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EFFICACY OF BIOCONTROL AGENTS AGAINST SOIL BORNE PATHOGENS UNDER *IN - VITRO* CONDITION

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ABSTRACT

In last one decades different research shown that bio control agents like *Trichoderma* sp. and *P. fluorescence* spp. are very effective to control different soil borne pathogens which causes different kind of diseases to the crops. These bio control agents are eco-friendly and balanced plant health managements. Hence, Laboratory experiment was conducted under *in vitro* condition at BTC, College of Agriculture Research Station Sarkanda, Bilaspur (IGKV) (Chhattisgarh), India, in the year 2018-19, to know antagonistic activity of four indigenous isolates of *Pseudomonas* and *Trichoderma.* Results indicated that *Trichoderma* strain T_3 and *Pseudomonas* strain P_{10} had good potential against *Sclerotium rolfsii* and *Rhizoctonia solani* under dual culture technique. These indigenous strains showed effective growth over *Sclerotium rolfsii* and *Rhizoctonia solani*. Other strains of *Trichoderma* strain T_{28} and *Pseudomonas* strain P_2 also resulted a good antagonistic activity over control.

(Key words: Bio control agents, *P. fluorescence, Trichoderma* sp., *Sclerotium rolfsii* and *Rhizoctonia solani*)

INTRODUCTION

In Indian under agriculture resources contributes 18% of gross domestic product (GDP) and it is important to maintain the balance between the growing food demand and growing population. However, a major of insect – pests, diseases and weeds brought down 40% decrease in yield cause around the world (Kaushik and Nirmalkar, 2020). Every year in India total loses is about 30% due to pests and diseases. Diseases are mainly caused by bacteria, fungus, nematodes and physiological disorders. *Sclerotium rolfsii* and *Rhizoctonia solani* are major soil borne plant pathogens, causing several diseases in the form of root rots, collar rots, leaf blight, stem blight, stem rot, leaf spot etc. on wide range of crops including field crops, vegetables, fruits and ornamental crops.

Management of these pathogens is done by cultural, chemical, using resistance varieties and biological control measure. However, it is seen that higher dose of chemicals brought deterioration in human health, environment groundwater and ecosystem. Further, unscientific use of pesticides has led to many problems i.e. detrimental effects on the non target organism's i.e. insect and birds involved in completion of pollination, insects and birds having predating habit and wild animals, development of resistance in insect- pest species, rejuvenation of once small pest into a major problem, food safety hazards and environmental hazards (Verma *et al*., 2023).

In the last few years, bio-insecticides are substituted to control pests and diseases in the place of chemical pesticides. The inhibitory effect of *Trichoderma* and *Pseudomonas* to control plant pathogens have been reported (Nirmalkar *et al*., 2020). These bio-control agents are acclaimed effective, eco-friendly, and cheap. For last few decades there is increase in the production in bio-control products because bio-control agents offer disease management alternatively with different mechanism of action than chemical pesticides. Bio control agents like *Trichoderma* spp. and *Psedumonas fluorescenes* are normally used for control several plant pathogens which cause many diseases. They show antagonistic effect against *S. rolfsii* and *Rhizoctonia solani* and also inhibit the mycelial growth. *Pseudomonas fluorescens* were identified for suppressing the peanut root and stem rot pathogen.

MATERIALS AND METHODS

Isolation of soil borne pathogens

Soil borne pathogen *Rhizoctonia solani* was isolated from sheath blight infected leaves of affected fields located at B.T.C. College of Agriculture and Research Station (IGKV), Bilaspur, Chhattisgarh (India) in year 2018-2019. Whereas, *Sclerotium rolfsii* was isolated from maize crop infected with stem rot disease. Infected leaves and stem were brought from fields and washed, cut into 5 mm segments including the advancing margins of infection. Infected bits were surface disinfected in 0.5% sodium hypochloride solution for 5 min followed by rinsed in sterilized water thrice. The segments were separately dried in between sheets of sterilized filter paper and placed in petridishes

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having on sterilized potato dextrose agar (PDA) impregnated with streptocycline (100 ppm), and incubated at 26±1°C (Dhingra and Sinclair, 1985). Pure culture was obtained by sub-culturing three – four times and maintained on culture slants under the refrigerated condition until required.

