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Antagonistic ability of fluorescent pseudomonads from Konkan soils

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Abstract
Six bacterial isolates were obtained from rhizosphere of brinjal, papaya, tomato, okra and Chilli from different pockets of Konkan region. The results of cultural and biochemical tests -Gram staining, KOH test, Catalase test, Starch Hydrolysis, Gas formation test and Methyl red test revealed that all the six isolates were negative to Gram stain and Methyl red test and positive to other tests. In case of H_2S production test the two isolates *viz.*, Pf4 and Pf6 showed positive results while others showed negative results. All the isolates exhibited yellow colony pigmentation and emitted bluish green fluorescens on exposure to UV radiation. On the basis of these results, the isolates were identified as strains of *P. fluorescens*. The bio-control ability of all the strains was evaluated against three fungal – *Sclrerotia*, *Rhizoctonia*, *Fusarium* and one bacterial plant pathogen -*Ralstonia solanacearum*. The results revealed that the Pf1 strain isolated from brinjal rhizosphere was the most promising against *Fusarium*, *Rhizoctonia* and *Ralstonia* while Pf4 isolated from okra field was effective against *Sclerotium*.

Keywords: *Pseudomonas fluorescens*, *Fusarium*, *Rhizoctonia*, *Sclerotium*, antagonism, Konkan etc.

Introduction
In present era of eco-friendly crop protection, researchers are focusing on use of potential bio-control agents, botanicals and other such practices which will not only reduce the population of harmful pathogens but also will help to avoid hazardous effect of chemicals in crop ecology. So also, the present-day consumers are inclined to use pesticide-free, safer foods. The fluorescent pseudomonads are capable to produce phytohormones, siderophore and also involved in nitrogen fixation and phosphate solubilization. The anti-fungal metabolite 2,4-diacetyl phloroglucinol is crucial to *P. fluorescens*' capacity for biocontrol (Delany *et al*.,2000) [2]. Hence, exploration of location specific antagonistic strains of *P. fluorescens* would be worthwhile to combat against soil borne pathogens.

Materials and Methods
Isolation and identification of bacterial strains
The method proposed by Vlassak *et al*., (1992) [18] was followed for isolation of soil borne bacteria. The solution of each soil sample was diluted within the range of 10−1 to 10−7 dilution. However, the dilutions within 10-5 to 10-7 were selected for isolation. A quantity of 0.1 ml of each of these dilutions was spread on Kings B agar medium in sterilized Petri plates. The Petri plates were then incubated at room temperature (28°C ± 2°C) for 24-72 hours. Each dilution was maintained in three replicates. The plates were monitored for three days. The colonies with yellow pigmentation and fluorescence under ultraviolet light were selected and streaked on NA medium. The isolates were designated as Pf1, Pf2, Pf3, etc. Gram staining and biochemical tests such as KOH solubility test, Catalase test, Starch Hydrolysis, Gas production from glucose, Hydrogen Sulphide (H2S) and Methyl Red test were performed for identification of the isolates.

In vitro efficacy of bacterial isolates against soil borne fungi (*Fusarium, Rhizoctonia Sclerotium and Ralstonia*)
The six bacterial strains were tested against three soil borne fungi *viz*. *Rhizoctonia bataticola*, *Fusarium oxysporum* f. sp *lycopersici* and *Sclerotium rolfsii* using the dual culture technique and against *Ralstonia solanacearum* by paper disc method. Each strain served as a treatment. Pf1, Pf2, Pf3, Pf4, Pf5 and Pf6 strains served as treatments T1, T2, T3, T4, T5 and T6 respectively. Three replications were maintained per treatment. Percent mycelium inhibition was calculated using

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

Where I = Percent inhibition. C = Radial growth of test pathogen (mm) in control, T = Radial growth of test pathogen (mm) in treatment

Experimental Results

Table 1: Bacterial strains isolated from selected localities of Konkan region.

