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Bio-efficacy of *Pseudomonas fluorescens* isolates against foliar and soil borne pathogens

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Abstract
This study assessed the antagonistic activity of five *Pseudomonas fluorescens* isolates (PF1-PF5) against key foliar and soil-borne pathogens, including *Fusarium oxysporum* f. sp. *ciceri*, *Colletotrichum capsici*, *Alternaria alternata*, and *Rhizoctonia bataticola*, using the dual culture method. The isolates showed significant variation in their ability to suppress pathogen growth. Among them, PF4 was most effective against *F. oxysporum f. sp. ciceri*, followed by PF5 and PF3, achieving inhibition rates of 70.96% and 70.67%, respectively. *For R. bataticola*, PF4 again showed the highest inhibition (72.50%), followed by PF5 (70.00%). *Against C. capsici*, PF4 recorded the greatest suppression (72.50%), with PF5 close behind at 69.48%. In the case of *A. alternata*, PF5 was the most effective, inhibiting up to 73.38% of pathogen growth. Overall, all *P. fluorescens* isolates significantly outperformed the control, with isolates PF1 through PF5 demonstrating strong and consistent biocontrol activity. These outcomes align with previous research supporting the role of *P. fluorescens* in biological disease management, attributed to mechanisms like antifungal compound production, competitive exclusion, and rhizosphere colonization. The findings underscore the potential of isolates PF4 and PF5 as promising biocontrol agents for managing diseases such as anthracnose, leaf blight, wilt, and dry root rot in.

Keywords: *Pseudomonas fluorescens*, *Fusarium oxysporum* f. sp. *ciceri*, *Colletotrichum capsici*, *Rhizoctonia bataticola*, *Alternaria alternata*, dual culture, biocontrol

1. Introduction
The biological control of foliar and soil-borne plant pathogens by the presented microorganisms has been researched more than 80 years and rhizospheric microorganisms are perfect for use as biocontrol agents since the rhizosphere gives the bleeding edge resistance to root against assault by pathogens (Suprapta, 2012). The genus *Pseudomonas* comprises a wide range of ubiquitous metabolically versatile microorganisms found in diverse ecosystems, including water, soil, and the rhizosphere. It is a common gram negative, rod-shaped bacterium. As its name implies, it secretes a soluble greenish fluorescent pigment called fluorescein, particularly under conditions of low iron availability. *Pseudomonas* possess many traits that make them well suited as biocontrol and growth-promoting agents. These include the ability to [1] grow rapidly *in vitro* and to be mass produced; [2] rapidly utilize seed and root exudates; [3] colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; [4] produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances); [5] compete aggressively with other microorganisms; and [6] adapt to environmental stresses. In addition, *Pseudomonas* are responsible for the natural suppressiveness of some foliar to soilborne pathogens. The biocontrol capacity of a microbe can result from production of antibiotic compounds, or enzymes capable of fungal cell wall lysis, depletion of iron from the rhizosphere, induced systemic resistance, and competition for niches with pathogens within the rhizosphere. From a biocontrol perspective, *Pseudomonas fluorescens* is the most important and extensively characterized species belonging to this genus.

2. Materials and Methods
The present investigations entitled "Bio-efficacy of *Pseudomonas fluorescens* isolates for management of foliar and soil borne pathogens" was conducted in Plant Pathology

Laboratory, College of Agriculture, Nagpur during the year 2024-2025. Five isolates of *Pseudomonas fluorescens* were obtained from different districts of Maharashtra (Amravati, Akola, Nagpur, Bhandara, and Buldhana) using the serial dilution method and confirmed by morphological and biochemical tests. Dual culture assays were conducted to evaluate the antagonistic potential of these *Pseudomonas fluorescens* isolates against four pathogens (*F. oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola*, *Colletotrichum capsici and Alternaria alternata.*

2.1 Dual culture of *Pseudomonas fluorescens* against foliar and soil borne pathogens

Mycelium disc of 5 mm of the pathogens were cut and placed at the centre of Petri plates containing a combined medium of King's B and Potato Dextrose Agar (PDA). A square of 2 cm is drawn around the mycelium disc using a bacteria inoculating needle containing a loop of freshly cultured *Pseudomonas fluorescens*. This procedure is repeated with all the five isolates of *Pseudomonas fluorescens* against each of the pathogen. The plates were then incubated at 28°C for three to seven days. A control plate, inoculated with the pathogen alone in the absence of any antagonistic bacteria was also maintained. All treatments, including the control, were performed in four replications. After the incubation period, the percent inhibition of radial growth of the pathogen by *P. fluorescens* isolates, in comparison to the control was calculated using the formula described by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