Collection of bio-control agents

Cultures of all strains of bio control agent were obtained from State Bio control Laboratory, Thakur Chhedilal Barrister College of Agriculture and Research Station, Sesal Farm, Chorbhatti, Bilaspur (C.G.).In general, petridishes were poured with 15-20 ml sterilized melted and cooled potato dextrose agar medium (PDA). This was supplemented with streptomycin, in order to check the bacterial contamination prior to the pouring. Wherever, growth studies were conducted in 7 mm disc (always kept in inverted position) of the actively growing fungi for inoculating the medium in petridishes. Four replications with five treatments i.e. strains of biocontrol P_2 and P_{10} from *Pseudomonas fluorescens* strains and T_3 and T_{28} from *Trichoderma harzianum* strains with one control were maintained under complete randomized design (CRD) with different factors as per the requirement. The inoculated plates were incubated in BOD incubator at 25 ± 2^0C for a period of 3 to 5 days.

Dual culture interaction

In vitro, the antagonistic activity of different strains of *Trichoderma* spp. and *Pseudomonas fluorescens* against *Rhizoctonia solani*, *Sclerotium rolfsii* was studied by dual culture technique (Kcuk and Kivane, 2003). A mycelial disc (7 mm diameter), obtained from the peripheral region of 7 days old cultures test pathogens i.e. *Rhizoctonia solani*, *Sclerotium rolfsii* and bio control agents i.e. *Trichoderma* spp. and *Pseudomonas fluorescens* were placed simultaneously on the periphery, about 1 cm from the edges of the petridishes (90 mm diameter) at opposite sides. Four replications were maintained for each treatment. The petridishes containing sterilized PDA inoculated with the test pathogen alone served as control. Petridishes having dual culture of *Trichoderma* and test pathogens were incubated at 25 ±2°C and measurements were taken after every 24 hours. Whereas, petridishes having dual culture of *Pseudomonas fluorescens* and test pathogens were incubated at 28 ±2°C. The percentage inhibition growth (mm) of test pathogens was recorded.

Dual culture interaction: $\mathbf{I = (C - T) \times 100 / C}$

Where I = % inhibition in mycelia growth; C = Growth of pathogen in control plates;

T = Growth of pathogen in dual culture plates.

Statistical analysis

In vitro experiment was conducted in completely randomized design with four replications and data recorded from different experiments conducted under *in vitro* conditions were analyzed.

RESULTS AND DISCUSSION

Data presented in Table 1 on antagonistic activity of bio-control agent against *Rhizoctonia solani* indicates that after 72 hours of incubation the growth of *Rhizoctonia solani* was significantly inhibited, by different strains of bio-control agents i.e. *Trichoderma* and *Pseudomonas* . Radial mycelial growth of *Trichoderma* strain (T3-58.50 mm) was significantly higher followed by *Pseudomonas* strain (P10-58.00 mm), *Trichoderma* starin (T28-54.12 mm) and *Pseudomonas* strain (P2-53.12 mm). Whereas, after 120 hours of incubation maximum inhibition of *Rhizoctonia solani* was recorded from *T. harzianum* (T3-60.12 mm) followed by *Pseudomonas flurorescens* (stain P10-58.37 mm) *Trichoderma* starin (T28-55.62 mm) and *Pseudomonas* strain (P2-54.37 mm).

Data from Table 1 also indicates that overall maximum mycelia growth inhibition percentage recorded in *Trichoderma harzianum* strain T_3 (66.66), *Pseudomonas flurorescens* strain P_{10} (64.86), *Trichoderma harzianum* strain T_{28} (61.80), and *Pseudomonas flurorescens* strain P_2 (60.69). Pal and Kaushik (2012) reported that *Trichoderma viride* was isolated from *Rhynchostylis retusa* and *Rhizoctonia solani* from *Aerides multiflora* an orchid. Dual culture method was followed to check the antagonistic activity and results revealed that *Trichoderma viride* inhibited the mycelial growth of *Rhizoctonia solani* by 79.08%.

