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In vitro antagonistic activity of fluorescent *Pseudomonas* isolates against *Rhizoctonia solani* and *Sclerotium rolfsii*

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Abstract
Fluorescent *Pseudomonas* as a biocontrol agent offers a promising alternative to manage soil borne plant pathogens. However, the production of an antimicrobial compound varies among cultivars of the same species, and this has hampered the commercialization (Notz *et al*., 2001). Secondary metabolites produced by fluorescent Pseudomonas play key roles in the suppression of various soilborne plant pathogens. However, the performance of this biocontrol agent varies depending on the environment and host plant species. In this study, *In vitro* antagonistic activity against phytopathogens by fluorescens Pseudomonas of the 5 isolates of *Pseudomonas* showed different rates of growth inhibitions of *R. solani* and *S. rolfsii*, ranging from 39.995 to 65.275% and 34.995to 68.325% respectively. Based on percentage of inhibition radial growth values, the antagonistic isolates in decreasing order were P141> P200> P66> P229> P260 and P66> P200> P141> P229> P260 against *R. solani* and *S. rolfsii* respectively The isolates P141 and P66 were found to have the maximum inhibiting effect on the growth of *R. solani* (65.275%) and *S. rolfsii* (68.325%) respectively.

Keywords: fluorescent pseudomonas, rhizoctonia solani, sclerotium rolfsii, antagonistic activity, biocontrol agent, isolation

Introduction
Agriculture over the past few decades is heavily dependent on the application of chemical inputs. However, many chemical pesticides are very toxic and thus result in contamination of environment. Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Compant *et al.*, 2005; Welbaum *et al.*, 2004) [1]. The introduction of *P. fluorescens* as a biocontrol agent offers a promising alternative to manage soilborne plant pathogens. However, the production of an antimicrobial compound varies among cultivars of the same species, and this has hampered the commercialization (Notz *et al.*, 2001) [6]. The studies of the ability to produce antibiotic secondary metabolites and their plant growth promoting potential are important not only for understanding their ecological roles in the rhizosphere and their interaction with plants, but also for any biotechnological applications. Biological control of plant pathogens by antagonistic micro organisms is a potential non-chemical means (Harman, 1991) [3] and is known to be a cheap and effective eco-friendly method for the management of crop diseases (Cook and Baker, 1983) [2]. Pseudomonas fluorescens is adapted to survival in soil and colonization of plant roots (Kiely *et al.*, 2006) [5].

Material Methods
In vitro screening for antagonistic activity against soil borne pathogens
The five isolates were also tested for their efficacy as biocontrol agent against the phytopathogens *Sclerotium rolfsii* and *Rhizoctonia solani* isolated from rice. Equal volume of sterilized potato dextrose agar (PDA) and King's B medium was mixed and poured in sterilized petri dishes. A heavy inoculum from an actively growing fluorescent *Pseudomonas* was streaked at 1cm away from the edges of the plate and the mycelial disc of the pathogens were placed at the centre of petriplates. Control plates were inoculated only with phytopathogens but not with *Pseudomonas* isolates. Percent inhibition of pathogens by *Pseudomonas* isolates over control was calculated by using the formula of Vincent (1947):
[(Growth of pathogen in control - Growth of pathogen with *Pseudomonas* isolate)/ Growth of pathogen in control]x 100.

Results and Discussion
In vitro antagonistic potential of different isolates of *Pseudomonas* was studied against fungal

plant pathogens *Rhizoctonia solani* and *Sclerotium rolfsii*. following dual culture method was assessed after 5 days of growth. There were differences in the antagonistic abilities of isolates of *Pseudomonas* against both the plant pathogens. (Table 1 and Fig 1)

All of the 5 isolates of *Pseudomonas* showed different rates of growth inhibitions of *R. solani* and *S. rolfsii*, ranging from 39.995 to 65.275% and 34.995to 68.325% respectively. Based on percentage of inhibition radial growth values, the antagonistic isolates in decreasing order were P141> P200> P66> P229> P260 and P66> P200> P141> P229> P260 against *R. solani* and *S. rolfsii* respectively The isolates P141 and P66 were found to have the maximum inhibiting effect on the growth of *R. solani* (65.275%) and *S. rolfsii* (68.325%) respectively while isolate P260 and P260 showed the lowest inhibitory effect on *R. solani* (39.995%) and *S. rolfsii* (34.995%) respectively. The study revealed that different isolates have different capacities as biological weapons in inhibiting the pathogens, even though all were *Pseudomonas* isolates. Over all *Pseudomonas* isolates were found to be more efficient against *S. rolfsii* than *R. solani* indicating that these isolates could therefore be exploited as potential candidates for development of biopesticides. Tiwari (2005) [7] also reported that isolate, isolated were from the rhizosphere of rice were found effective as antagonists to the fungal species of *Pyricularia grisea* and *Rhizoctonia solani*.

Table 1: Confrontation assay between *R. solani* and *S. rolfsii* with fluorescent *Pseudomonas* isolates

Isolate	% inhibition *of R. solani*	% inhibition of *S. rolfsii*
P66	62.660^{a}±1.000	68.325^{a}±0.555
P141	65.275^{a}±0.835	48.885^{b}±1.115
P200	64.440^{a}±1.110	66.105^{a}±0.055
P229	49.440^{b}±2.780	39.995^{c}±1.115
P260	39.995^{c}±1.115	34.995^{d}±0.555
Max	65.275^{a}±0.835	68.325^{a}±0.555
Min	39.995^{c}±1.115	34.995^{d}±0.555
CV	3.871	2.268
CD0.01%	8.791	4.703
CD 0.05%	5.604	3.009
Fcal	52.539	332.746