Where,

- I = Percentage inhibition over control
- C = Mycelial growth in control (mm)
- T = Mycelial growth in treatment (mm)

3. Results and Discussions

An experiment was conducted to identify efficient *P. fluorescens* isolates against foliar and soil borne plant pathogens using combined media (CM) plates through the dual culture technique. The results of the experiments were presented here under

3.1 Dual culture of *Pseudomonas fluorescens* against *F. oxysporum* f. sp. *Cicero:* The data presented in Table 1, Plate 1 and Fig. 1 indicated that there was a significant difference among the five *P. fluorescens* isolates in inhibiting the growth of *Fusarium oxysporum* f. sp. *ciceri.* At 3 days after inoculation (DAI), isolate PF4 recorded the least mycelial growth of the pathogen (13.75 mm), followed by PF5 (14.00 mm) and PF3 (14.75 mm). At 5 DAI, T_4 again showed the lowest pathogen growth (18.75 mm), followed by PF5 (19.00 mm) and PF3 (19.75 mm). Similarly, at 7 DAI, PF4, PF5, and PF3 maintained the lowest growth levels of the pathogen at 24.75 mm, 25 mm, and 26.75 mm, respectively. The highest growth of *F. oxysporum* f. sp. *ciceri* was observed in the untreated control. Among all the isolates tested, PF4 demonstrated the highest percentage inhibition of the pathogen, followed by PF5 and PF3, with inhibition rates of 70.96%, 70.67%, and 68.62%, respectively.

The present investigation is in agreement with the findings of Pandey *et al.*, (2017) [18] that the antagonistic activity of (Pf 18, Pf 4, Pf 20, Pf 19, Pf13, and Pf 14) was reduced by (80.1, 79.8, 76.4, 73, 72.6, and 70.3) per cent, respectively, as compared to the control.

Table 1: Antagonistic effect of *Pseudomonas fluorescens* isolates against *F. oxysporum* f. sp. *ciceri* by dual culture method

Isolates	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *F. oxysporum* f. sp. *ciceri* (mm)	Percent inhibition over control (%)	Mycelial growth of *F. oxysporum* f. sp. *ciceri* (mm)	Percent inhibition over control (%)	Mycelial growth of *F. oxysporum* f. sp. *ciceri* (mm)	Percent inhibition over control (%)
PF1	15.50	56.94	22.75	60.26	29.75	65.10
PF2	19.50	45.83	30.50	46.72	37.75	55.72
PF3	14.75	59.02	19.75	65.50	26.75	68.62
PF4	13.75	61.80	18.75	67.24	24.75	70.96
PF5	14.00	61.11	19.00	66.81	25.00	70.67
Control	36.00		57.25		85.25	
F test	Sig		Sig		Sig	
SE (m ±)	0.52		0.53		0.46	
CD (P = 0.01)	2.12		2.14		1.90	

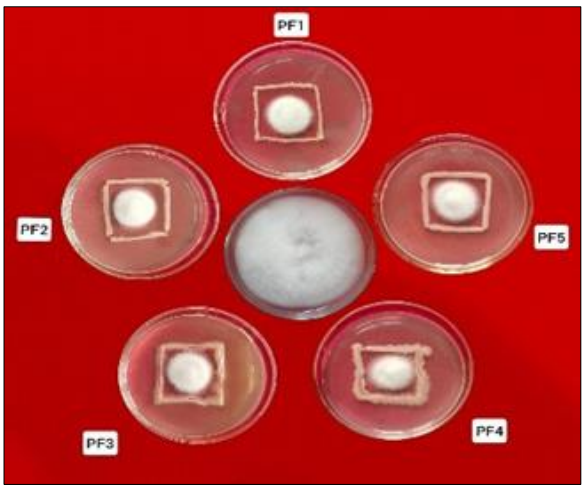

Plate 1: Efficacy of isolates of *Pseudomonas fluorescens* against *F. oxysporum* f. sp. *ciceri*