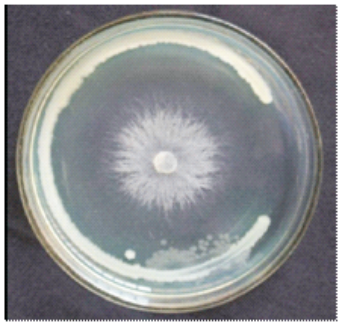
Singh *et al.* (2013) reported that the *Trichoderma harzianum* NBRI-1055 significantly suppressed the seedling blight of sunflower caused by *Rhizoctonia solani* and induced defence mechanism in host. Saeed *et al.* (2013) found that growth inhibition of *R. solani* was significantly higher with *T. asperllum* (74.4%) followed by *T. spp.* (70.0%) and *T. harzianum* (67.8%) under *in vivo* and *in vitro* condition *T. asperellum* was more effective in trial and showed a decrease in disease incidence with increase in concentration of antagonist, providing a control efficacy of 29.1 and 35.3% ($p<0.01$), when applied at a dose of 5 and 1 g l^{-1} in first trial ,respectively. *T. asperllum* showed maximum efficacy (54%).

Antagonistic activity of bio-control agent against soil borne pathogen (*Sclerotium rolfsii*)

Data presented in Table 2 on antagonistic activity of bio-control agent against *Sclerotium rolfsii* indicates that after 96 hours of incubation the growth of *Sclerotium rolfsii* was significantly inhibited by different strains of bio-control agents i.e. *Trichoderma* and *Pseudomonas*. Radial mycelial growth of *Trichoderma* starin (T_3 - 65.12 mm) was significantly higher followed by *Pseudomonas* strain (P_{10}- 60.00 mm), *Trichoderma* starin (T_{28} - 56.87 mm) and *Pseudomonas* strain (P_2 - 56.50 mm). Whereas, after 144 hours of incubation maximum inhibition of *S. rolfsii* was recorded from *T. harzianum* (T_3 - 67.63 mm) followed by *Pseudomonas flurorescens* (strain P_{10} - 61.87 mm) *Trichoderma* strain (T_{28} - 58.62 mm) and *Pseudomonas* strain (P_2 - 57.75 mm).

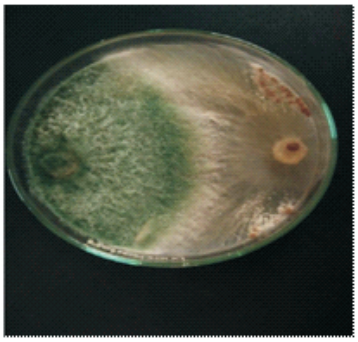
Data from Table 2 also indicates that overall maximum mycelial growth inhibition percentage recorded in *Trichoderma harzianum* strain T_3 (75.13), followed by *Pseudomonas fluorescence* strain P_{10} (68.75), *Trichoderma harzianum* strain T_{28} (65.13) and *Pseudomonas* strain P_2

