS45
47	S46	Urad	Mursheti	Pauri	1800	No	-
48	S47	Amaranth	Buranshi	Pauri	1650	No	-
49	S48	Maize	Buranshi	Pauri	1700	No	-
50	B1	Soybean	Kafligair	Bageshwar	1245	Yes	PB1
51	B2	Colocasia	Kafligair	Bageshwar	1245	No	-
52	B3	Barnyard	Bharadi	Bageshwar	1522	No	-
53	B4	Ginger	Vijaypur	Bageshwar	1530	Yes	PB4
54	B5	Marigold	Vijaypur	Bageshwar	1530	No	-
55	B6	Raddish	Vijaypur	Bageshwar	1520	No	-
56	B7	Urad	Sama	Bageshwar	2500	No	-
57	U1	Soybean	Purola	Uttarkashi	1500	Yes	PU1
58	U2	Rajma	Purola	Uttarkashi	1500	Yes	PU2
59	U3	Beefsteak plant	Mainjni	Uttarkashi	2059	No	-

60	A1	Beans	Kausani	Almora	1890	Yes	PA1
61	A2	Cabbage	Kausani	Almora	1890	No	-
62	A3	Summer squash	Ranikhet	Almora	1869	No	-
63	A4	Pumpkin	Ranikhet	Almora	1869	No	-
64	A5	Cucumber	Jageshwar	Almora	1870	Yes	PA5
65	CH1	Torai	Bhadura	Chamoli	1500	No	-
66	CH2	Pumpkin	Bhadura	Chamoli	1500	Yes	PCH2
67	CH3	Brinjal	Malari	Chamoli	3050	Yes	PCH3
68	CH4	Cucumber	Malari	Chamoli	3050	No	-
69	CH5	Okra	Mana	Chamoli	3200	Yes	PCH5
70	CH6	Bottle gourd	Mana	Chamoli	3200	No	-

Table 3. Antagonistic activity and performance of fluorescent *Pseudomonas* isolates against *F. oxysporum* f. sp. *lycopersici, R. solani* and *S. rolfsii*

S. No.	*Pseudomonas* isolate code	% Inhibition of *F. oxysporium* f. sp. *lycopersici*	% Inhibition of *R. solani*	% Inhibition of *S. rolfsii*	*F. oxysporum* f. sp. *lycopersici*	*R. solani*	*S. rolfsii*
1	PS1	59.26*± 0.98	55.19*± 0.37	19.26*± 1.61	++ ve	++ ve	+ ve
2	PS2	67.04*± 0.98	55.93*± 0.37	13.33*± 1.11	++ ve	++ ve	+ ve
3	PB1	75.93*± 0.98	77.04*± 0.74	17.41*± 0.74	+++ ve	+++ ve	+ ve
4	PS10	29.63*± 0.98	44.82*± 0.37	31.85*± 0.98	+ ve	++ ve	++ ve
5	PS14	42.59*± 0.98	50.00*± 1.28	22.22*± 1.28	++ ve	++ ve	+ ve
6	PB4	79.26*± 0.74	87.04*± 0.98	55.19*± 0.98	+++ ve	+++ ve	++ ve
7	PS21	10.37*± 0.74	61.48*± 1.61	41.11*± 1.28	+ ve	++ ve	++ ve
8	PS29	12.22*± 0.64	57.41*± 0.37	33.33*± 1.28	+ ve	++ ve	++ ve
9	PS32	36.67*± 0.64	56.30*± 0.74	11.85*± 0.74	++ ve	++ ve	+ ve
10	PS33	32.59*± 0.74	60.74*± 0.98	15.93*± 0.74	++ ve	++ ve	+ ve
11	PS35	65.56*± 0.64	51.11*± 0.64	13.33*± 1.28	++ ve	++ ve	+ ve
12	PS37	42.59*± 0.98	71.48*± 0.74	31.85*± 0.98	++ ve	+++ ve	++ ve
13	PS40	77.04*± 0.74	54.82*± 0.74	86.67*± 1.70	+++ ve	++ ve	+++ ve
14	PS42	49.26*± 0.74	52.22*± 1.28	21.85*± 0.98	++ ve	++ ve	+ ve
15	PS43	67.04*± 0.37	53.33*± 1.28	53.33*± 1.70	++ ve	++ ve	++ ve
16	PS45	32.96*± 0.37	50.00*± 1.70	64.07*± 1.61	++ ve	++ ve	++ ve
17	PU1	51.85*± 0.98	56.67*± 1.11	77.04*± 0.74	++ ve	++ ve	+++ ve
18	PU2	67.04*± 0.98	94.44*± 3.21	21.11*± 0.64	++ ve	+++ ve	+ ve
19	PA1	11.85*± 0.74	52.22*± 1.11	75.56*± 1.28	+ ve	++ ve	+++ ve
20	PA5	37.04*± 0.98	60.74*± 0.98	34.07*± 0.74	++ ve	++ ve	++ ve
21	PCH2	66.30*± 0.37	75.56*± 1.28	13.33*± 1.28	++ ve	+++ ve	+ ve
22	PCH3	60.37*± 0.74	65.19*± 0.98	26.30*± 0.98	++ ve	++ ve	+ ve
23	PCH5	11.85*± 0.74	52.59*± 1.61	42.59*± 0.98	+ ve	++ ve	++ ve
24	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00			
	SE (d)	1.11	1.69	1.61			
	$C.D_{(0.05)}$	2.23	3.42	3.24			

