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IDENTIFICATION OF ANTAGONISTIC ACTIVITY OF *PSEUDOMONAS* SP. ISOLATES AGAINST SOIL BORNE PLANT PATHOGENS *IN VITRO*

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KEYWORDS

F. oxysporum
S. rolfsii
A. flavus
Aspergillus niger

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ABSTRACT

The *Pseudomonas fluorescens* isolates were tested for identification of antagonistic property against the plant pathogens *viz*. *Aspergillus niger, A. flavus, Colletotrichum* sp., *Fusarium* sp., *Macrophomina phaseolina, Sclerotium rolfsii*, *Rhizoctonia* sp. *in-vitro*. the mycelial growth of all pathogens was significantly reduced by *P. fluorescens* isolate (Shiware) in dual culture over control. The antagonist showed maximum i.e. 60.37 per cent of mycelial growth inhibition of *F. oxysporum* followed by *A. niger* and *M. phaseolina*, where 58.15 and 52.22 per cent of growth inhibition was recorded, respectively. While, *R. solani, C. capsici* and *S. rolfsii* showed 44.82, 44.65 and 30.37 per cent growth inhibition, respectively. The another *P. fluorescens* isolate (Pimple) showed significant reduction in the radial diameter of all the pathogens over control in dual culture. The *A. niger* and *C. capsici* showed maximum extent of mycelial growth inhibition *i.e.* 52.78 and 52.15 per cent, respectively, followed by *F. oxysporum* f. sp. *ciceri* and *A. flavus* where 49.90 and 46.30 per cent of growth inhibition was recorded. While, *S. rolfsii* and *R. solani* showed 41.67 and 37.78 per cent growth inhibition, respectively.

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INTRODUCTION

The genus *Pseudomonas* is one of the best-studied bacterial groups and is found almost everywhere, in soil, water, plants and animals. In most cases it is non pathogenic and in fact can be beneficial in the biological control of soilborne plant pathogens and in the bioremediation of pollutants. *Pseudomonas* spp. received great attention as biocontrol agent because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites (Chin-A-Woeng *et al.,* 2001; Raaijmaker *et al.,* 2002). The mechanisms through which *Pseudomonas* spp. control plant diseases involve (i) competition for niches and nutrients, (ii) antibiosis, (iii) predation and (iv) induction of plant defence responses. (Rakh, *et al.,* 2011). Jayaswal *et al.,* (1990) investigated the effectiveness of *Pseudomonas* against *Macrophomina phaseolina, Fusarium, Helminthosporium, A. flavus* and *Penicillium*. Siddiqui *et al.,* (2005) studied the effectiveness of *Pseudomonas* against *Fusarium, Rhizoctonia, Sclerotium, Pythium* pathogens and reported the *Pseudomonas* isolates managed all soil borne pathogens.

Singh *et al*., (2011) evaluated for antifungal activity of *Pseudomonas* against *F. oxysporum*, *A. niger*, *A. flavus*, *A. alternata* and *Erysiphe cruciferarum*. They reported that *Fusarium* showed maximum extent of inhibition (51.76 %) followed by *A. niger* (50.14 %), and least by *E. cruciferarum* (22.27 %). Patel *et al.,* (2011) studied antagonistic effect of four strains of *Pseudomonas fluorescence viz*., BHUPf 4, BHUP 6, BHUP 5 and BHUPsb against *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum*. They found that all the antagonistic strains of *Pseudomonas* restricted the mycelial growth of *R. bataticola* and *S. sclerotiorum* significantly and among them the strain BHUPf4 showed significantly highest mycelial growth inhibition against both fungus. Rakh (2011) isolated 11 *Pseudomonas* spp., from rhizospheric soil, were evaluated for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium identified as, *Pseudomonas* cf. *monteilii* 9, showed highest antagonistic activity against the pathogen *S. rolfsii*. He reported, the *Pseudomonas* cf. *monteilii* 9 inhibited the *S. rolfsii* to up 94 % in terms of dry weight. Vanitha *et al.,* (2014) isolated ten different strains of *P. fluorescens* from coleus rhizosphere and were screened against *M. phaseolina* (Tassi) Goid, (the causal organism of coleus root rot) and indicated that among them Pf1 strains recorded maximum inhibition of mycelial growth against control.Toppo and Tiwari (2015) examined *Pseudomonas* spp. for their antagonistic effect on a fungal pathogen *Fusarium* spp., *Rhizoctonia* spp. and *Colletotrichum* spp. in *in-vitro* plate assay and *in-vivo* green house condition reported that PKJ25 was the most active isolate and significantly suppressed the vegetative growth of all the test fungi by restricting the hyphal growth of *Rhizoctonia* spp., *Fusarium* spp. and *Colletotricum* spp. to 0.73, 1.54 and 2.01cm with 91.20%, 78.90% and 77.67% inhibition.