Sr. No.	Place of isolation	Crop	Isolate code
1.	CES, Wakawali	Brinjal	Pf1
2.	Kelve, Palghar	Papaya	Pf2
3.	Dapoli, Ratnagiri	Tomato	Pf3
4.	Mithbav, Sindhudurg	Okra	Pf4
5.	Javade, Ratnagiri	Chilli	Pf5
6.	Bhilawadi, Raigad	Brinjal	Pf6

Table 2: Confirmatory tests for Pseudomonas fluorescens isolates

Test	Isolate					
	Pf1	Pf2	Pf3	Pf4	Pf5	Pf6
Gram staining	-ve	-ve	-ve	-ve	-ve	-ve
Fluorescence production	+ve	+ve	+ve	+ve	+ve	+ve
KOH test	+ve	+ve	+ve	+ve	+ve	+ve
Catalase test	+ve	+ve	+ve	+ve	+ve	+ve
Starch Hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve
H_2S production	-ve	+ve	-ve	+ve	-ve	-ve
Gas Production from glucose	+ve	+ve	+ve	+ve	+ve	+ve
Methyl Red	-ve	-ve	-ve	-ve	-ve	-ve

The negative reaction of all the six isolates to Gram staining test indicated that they are Gram negative bacteria. The findings showed that every test isolate responded positively to the KOH solubility test by forming the strands of viscid material. All the 6 isolates obtained in present study showed greenish-blue fluorescence production under UV light. When a few drops of 3 percent hydrogen peroxide were applied to the slides smeared with each bacterial culture, gas bubbles were formed. All the isolates produced clear golden zone around the colonies on starch medium when stained with Lugol's iodine after 7 days of incubation at 28±2ºc. Thus, bacteria showed positive reaction to the test. It was found that out of 6 bacterial isolates obtained from local soils only 2 isolates, Pf2 and Pf4 produced gas which reacted with lead acetate strips to form a precipitate at the tips of the strips indicating release of H2S gas which depicted positive reaction. Other isolates did not produce the gas and therefore the subsequent reactions were not observed. Air bubbles in the inverted Durham's tube indicated gas production when bacteria were cultured in nutrient broth containing 2% glucose. All the isolated strains generated gas from glucose and showed positive reaction. All the isolates retained normal colour of medium (yellow) after 4 days of incubation indicating alkalinity and thus showed negative reaction. On the basis of the results of all the above tests it can be concluded that the isolates obtained from soil samples from different pockets of Konkan region were the strains of P. fluorescens.

Table 3: Antagonistic effect of different strains of Pf on radial mycelium growth and percent mycelium inhibition of the different pathogens

Treatments	Control (Radial mycelium growth)	Pf1		Pf2		Pf3		Pf4		Pf5		Pf6		Mean	
		*Radial mycelium growth	percent mycelium inhibition	Radial mycelium growth	percent mycelium inhibition	Radial mycelium growth	percent mycelium inhibition	Radial mycelium growth	percent mycelium inhibition	Radial mycelium growth	percent mycelium inhibition	Radial mycelium growth	percent mycelium inhibition	Radial mycelium growth	percent mycelium inhibition
Sclerotium rolfsii	90.00	39.00	56.67	42.50	52.78	40.00	55.56	38.00	57.78	47.00	47.78	42.00	53.33	48.36	46.26
Rhizoctonia bataticola	90.00	39.00	56.67	60.00	33.33	42.50	52.78	52.50	41.67	39.50	56.11	47.50	47.22	53.00	41.11
Fusarium oxysporum f. sp. *lycopersici*	90.00	31.00	65.56	33.00	63.33	34.50	61.67	37.00	58.89	35.50	60.56	39.00	56.67	42.86	52.37
Mean	90.00	36.33	59.63	45.17	49.81	39.00	56.67	42.50	52.78	40.67	54.81	42.83	52.41		
	Pf		Pathogen		Interaction										
Sem	1.58		1.03		2.73										
CD@1%	5.97		3.91		10.34										

* Indicates mean of three replications in mm

It is apparent from data presented in Table no 3 that the strain Pf1 caused maximum reduction of radial growth (36.33 mm) of all the three test pathogens and statistically at par with Pf strain 3 (39.00 mm) and Pf strain 5 (40.67 mm) along with higher percent mycelial inhibition 59.63, 56.66 and 54.81 respectively. All these treatments were superiorly significant over the rest of treatments.