Key words: + <30 %, ++ 30-70 %, +++ >70 %, *Significant at 5% level of significance as compared to Control

Table 4. Performance of fluorescent *Pseudomonas* isolates towards different biochemical tests (Gram's staining, catalase test, KOH test, starch hydrolysis, and ammonification test), along with evaluation for biocontrol traits including HCN production, phosphate solubilization, and siderophore production.

S. No.	*Pseudomonas* isolate code	Biochemical Tests					Biocontrol Traits		
		Gram's straining	Catalase test	KOH test	Starch hydrolysis	Ammonification test	HCN Production	Phosphate Solubilisation	Siderophore Production
1	PS1	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
2	PS2	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	PB1	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4	PS10	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
5	PS14	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
6	PB4	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7	PS21	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
8	PS29	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
9	PS32	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
10	PS33	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
11	PS35	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve
12	PS37	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
13	PS40	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
14	PS42	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
15	PS43	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
16	PS45	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
17	PU1	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
18	PU2	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
19	PA1	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
20	PA5	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
21	PCH2	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
22	PCH3	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
23	PCH5	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

+ = Positive reaction, - = Negative reaction

Table 5. Evaluation of *Pseudomonas* isolates applied through seed biopriming for wilt disease suppression in tomato caused by *Fusarium oxysporium* f. sp. *lycopersici* under glass house conditions

S. No.	Treatments	Germination (%)	PDI (%)		PDR (%)
			30 DAS	*60 DAS	
1	T1	100.00*± 0.00	0.00± 0.00	0.00± 0.00	100.00*± 0.00
2	T2	53.33*± 3.33	60.00*± 5.57	100.00*± 0.00	0.00± 0.00
3	T3	76.67*± 3.33	20.00*± 0.00	30.00*± 0.00	70.00*± 0.00
4	T4	83.33*± 3.33	23.33*± 3.33	26.67*± 3.33	73.33*± 3.33
5	T5	93.33*± 3.33	06.67*± 3.33	10.00*± 0.00	90.00*± 0.00
6	T6	70.00*± 0.00	16.67*± 3.33	33.33*± 3.33	66.67*± 3.33
7	T7	86.67*± 3.33	13.33*± 6.66	16.67*± 3.33	83.33*± 3.33
SE(d)		3.98	5.63	3.09	3.09
$C.D_{(0.05)}$		8.63	12.20	6.68	6.68

Where, PDI = Percent Disease Incidence, PDR = Percent Disease Reduction over Negative control
*Significant at 5% level of significance