Management of soil borne plant pathogens is very difficult task so the present work was conducted with the need to identify the prevalent and dominant bacterial antagonist *Pseudomonas* sp. isolates from different physiographic regions of western Maharashtra to use them as biocontrol agents against soil borne plant pathogens.

MATERIALS AND METHODS

Method of isolation plant pathogens and antagonists:

The plant pathogens and antagonists were isolated from soil samples collected from western Maharashtra by the soil dilution plate technique and spread plate method (Johnson & Curl, 1972) which is briefly described here.

Ten gram of representative soil sample was transferred to an Erlenmeyer flask containing 100 ml sterile distilled water and mixed thoroughly by shaking for 5 to 10 minutes. 10 ml samples were immediately drawn from the suspension in motion and transferred to 90 ml sterile water blank and shaken for one minute. From each suspension serial dilutions were prepared to obtain the desired dilution. One ml of the final dilution was transferred aseptically to a Petri dish and 15-18 ml of medium was added to the Petri dish. Media used for isolation were Potato dextrose agar, nutrient agar and *Pseudomonas* were maintained in pure form in King's B medium by transferring in fresh medium periodically and storing in refrigerator at 4°C. The dish was rotated by hand in different directions so as to disperse it uniformly in each media. When the medium was solidified, the Petri dishes were incubated at 28 ± 2 ^{0}C for 7 days, before they were taken for counting their populations. Single colonies of each microorganisms were picked up, numbered and maintained on respective agar medium slants for further studies.

Dual plate method

The *Pseudomonas fluorescens* isolates were tested for antagonistic property against the plant pathogens *viz*. *Aspergillus niger, A. flavus, Colletotrichum* sp., *Fusarium* sp., *M. phaseolina, S. rolfsii*, *Rhizoctonia* sp., etc. by dual plate method (Haung and Hoes (1976). Fungal culture was grown on potato dextrose agar (PDA) at 28^{0}C for 3 days. A fungal disc (5 mm dia.) was removed from PDA and placed centrally on prepoured PDA: nutrient agar (1:11, v/v) plate. Bacterial cultures were spot inoculated towards the edge of the plates, which were then incubated at 28^0 C for 2-4 days. Inhibition of fungal mycelium around the bacterial colony was scored after 4 days and calculated by using the following formula (Mital and Johri 2007, Jayaswal *et al.* 1990).

$$\text{Percent Growth Inhibition (\%)} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 = Radial growth measurement of the pathogen in control (mm)

R_2 = Radial growth of the pathogen in the presence of bioagent (mm).

RESULTS AND DISCUSSION

Antagonism of *Pseudomonas fluorescens* isolate (Shiware)

The results given in Table 1 (Plate 1) revealed that the mycelial growth of all pathogens was significantly reduced by *Pseudomonas* in dual culture over control. The antagonist showed maximum extent of mycelial growth inhibition of *F. oxysporum i.e.* 60.37 per cent. It was followed by *A. niger* and *M. phaseolina*, where 58.15 and 52.22 per cent of growth inhibition was recorded, respectively.

Plant pathogens

However, *R. solani, C. capsici* and *S. rolfsii* showed 44.82, 44.65 and 30.37 per cent growth inhibition, respectively. *A. flavus* recorded lowest growth inhibition *i.e.* 24.63 per cent among all pathogens screened in dual culture.

The zone of inhibition was also observed in case of *A. niger, F. oxysporum* f. sp. *ciceri*, *M. phaseolina* and *R. solani* in dual culture.

Antagonism of *Pseudomonas fluorescens* isolate (Pimple)

The results given in Table 2 revealed that the antagonist *Pseudomonas* showed significant reduction in the radial diameter of all the pathogens over control in dual culture.

The *A. niger* and *C. capsici* showed maximum extent of mycelial growth inhibition *i.e.* 52.78 and 52.15 per cent, respectively, followed by *F. oxysporum* f. sp. *ciceri* and *A. flavus* where 49.90 and 46.30 per cent of growth inhibition was recorded. While, *S. rolfsii* and *R. solani* showed 41.67 and 37.78 per cent growth inhibition, respectively.

The minimum growth inhibition (36.48 %) was observed in *M. phaseolina*. The zone of inhibition was also observed in the dual culture in case of *F. oxysporum* f. sp. *ciceri, M. phaseolina* and *R. solani*.

These results are supported with the findings of following workers.