Radial growth of the pathogen *Fusarium oxysporum* f. sp.

lycopersici (42.86 mm) was very much restricted by the different strains of Pf than other pathogens and showing significantly superior are the rest of the treatments along with the higher per cent mycelium inhibition (52.37%). *Fusarium oxysporum* f. sp. *lycopersici* was followed by *Sclerotium rolfsii* with 48.36 mm radial mycelium growth diameter with 46.26% mycelium inhibition. The pathogen *Rhizoctonia bataticola* was showing highest radial mycelium growth (53.00%) and lowest per cent mycelium inhibition (41.11%). Interaction effects of different strains of Pf against different pathogens were found statistically significant. The combination of Pf1 strain was used against pathogen *Fusarium oxysporum* f. sp. *lycopersici* shows less radial mycelial growth (31 mm) and highest per cent inhibition (65.56%).

Table 4: *In vitro* efficacy of *P. fluorescens* isolates against *Ralstonia solanacearum*

Treatment	Isolate	Zone of inhibition(mm)*
T_1	Pf1	19.33
T_2	Pf2	11.33
T_3	Pf3	18.67
T_4	Pf4	9.33
T_5	Pf5	18.33
T_6	Pf6	9.67
T_7	Control	0.00
	S.Em. ±	0.31
	CD @ 1%	1.30

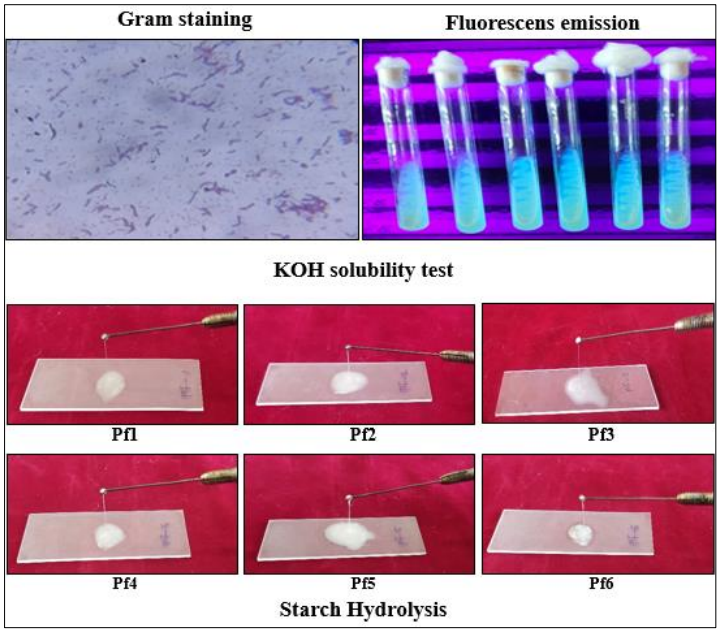
* Mean of three replications.

It is revealed from the data presented in Table 4 that the range of inhibition zone formed by *P. fluorescens* isolates against *R. solanacearum* was 9.33 to 19.33 mm. The *P. fluorescens* strain designated as Pf1 (T_1-19.33mm) was numerically superior to all the isolates but statistically at par with Pf3 (T_3-18.67mm) and Pf5 (T_5-18.33mm) while Pf6 (T_6-9.67mm) was at par with Pf4 (T_4-9.33mm) with least zone of inhibition.While Pf2 (T_2) portrayed very meagre (11.33mm) zone of inhibition.

