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Evaluation of Biocontrol Potential of *Trichoderma* spp. and *Pseudomonas* spp. against *Rhizoctonia solani* under *in-vitro* condition

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Abstract
The test antagonists *Trichoderma* spp. and *Pseudomonas* spp. were tested against test pathogen *Rhizoctonia solani* and they were grown on the same plate to test the antagonistic activity. About 15 to 20 ml of melted and cooled PDA medium. and the King's B medium was poured in to Petri plates and allowed to solidify. Fungal disc of the antagonist of was placed at one end of media on Petri plate. In dual culture test, maximum inhibition in radial growth of *Rhizoctonia solani* was obtained with *Trichoderma viride* (67.60 %) followed by *Trichoderma harzianum* (60.00%). *Pseudomonas* spp. was also inhibited the radial growth of *Rhizoctonia solani* by (70.40%).

Keywords: Biocontrol potential, *Trichoderma* spp., *Pseudomonas* spp.

Introduction
R. solani (R. solani JG Kuhn) [teleomorph Thanatephorus cucumeris (Frank) Donk] is an important soil born pathogen with a necrotrophic life style that persist in the soil for extended periods by producing sclerotia a resistant survival structure. This fungus is a complex with more than 100 species that causes severe damage to many economically important agricultural and horticultural crops as well as trees worldwide. The control of *R. solani* becomes difficult because of high survival rate of sclerotia, it's extremely broad host range and its ecological behaviour. Therefore, the strategies to control *R. solani* are limited because no cultivar is found to be complete resistance. Hence, agronomic controls such as crop rotation are heavily relied upon to fight this disease, though the polyphagous habit of some isolates can include commonly rotated crop species. Broad spectrum fungicides are also available but they have high toxicity and not eco-friendly. Moreover, chemical control methods may not be feasible nor economical for the control of many soil-borne pathogens. Hence biocontrol strategy offers an environmentally friendly alternative to protect plants from this soil born fungi.

Material and Methods
Antagonistic effect of *Trichoderma* spp.
The antagonistic activity of two isolates of *Trichoderma* spp. was evaluated against *Rhizoctonia solani*by dual culture technique. *T. viride* and *T. harzianum* were obtained from Department of Plant Pathology. An amount of 20 ml sterilized melted PDA was poured in 90 mm diameter petriplates. After solidification of medium, 5 mm disc of the antagonist and the test pathogen were separately cut with the help of a sharp sterilized cork borer from the edge of 4 days old culture and placed in straight line at distance of 5 mm from the edge. Without antagonist served as control. Six replications were maintained. The inoculated petriplates were incubated at 27±2 ºC. Observation was made on the radial growth of the antagonist and test pathogen when the fungus in control plate reached to rim of the plate. The per cent growth inhibition of the test pathogen in presence of antagonist was calculated over control as below.

$$\% \text{ Growth inhibition} = \frac{\left[\text{Growth of test pathogen in control plate}\right] - \left[\text{Growth of test pathogen in presence of } \textit{Trichoderma} \text{ spp}\right]}{\text{Growth of test pathogen in control plates}} \times 100$$

Antagonistic effect of *Pseudomonas* spp.

Pseudomonas fluorescens isolates tested for their anagonistic ability against *Rhizoctonia solani*, causing web blight disease on groundnut. The King's Bmedium @ 20 ml per petri plate (90 mm) were poured and allowed to solidify. A 5 mm mycelial disc from young growing edge of the test fungus were cut and placed at one side of the petriplate. The *Pseudomonas* isolates whose inhibition ability need to be tested were streaked at a perpendicular to the test fungus roughly at a distance of 10-15 mm and incubated at 27±2°C for 7 days and percentage inhibition of test fungus were calculated. Six replicated plates were maintained for each isolate. The per cent inhibition of test fungus was expressed in comparison to the control plates.

$$\% \text{ Growth inhibition} = \frac{\left[\begin{array}{l}\text{Growth of test pathogen}\\ \text{in control plate}\end{array}\right] - \left[\begin{array}{l}\text{Growth of test pathogen in presence}\\ \text{of } \int \textit{ Pseudomonas} \text{ spp}\end{array}\right]}{\text{Growth of test pathogen in control plates}} \text{x100}$$

Result

Antagonistic effect of *Trichoderma* spp. against *Rhizoctonia solani* under *in-vitro* condition.