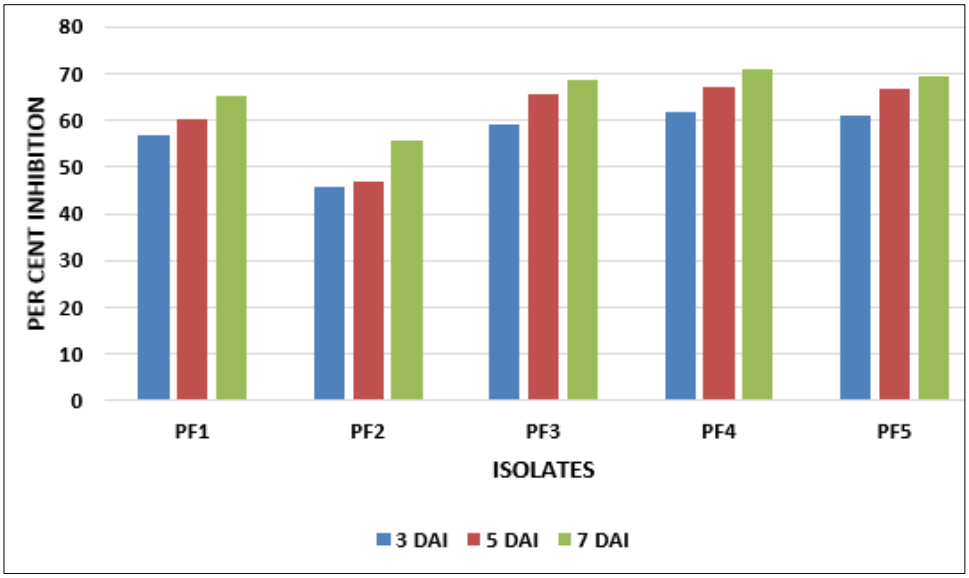

Fig 1: Per cent growth inhibition of *F. oxysporum* f. sp. *ciceri* by isolates of *P. fluorescens*

3.2 Dual culture of *Pseudomonas fluorescens* against *Rhizoctonia bataticola*

The data presented in Table 2, Plate 2 and Fig 2 reveal a significant variation among the five *Pseudomonas fluorescens* isolates in suppressing the growth of *Rhizoctonia bataticola*. At 3 days after inoculation (DAI), the lowest mycelial growth was recorded in treatment PF4 (13.75 mm), followed closely by PF5 (16.50 mm) and PF3, PF1 (16.75 mm). At 5 DAI, *R. bataticola* growth remained lowest in PF4 (16.25 mm), followed by PF5 (19.25 mm) and PF1 (19.75 mm). Similarly, at 7 DAI, treatment PF4 continued to be the most effective, showing the least mycelial growth at 24.75 mm, while PF5 and PF1 recorded 27.00 mm and 27.75 mm, respectively. The control treatment exhibited the maximum fungal growth throughout the observation period. Among all *P. fluorescens* isolates, PF4 demonstrated the highest percentage inhibition of *R. bataticola* growth compared to the control, with 72.50%, followed by PF5 (70.00%) and T_5 (69.16%).These findings are consistent with those of Singh *et al.* (2014), who studied the isolation and characterization of biocontrol microbes for development of effective microbial consortia for managing *Rhizoctonia bataticola* root rot of cluster bean under hot arid climatic conditions and found that the most effective consortium, comprising *Trichoderma afroharzianum* 5F, *Pseudomonas fluorescens* 131B, *Bacillus licheniformis* 223B, and *Bacillus subtilis* 236B achieved a 76.5% disease control.