The antifungal activity of *Pseudomonas* was evaluated by Singh *et al.* (2011) against *F. oxysporum*, *A. niger*, *A. flavus*, *A. alternata* and *Erysiphe cruciferarum* found maximum extent of inhibition of *Fusarium* (51.76 %) followed by *A. niger* (50.14

Table 1: *In vitro* evaluation of antagonism of *Pseudomonas fluorescens* isolate (Shiware) against soil borne plant pathogens

Sr. No.	Soil borne plant pathogens	Mycelial growthof pathogenin dual culture* (mm)	Mycelial growthof pathogen in control* (mm)	Mycelial Growth Inhibition (%)
1	*Aspergillus flavus*	67.83	90.00	24.63
2	*Aspergillus niger*	37.67	90.00	58.15
3	*Colletotrichum capsici*	29.33	53.00	44.65
4	*Fusarium oxysporum* f. sp. *ciceri*	35.67	90.00	60.37
5	*Macrophomina phaseolina*	43.00	90.00	52.22
6	*Rhizoctonia solani*	49.67	90.00	44.82
7	*Sclerotium rolfsii*	62.67	90.00	30.37
	SE ±	1.59	-	1.91
	CD	4.81	-	5.81

*Each value is mean of three replication

Table 2: *In vitro* antagonism of *Pseudomonas fluorescens* isolate (Pimple) against soil borne plant pathogens.

Sr. No.	Soil borne plant pathogens	Mycelial growth of pathogen in dual culture*(mm)	Mycelial growthof pathogenin control*(mm)	Mycelial Growth Inhibition(%)
1	*Aspergillus flavus*	48.33	90.00	46.30
2	*Aspergillus niger*	42.50	90.00	52.78
3	*Colletotrichum capsici*	27.83	58.17	52.15
4	*Fusarium oxysporum* f. sp. *ciceri*	42.17	84.17	49.90
5	*Macrophomina phaseolina*	57.17	90.00	36.48
6	*Rhizoctonia solani*	56.00	90.00	37.78
7	*Sclerotium rolfsii*	52.50	90.00	41.67
	SE ±	1.75	-	2.19
	CD	5.31	-	6.63

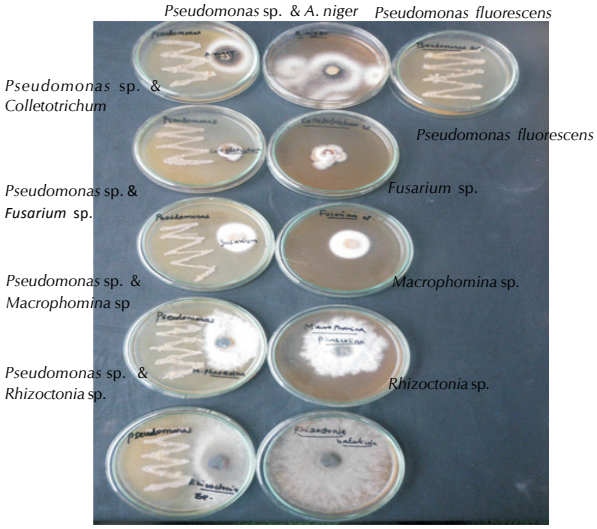

Plate 1: Antagonism of *Pseudomonas fluorescens* isolate (Shiware) against soil borne Plant pathogens

%) and least by *E. cruciferarum* (22.27 %) by the bioagent.

Similarly, Toppo and Tiwari (2015) examined *Pseudomonas* spp. against *Fusarium* spp., *Rhizoctonia* spp. and *Colletotrichum* spp. in *in-vitro* and *in-vivo* reported that PKJ25 was the most active isolate and significantly suppressed the vegetative growth of all the test fungi by restricting the hyphal growth of *Rhizoctonia* spp., *Fusarium* spp. and *Colletotricum* spp. to 0.73, 1.54 and 2.01cm with 91.20%, 78.90% and 77.67% inhibition.

The similar results on the effectiveness of *Pseudomonas* were obtained by the earlier research workers against *Fusarium, Rhizoctonia, Sclerotium, Pythium* (Siddiqui *et al.,* 2005;) *Macrophomina phaseolina, Fusarium, Helminthosporium, A. flavus, Penicillium* (Jayaswal *et al.,* 1990); *Macrophomina phaseolina* (Kumar *et al.,* 2007; Vanitha *et al.,* 2014); *S. rolfsii* (Rakh, 2011) and on *Rhizoctonia bataticola, Sclerotinia sclerotiorum* (Patel *et al.*, 2011).

Thus the results are in conformity with the research work carried out by earlier research workers.

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