In vitro antagonistic potential of two different isolates of *Trichoderma* spp. (*Trichoderma viride and Trichoderma harzianum)* was studied against fungal plant pathogens *Rhizoctonia solani* following dual culture method and was assessed after 7 days of growth. Observations were recorded on growth of the interacting fungi from which % inhibition was calculated. Percent inhibition was also then converted to 1-5 scale which indicated the reaction type. 1:*Trichoderma* completely overgrew the pathogen and covered the entire medium surface; 2: *Trichoderma* overgrew at least two thirds of the medium surface; 3:*Trichoderma* and pathogen each colonized approximately one half of the medium surface (more than one third and less than two thirds) and neither organism dominate to each other; 4: The pathogen colonized at least two thirds of the medium surface and appeared to withstand encroachment by *Trichoderma*; 5: The pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface

Bipartite interaction using both the *Trichoderma isolates* (*Trichoderma viride and Trichoderma harzianum)* exhibited significant inhibition of *Rhizoctonia solani* and 67.00% and 60.00% respectively as compared to control. A clear visible inhibitory zone was observed in the region of confluence between species of *Trichoderma* and *R. solani*. Inhibitory effects on *Rhizoctonia solani* was more pronounced in bipartite interaction with *Trichoderma viride* (29.16 mm) which was less with *Trichoderma harzianum* exhibiting less preference to mycoparasitize *R. solani* (36.00 mm). *Trichoderma viride* isolate used in our present investigation was identified more effective in inhibiting the growth of *R solani* as compared to *T. harzianum*. Earlier reports of Dutta and Kalha (2011) [2] that isolates of *T. viride* were more effective than isolates of *T. harzianum and by* Kumar and Tripathi (2011) [7] that *Trichoderma* significantly checked the growth of *Rhizoctonia solani* are in support to the observation during the present investigation. Besides this several lines of evidence indicate the antagonistic / mycoparasitic behavior of different species of *Trichoderma*. Kapil and Kapoor (2005) [5] reported that the culture filtrate of *T. viride* inhibited the mycelial growth of *Sclerotinia sclerotiorum* due to production of antibiotic like substance. Lee and Wu (1984) [8] observed that *T. viride* produced metabolites that inhibited the mycelial growth of *Sclerotinia sclerotiorum*. Elad *et al.* (1982) [3] reported that the isolates of *T. harzianum*, which were found to differ in their ability to attack *Sclerotium rolfsii*, *Rhizoctonia solanii* and *P. aphanidermatum*, also differed in the levels of mycolytic enzymes produced by them.The antagonistic fungus *Trichoderma* sp. led to break the outer shell of sclerotia causing its destruction along with several histological changes such as degeneration and decay of cytoplasmic content, deformation and lysis of cell wall of hypha (Rawat and Tewari, 2011) [10]. Seventy *Trichoderma* isolates collected from different regions of Morocco were tested for their capacity to inhibit *in vitro* mycelia growth of *Sclerotium rolfsii* (Khattabi *et al*, 2004) [6]. Four of these isolates (Nz, Kb2, Kb3 and Kf1) showed good antagonistic activity against *S. rolfsii* and were also highly competitive in natural soil. These isolates would therefore be candidates for development in biological control. However, Shalini and Kotasthane (2007) [11] screened seventeen *Trichoderma* strains against *Rhizoctonia solani in vitro*. All strains including *T.harzianum, T.viride* and *Trichoderma aureoviride* were more or less inhibited the growth of *R. solani*.

Table 1: Antagonistic effect of *Trichoderma* spp. against *Rhizoctonia solani* under *in-vitro* condition.

S. No.	*Trichoderma* spp.	Radial growth (mm)		Inhibition (%)
		Trichoderma spp.	*R. solani*	
1	*Trichoderma viride*	43.18	29.16	67.60 (1)
2	*Trichoderma harzianum*	54.04	36.00	60.00 (2)
3	Control	90.00	90.00	00.00
	SEm±	0.88	1.10	
	CD(P=0.05)	2.66	3.33	

Average of six replications; Figures in parenthesis indicate reaction type as a measure of mycoparasitism or antagonism.