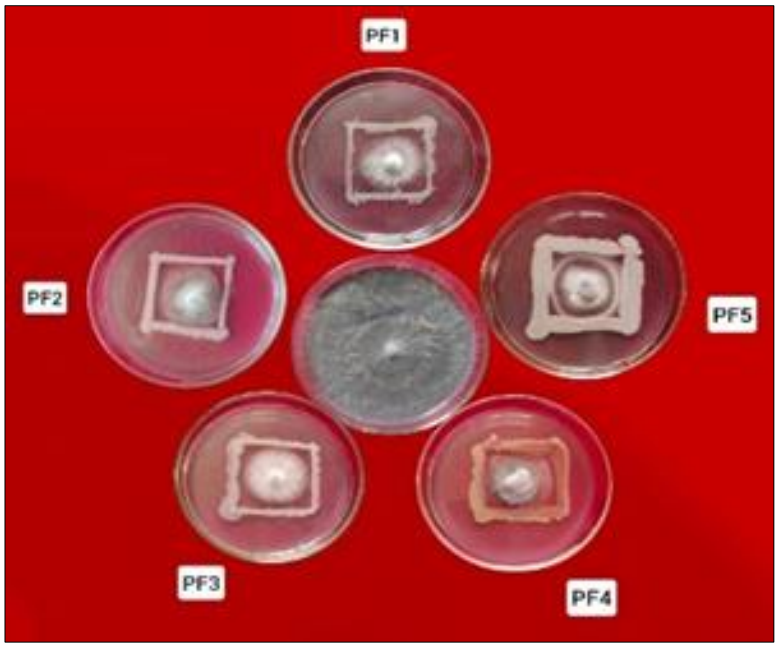

Plate 2: Efficacy of isolates of *Pseudomonas fluorescens* against *Rhizoctonia bataticola*

Table 2: Antagonistic effect of *Pseudomonas fluorescens* isolates against *Rhizoctonia bataticola* by dual culture method

Isolates	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *R. bataticola* (mm)	Percent inhibition over control (%)	Mycelial growth of *R. bataticola* (mm)	Percent inhibition over control (%)	Mycelial growth of *R. bataticola* (mm)	Percent inhibition over control (%)
PF1	16.75	53.14	19.75	64.57	27.75	69.16
PF2	20.25	43.35	24.25	56.50	32.25	64.16
PF3	16.75	53.14	20.75	53.63	28.75	68.05
PF4	13.75	61.53	16.75	62.78	24.75	72.50
PF5	16.50	54.22	19.25	65.47	27.00	70.00
Control	35.75		55.75		90.00	
F test	Sig		Sig		Sig	
SE (m ±)	0.45		0.48		0.42	
CD (P = 0.01)	1.84		1.95		1.73	

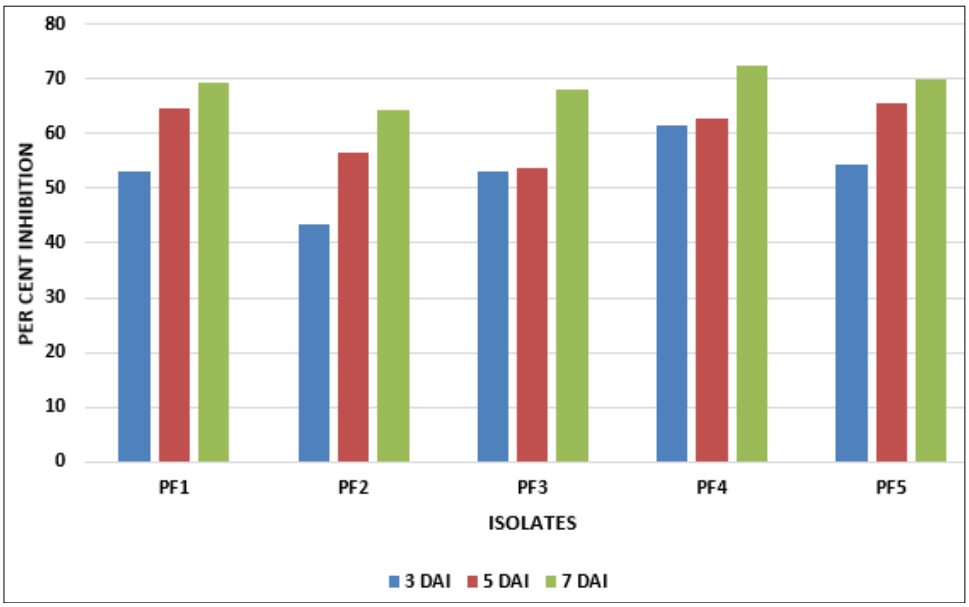

Fig 2: Percent growth inhibition of *Rhizoctonia bataticola* by isolates of *P. fluorescens*

3.3 Dual culture of *Pseudomonas fluorescens* against *Colletotrichum capsici*

The data presented in Table 3, Plate 3 and Fig 3 show a significant variation among the five *Pseudomonas fluorescens* isolates in suppressing the growth of *Colletotrichum capsici*. At 3 days after inoculation (DAI), the lowest mycelial growth was observed in treatment PF4 (12.75 mm), followed by PF5 and PF3 (13.00 mm). By 5 DAI, PF4 continued to show the least mycelial growth (15.75 mm), with PF5 (18.25 mm) and PF3 (20.00 mm) following. At 7 DAI, PF4 again recorded the minimum growth (22.75 mm), while PF5 and PF3 measured 25.25 mm and 27.00 mm, respectively. In contrast, the control treatment consistently exhibited the maximum mycelial growth throughout the evaluation period. Among all *P. fluorescens* isolates, PF4 demonstrated the highest percentage of growth inhibition against *C. capsici* (72.50%), followed by PF5 (69.48%) and PF3 (67.37%).