DAS=Days after sowing

*60 DAS= All plants in T2 (Negative control) wilted/died

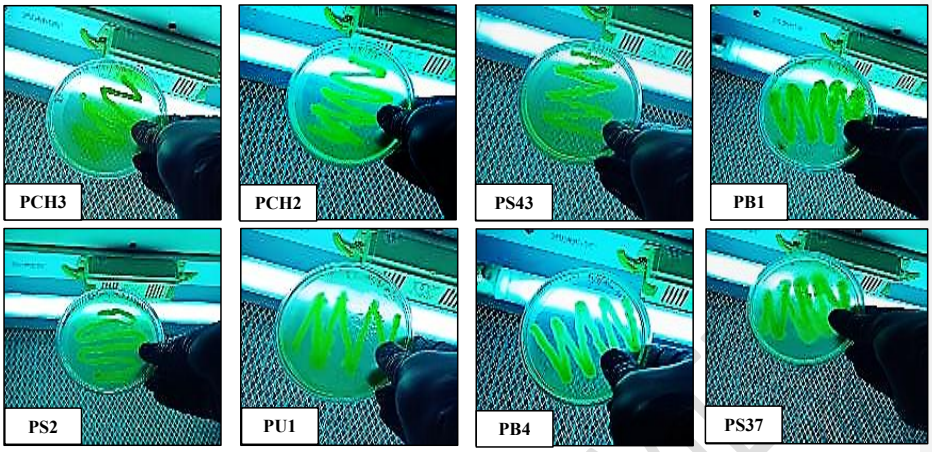

Fig. 1 Bacterial isolates exhibiting green fluorescence under UV light on King's B medium

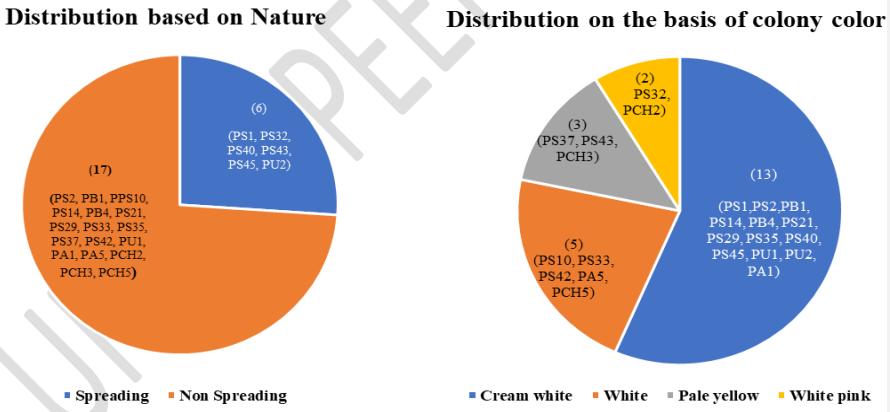

Fig. 2a Morphological characteristics of *Pseudomonas* isolates

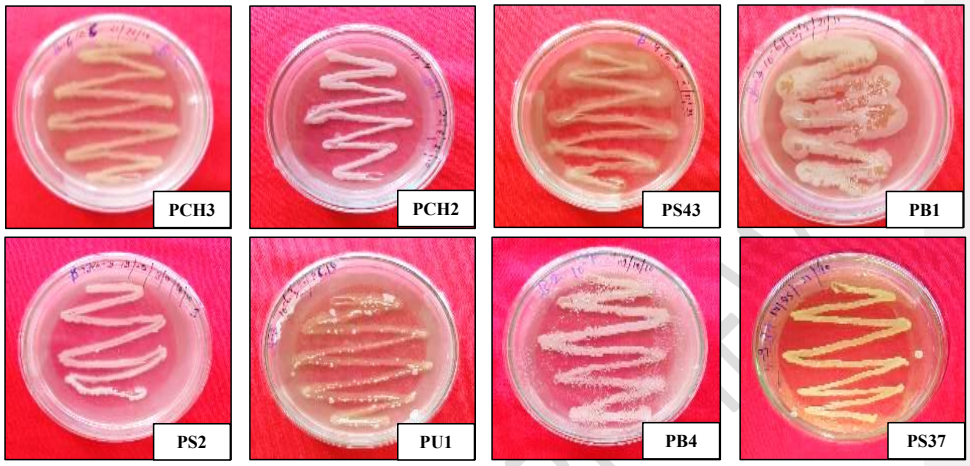

Fig. 2b Pure culture of *Pseudomonas* isolates producing slimy, cream white, white, pale yellow, and white pink colonies on King's B medium.