Fig 1: Antagonistic effect of *Trichoderma* spp. against *Rhizoctonia solani* under *in-vitro* condition.

Antagonistic effect of *Pseudomonas* spp. against *Rhizoctonia solani* under *in-vitro* condition.

In vitro antagonistic potential of *Pseudomonas* sp. was studied against fungal plant pathogens *Rhizoctonia solani* following dual culture method and was assessed after 3 days of bipartite interaction. The data are presented in Table 5 revealed that isolates of *Pseudomonas* sppin dual culture significantly inhibited mycelial growth of *Rhizoctonia solani*

(70.00%). A clear zone of inhibition in te region of confluence between the two (*Pseudomonas* spp vs. *Rhizoctonia solani) was observed*. Inhibition was recorded by *Pseudomonas* spp (70.4%). Several lines of evidence are in support for our present observation on inhibitory behavior as imposed by fluorescent *Pseudomonas* on *R solani*.

Tripathi and Johri (2002) [14] studied the biocontrol potential of fluorescent *Pseudomonas* isolated from rhizosphere of pea and wheat *in vitro* and *in vivo* against maize sheath blight caused by *Rhizoctonia solani*. They found some isolates to possess multiple disease control potential, while some others exhibited biocontrol potential against specific pathogens, which indicated that *fluorescent pseudomonads* are diverse with respect to their biocontrol potential. Ahmadzadeh *et al.*, (2004) [1] reported that antagonistic rhizobacteria, more specifically *fluorescent pseudomonads* and certain *Bacillus* species possessed the ability to inhibit fungal and bacterial root diseases of agricultural crops. Tiwari and Thrimurthy (2007) [13] reported that twenty-one isolates of *Pseudomonas fluorescens* were isolated from the rhizosphere of rice, maize, wheat, chickpea, mung, urd, soybean and sunflower from Raipur and Bastar regions. Among these seven isolates which showed bright fluorescence under UV light were further tested. The isolates showed positive response of siderophore production and plant growth promoting activity on rice cv. Bamleshwari. Among the isolates PFR 1 and PFR 2 were found significantly superior to control in increasing the shoot length and root length. *In vitro* evaluation of the *P. fluorescens* isolates also confirmed their antagonistic ability against both *Pyricularia grisea* and *Rhizoctonia solani* in dual culture tests. Pure culture of *P. aeruginosa* was obtained from the soil and studied for siderophore production. The antifungal activity of the strain against three phytopathogenic fungi, *viz. F. moniliformae*, *Altenaria solani* and *Helminthosporoum halodes* was assayed by poison food technique. Inhibition of these fungal pathogens appeared to be due to production of antifungal secondary metabolites by *P. aeruginosa* (Kanika Sharma *et al.*, 2007) [4]. Tiwari and Thrimurty (2009) [12] reported *Pseudomonas fluorescens* significantly inhibited the growth of *Rhizoctonia solani*. Pandey and Pundhir (2013) [9] also found similar result.

Table 2: Antagonistic effect of *Pseudomonas* spp. against *Rhizoctonia solani* under *in-vitro* condition

S. No.	Treatment	Radial growth of *Rhizoctonia solani* (mm)	Inhibition (%)
1	*Pseudomonas* spp.	26.64	70.40
2	Control	90.00	00.00
	SEm±	0.34	
	CD(P=0.05)	1.08	

Average of six replications

Fig 2: Antagonistic effect of *Pseudomonas* spp. against *Rhizoctonia solani* under *in-vitro* condition

Conclusion

It can be concluded that the present study revealed the antagonistic property of *Trichoderma* spp. and *Pseudomonas* spp. against *Rhizoctonia solani*. The biocontrol agents work by triggering the plant's natural defence system to protect it from more harmful pests and diseases or by competing with pathogens for space and nutrients. Because these bacteria and fungi are not toxic to other organisms, they pose a low risk to the environment. The above proposed study will provide us in depth knowledge on the influence of the biocontrol agents like *Trichoderma* spp. and *Pseudomonas* spp on the control of the various diseases of field crop biologically. using biocontrol agents which are very cost effective and ecofriendly in the present contest of sustainable agriculture.

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