These findings are consistent with previous research of Charumathi *et al.* (2020) who evaluated 20 isolates of *Pseudomonas fluorescens* against *C. capsici* where Pf 1 native isolate was able to inhibit mycelial growth of the pathogen followed by Pf 11 and Pf 2. In dual culture assay, Pf 1 showed 93.41% inhibition whereas Pf 11 produced 76.81% inhibition of *C. capsici*.

Table 3: Antagonistic effect of *Pseudomonas fluorescens* isolates against *Colletotrichum capsici* by dual culture method

Isolates	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *C. capsici* (mm)	Percent inhibition over control (%)	Mycelial growth of *C. capsici* (mm)	Percent inhibition over control (%)	Mycelial growth of *C. capsici* (mm)	Percent inhibition over control (%)
PF1	18.50	46.76	25.50	54.66	33.00	60.12
PF2	15.75	54.67	22.75	59.55	29.75	64.05
PF3	13.00	62.58	20.00	64.44	27.00	67.37
PF4	12.75	63.30	15.75	72.00	22.75	72.50
PF5	13.00	62.58	18.25	67.55	25.25	69.48
Control	34.75		56.25		82.75	
F test	Sig		Sig		Sig	
SE (m ±)	0.43		0.47		0.45	
CD (P = 0.01)	1.75		1.92		1.86	