Discussion

The results of Gram staining are in concurrence with results of Deshwal *et al*., 2013 [3]; Priyanka *et al*., 2017 [13]; Singh *et al*., 2017) [14]. The results obtained from KOH test are in congruence with results of Kipgen and Bora (2017) [7] and Vignesh *et al*. (2021) [16]. The results of fluorescent emission test are in agreement with those of Manasa *et al.* (2017) [9] and Meera and Balabaskar (2021) [16] while results of catalase test are in accordance with the findings of Manasa *et al*. 2017 [9]; Singh *et al*., 2017 [14] and Lamani and Kulkarni, 2019 [8]. The results obtained in the starch hydrolysis test are similar to Joseph *et al*. (2007) [4] and Manasa *et al*. (2017) [9]. The results obtained in the H2S production test are similar to those of Vinay *et al*. (2016) [17] and (Manasa *et al*., 2017) [9]. The results of methyl red test resemble to the findings of Kapali *et al*. (2016) [6] and Singh *et al*. (2017) [14].

The results obtained in antagonistic study of *P. fluorescens* strains against *F. oxysporum* are in close consonance with the results reported by Kandoliya *et al*. (2012) [5] and Vignesh *et al*. (2021) [16] while against *R. bataticola* are in agreement with Suman *et al*. (2015) [15] and Mezeal (2014) [19]. The results obtained in antagonistic study of *P. fluorescens* strains against *S. rolfsii* are in are in close proximity with those of Prasad *et al*. (2013) [12] and Avinash *et al*. (2015) [20] while that of against *R. solanacearum* are in close proximity with those of Basha *et al*. (2017) [1] and Perumal *et al*. (2021) [11].