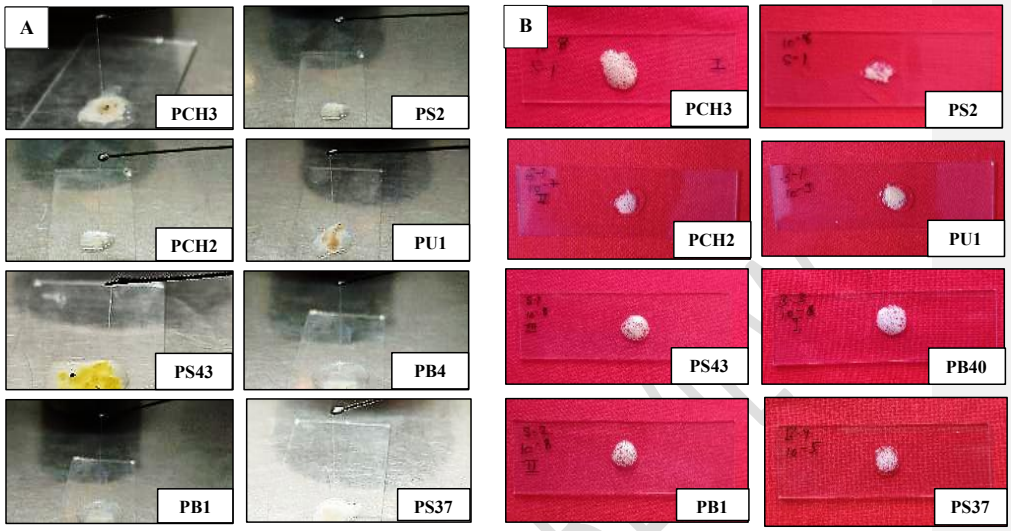

Fig. 3 Biochemical characterization of fluorescent *Pseudomonas* isolates (A) KOH test (B) Catalase test

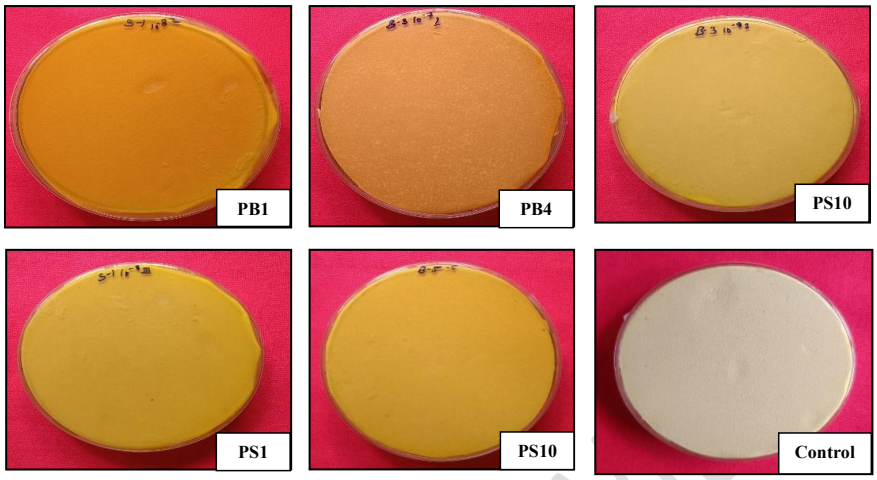

Fig. 4 Evaluation of fluorescent *Pseudomonas* isolates for HCN production. Change of white coloured filter paper from yellow to orange indicates positive reaction.

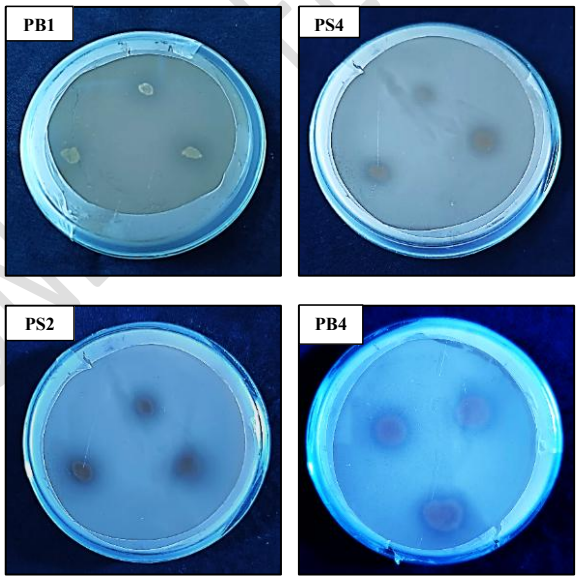

Fig. 5 Evaluation of fluorescent *Pseudomonas* isolates for phosphate solubilization on Pikovskaya's agar medium. Phosphate solubilizers were screened with a clear zone of solubilization around the bacterial growth.