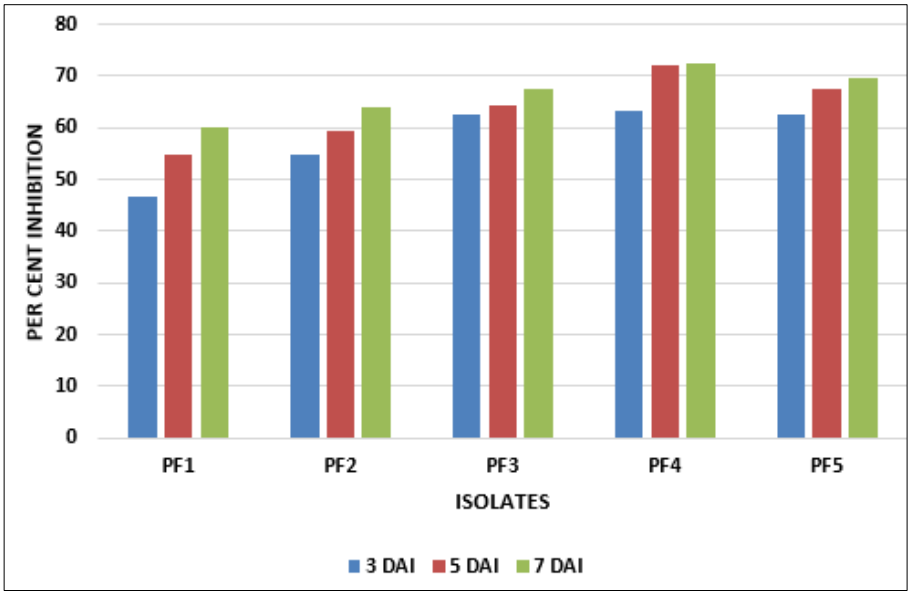

Fig 3: Percent growth inhibition of *C. capsici* by isolates of *P. fluorescens*

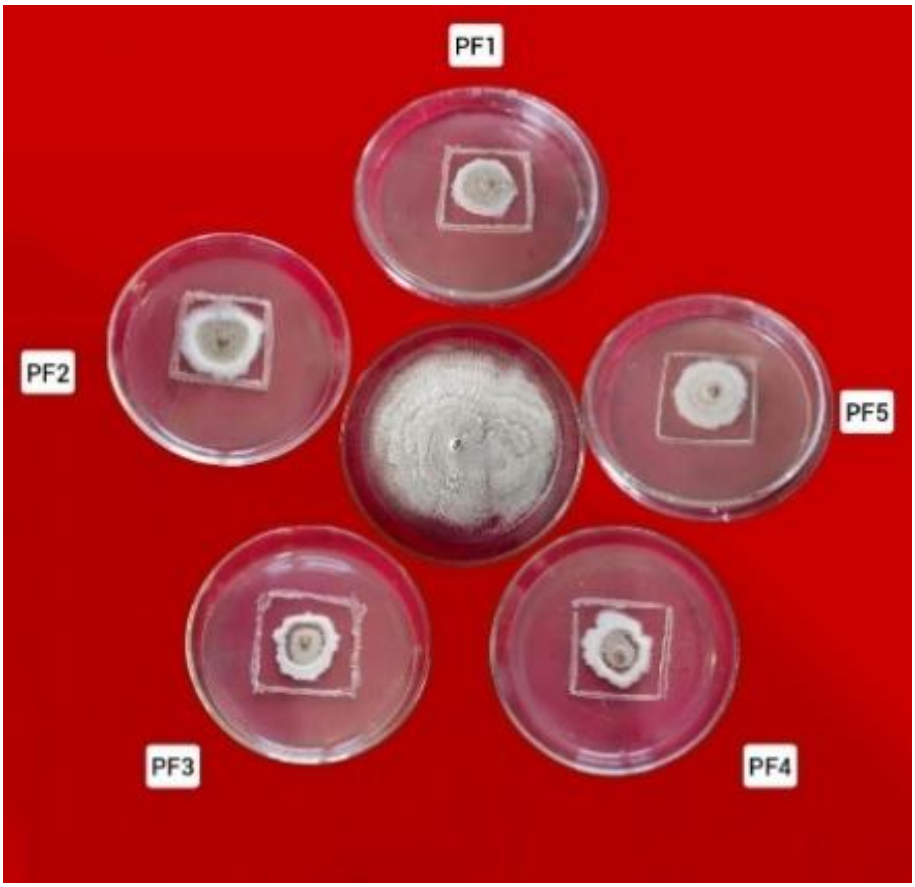

Plate 3: Efficacy of isolates of *Pseudomonas fluorescens* against *Colletotrichum capsici*

Dual culture of *Pseudomonas fluorescens* against *Alternaria alternata*

Table 4, Plate 4 and Fig 4 reveal that the five isolates of P. fluorescens significantly differ in their ability to suppress the growth of *Alternaria alternata*. At 3 days after inoculation (DAI), the lowest mycelial growth of *A. alternata* was observed in treatment PF4 (13.75 mm), followed by PF5(14.25 mm) and PF3 (15.50 mm). Similarly, at 5 DAI, PF4 again showed the least growth (17.75 mm), with PF5 (19.00 mm) and PF3 (20.50 mm) following. By 7 DAI, *A. alternata* growth remained lowest in PF4 (23.75 mm), followed by PF5 (26.25 mm) and PF3 (27.50 mm). The control treatment consistently showed the highest fungal growth. Among the isolates, PF4 demonstrated the greatest inhibition percentage against *A. alternata*, achieving 73.38%, followed by PF5 (70.59%) and PF3 (69.18%). These findings align with previous research of Maurya *et al.*-*In vitro* evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen where *Pseudomonas fluorescens* strains Pf 07 were found most effective and shown maximum inhibition of mycelial growth of *Alternaria alternata* (48.13%).

Table 4: Antagonistic effect of *Pseudomonas fluorescens* isolates against *Alternaria alternata* by dual culture method

Isolates	3 DAI		5 DAI		7 DAI	
	Mycelial growth of *A. alternata* (mm)	Percent inhibition over control (%)	Mycelial growth of *A. alternata* (mm)	Percent inhibition over control (%)	Mycelial growth *A. alternata* (mm)	Percent inhibition over control (%)
PF1	17.00	54.36	22.00	62.23	29.00	67.50
PF2	20.00	46.30	26.25	54.93	33.25	62.74
PF3	15.50	58.38	20.50	64.80	27.50	69.18
PF4	13.75	63.08	17.75	69.53	23.75	73.38
PF5	14.25	61.74	19.00	67.38	26.25	70.59
Control	37.25		58.25		89.25	
F test	Sig		Sig		Sig	
SE (m ±)	0.46		0.49		0.44	
CD (P = 0.01)	1.87		1.99		1.79	