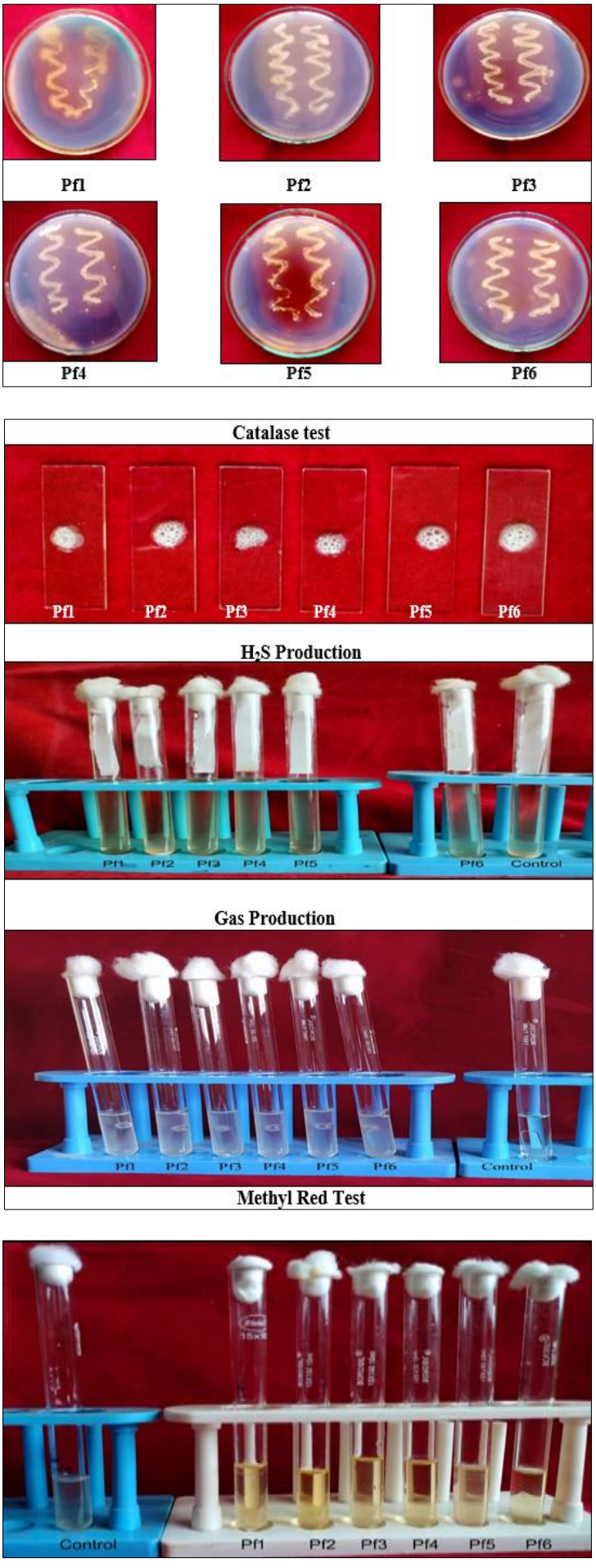


Plate I: Confirmatory tests for isolated *Pseudomonas fluorescens* strains

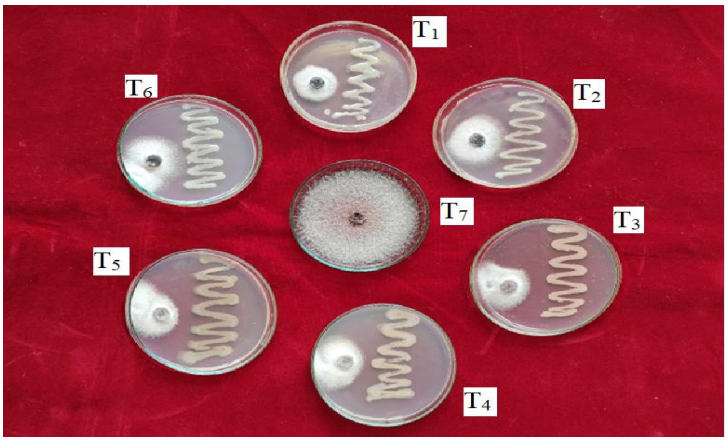


Plate II: *In vitro* efficacy of *Pseudomonas fluorescens* strains against *F. oxysporum* f. sp. *lycopersici*

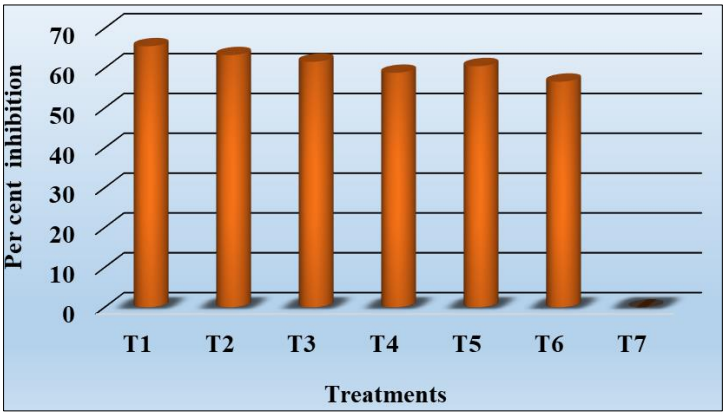


Fig 1: *In vitro* efficacy of *P. fluorescens* strains against *F. oxysporum* f. sp. *lycopersici*

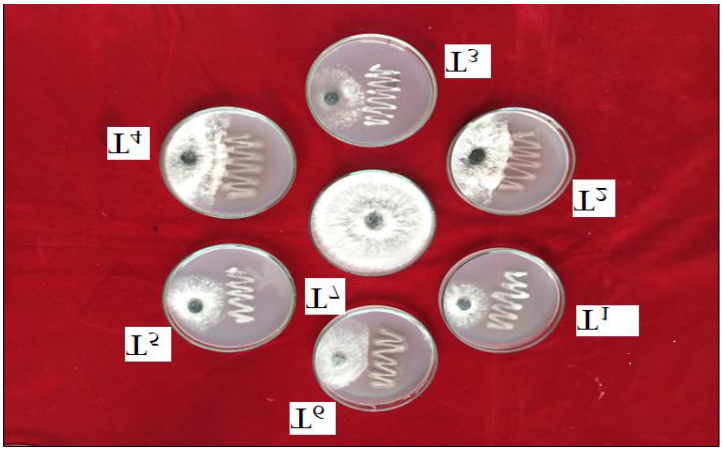


Plate III: *In vitro* efficacy of *Pseudomonas fluorescens* strains against *R. bataticola*