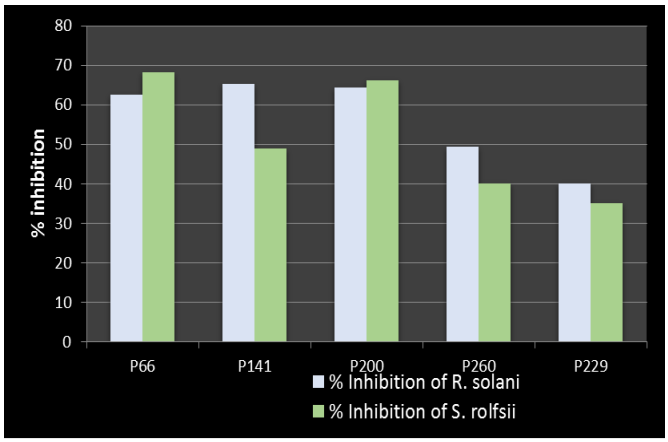


Fig 1: % Inhibition of fluorescent *pseudomonas* isolates against *R. solani* and *S. rolfsii*

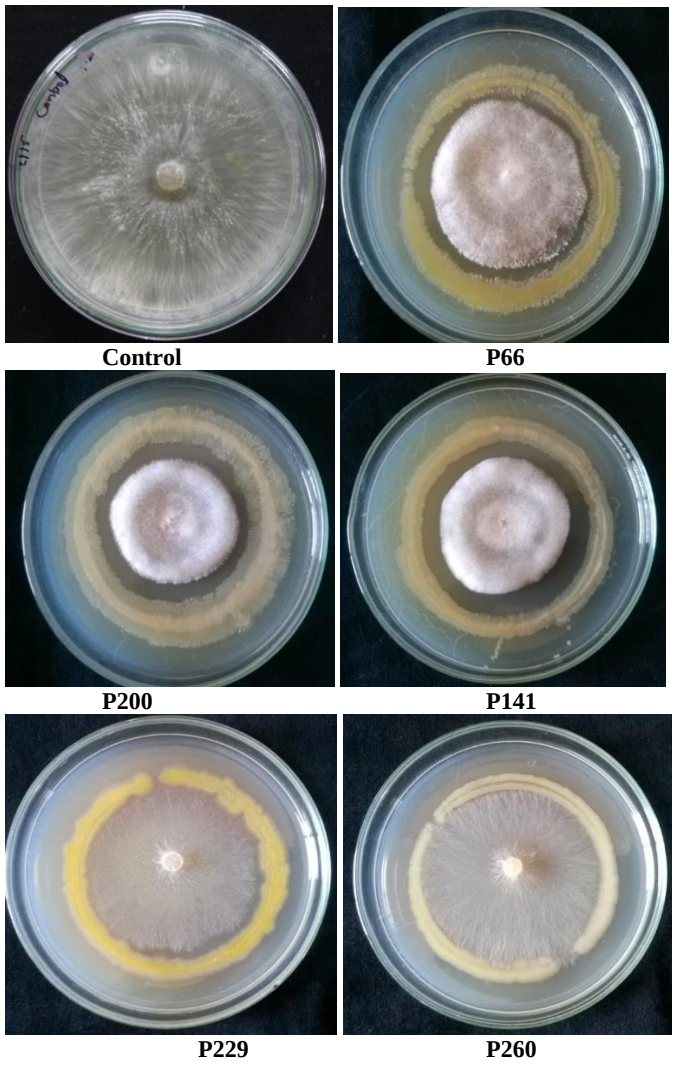
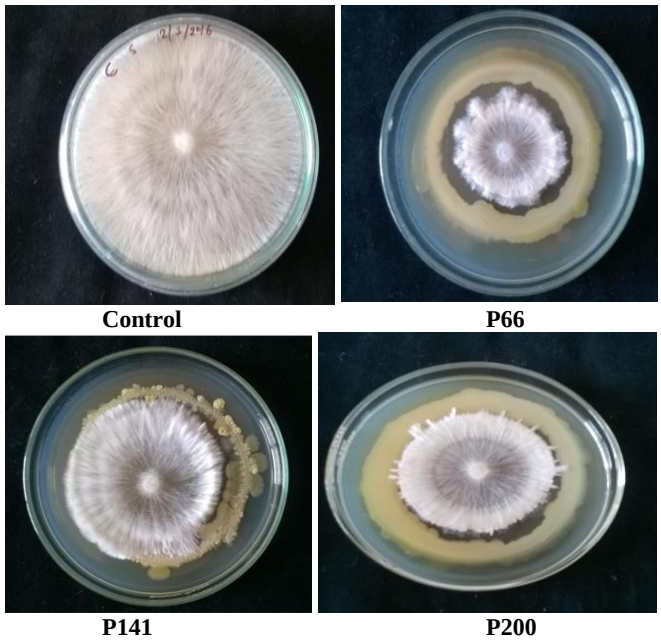


Plate 1: Confrontation plate assay between *Rhizoctonia solani and* fluorescent *Pseudomonas isolates*



P229 **P260**

Plate 2: Confrontation plate assay between *Sclerotium rolfsii and* fluorescent *Pseudomonas isolates*

Conclusion

All of the 5 isolates of *Pseudomonas* showed different rates of growth inhibitions of *R. solani* and *S. rolfsii*, ranging from 39.995 to 65.275% and 34.995to 68.325% respectively. Based on percentage of inhibition radial growth values, the antagonistic isolates in decreasing order were P141> P200> P66> P229> P260 and P66> P200> P141> P229> P260 against *R. solani* and *S. rolfsii* respectively The isolates P141 and P66 were found to have the maximum inhibiting effect on the growth of *R. solani* (65.275%) and *S. rolfsii* (68.325%) respectively.

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