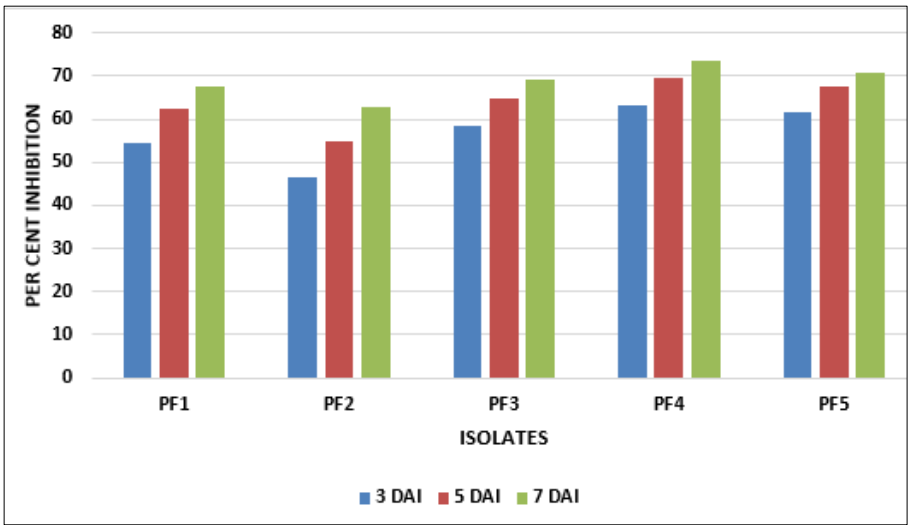

Fig 4: Percent growth inhibition of *A. alternata* by isolates of *P. fluorescens*

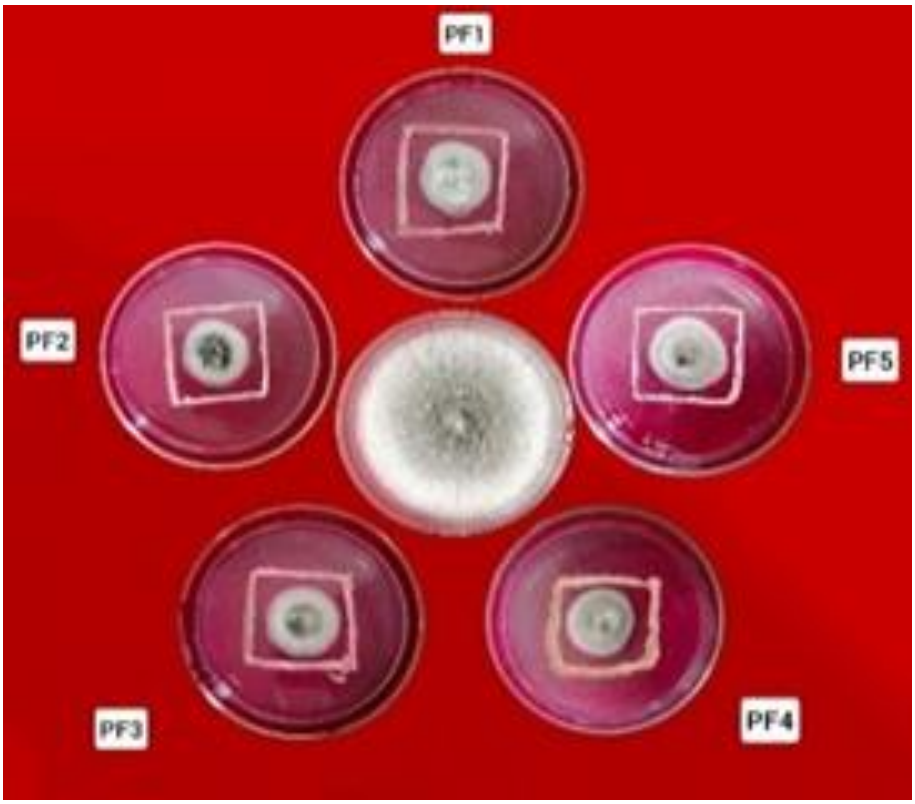

Plate 4: Efficacy of isolates of *P. fluorescens* against *Alternaria alternata*

Conclusion

Pseudomonas fluorescens isolate PF4 exhibited significant antagonism against all the four pathogens followed by the isolate PF5. The consistent inhibition across pathogens confirms *Pseudomonas fluorescens* to be an effective biocontrol agent against a range of foliar and soil-borne pathogens. Its antagonistic activity is attributed to the production of various bioactive compounds and its ability to colonize the rhizosphere and phyllosphere, effectively.

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