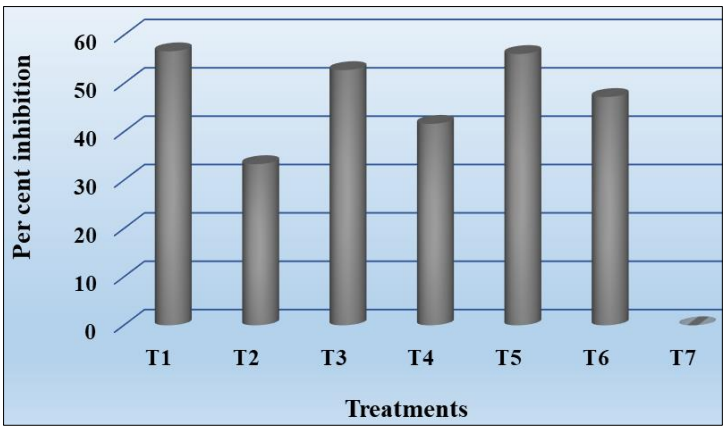


Fig 2: *In vitro* efficacy of *P. fluorescens* strains *against R. bataticola*

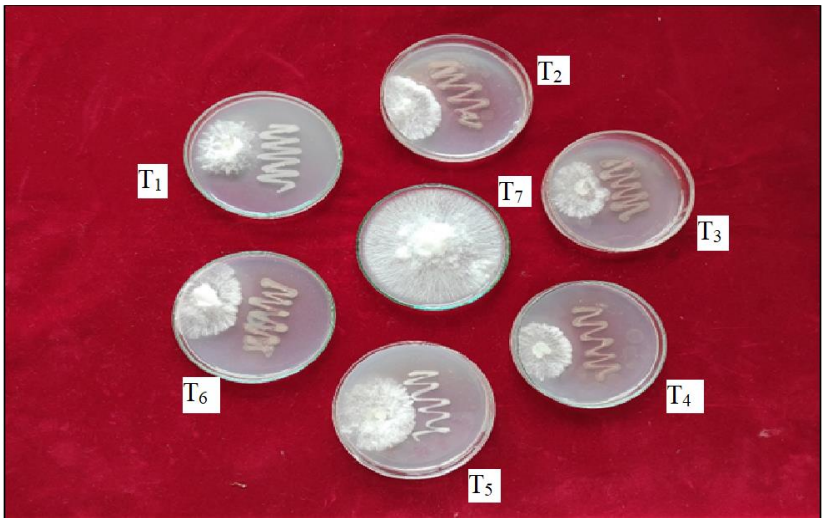


Plate IV: *In vitro* efficacy of *Pseudomonas fluorescens* strains against *S. rolfsii*

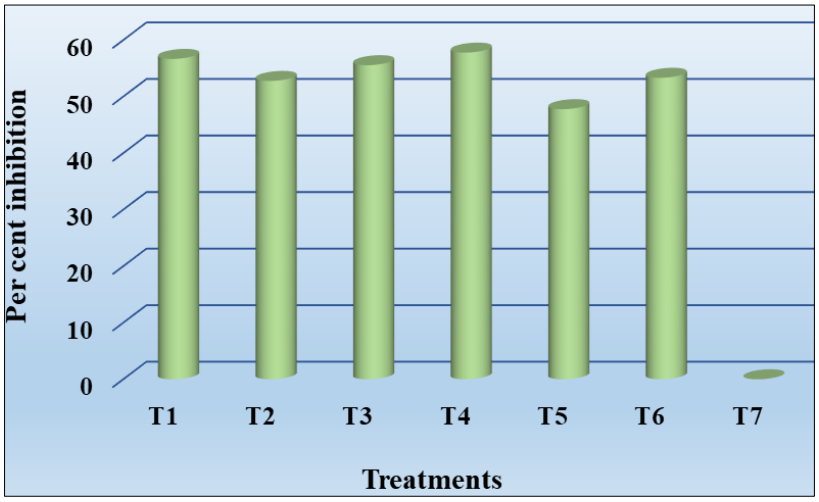


Fig 3: *In vitro* efficacy of *P. fluorescens* strains against *Sclerotium rolfsii*

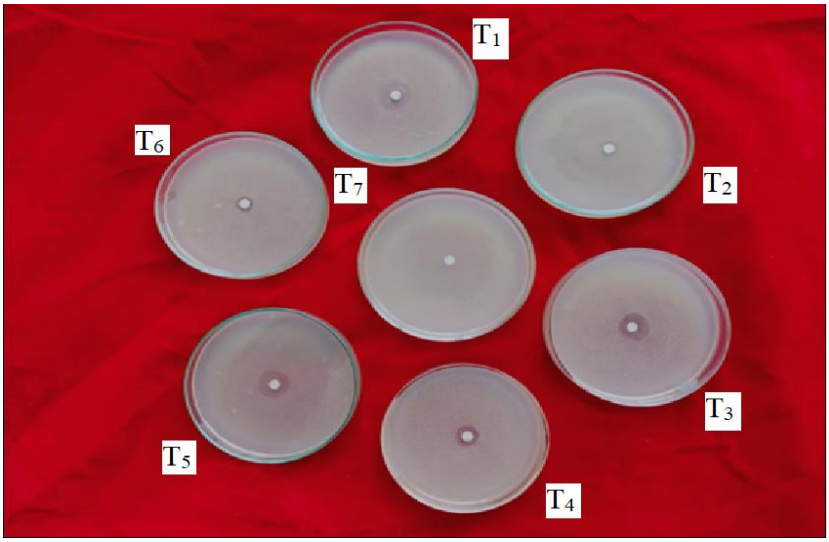


Plate V: *In vitro* efficacy of *Pseudomonas fluorescens* isolates against *Ralstonia solanacearum*

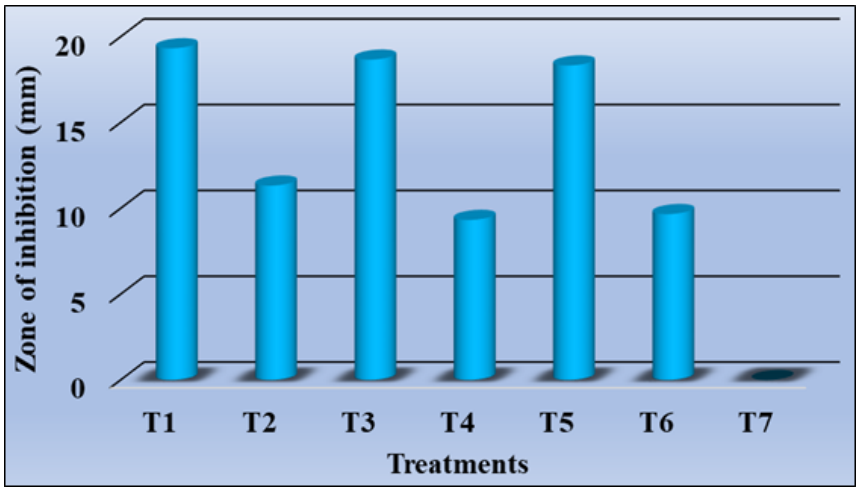


Fig 4: *In vitro* efficacy of *Pseudomonas fluorescens* isolates against *Ralstonia solanacearum*

Conclusion

The results of present study confirm that the local isolates of antagonists are more potent against the soil borne plant pathogens. This is due to the fact that local strains survive in the same ecological niche as that of the pathogen and therefore they are well acclimatized to the soil and environmental conditions. It is necessary to lay emphasis on isolation, augmentation and utilization of local antagonistic microbes to combat efficiently against soil borne pathogens in an eco-friendly manner.

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