Control

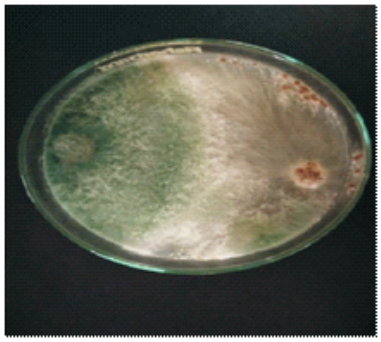


P. flurosence P_2 + *R. solani*

P. flurosence P_{10} + *R. solani*



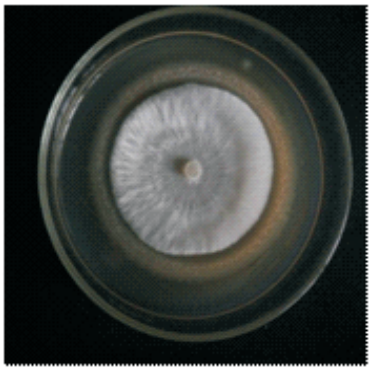
T. harzianum T_3 + *R. solani*



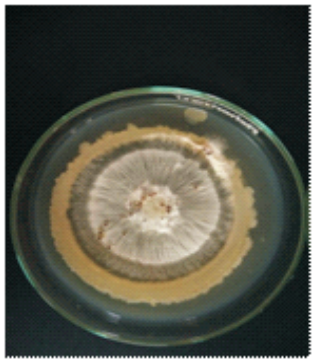
T. harzianum T_{28} + *R. solani*

Antagonistic activity of bio-control agents against *Scleortium rolfsii*

Control

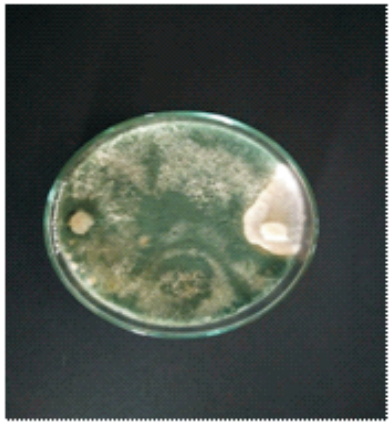


P. flurosence P_2 + *S. rolfsii*



P. flurosence P_{10} + *S. rolfsii*

T. harzianum T_3 + *S. rolfsii*



T. harzianum T_{28} + *S. rolfsii*

Figure 1. Antagonistic activity of bio-control agents against *Rhizoctonia solani*

Table 1. Mycelia growth of *Rhizoctonia solani* against bio-control agents

Bio-control agents	Designation	Mycelia growth (mm) after incubation hrs days^{-1}				Inhibition percent after 120 hrs
		72 hrs 3days^{-1}		120 hrs 5 days^{-1}		
		R. solani	*Trichoderma/ Pseudomon-as*	*R. solani*	*Trichoderma/ Pseudomonas*	
Trichoderma strain (T_3)	T1	31.50	58.50	30.00	60.12	66.66
Trichoderma strain (T_{28})	T2	36.00	54.12	34.37	55.62	61.80
Pseudomonas strain (P_2)	T3	36.87	53.12	35.37	54.37	60.69
Pseudomonas strain (P_{10})	T4	32.00	58.00	31.62	58.37	64.86
Control	T5	90.00	-	90.00	-	00.00
SEm (±)	1.26					
CD at 5%	3.69					

hrs- hours

Table 2. Mycelia growth of *Sclerotium rolfsii* against bio-control agents

Bio-control agents	Designation	Mycelia growth (mm) after incubation hrs days^{-1}				Inhibition percent after 144 hrs
		96 hrs 4days^{-1}		144 hrs 6 days^{-1}		
		R. solani	*Trichoderma/ Pseudomon-as*	*R. solani*	*Trichoderma/ Pseudomonas*	
Trichoderma strain (T_3)	T1	24.87	65.12	22.37	67.63	75.13
Trichoderma strain (T_{28})	T2	33.12	56.87	31.37	58.62	65.13
Pseudomonas strain (P_2)	T3	33.50	56.50	31.25	57.75	65.27
Pseudomonas strain (P_{10})	T4	30.00	60.00	28.12	61.87	68.75
Control	T5	90.00	-	90.00	-	00.00
SEm (±)	1.26					
CD at 5%	3.66					

hrs- hours

(65.27). Various worker also found similar trends. Nirmalkar *et al*. (2018) reported that *Pseudomonas flurorescens* and *T. harzianum* combination and alone were effective in managing the damping off in Solanaceous crops when compared with chemicals.

Bandyopodhyay *et al*. (2003) reported antagonistic activities of different *Trichoderma* strains against soil borne pathogen and found that *Trichoderma* strains inhibited the growth of *Rhizoctonia solani* by 73.3%, *Sclerotium* sp. by 66.6% and *R. bataticola* by 51.1%. Balasaheb (2015) reported that *P. flurorescens* and *Trichoderma harzianum* showed good potential in controlling stem rot of groundnut caused by *S. rolfsii* under pot culture. Singh *et al*. (2017) reported that collar rot disease of chickpea can be minimizing by the integration of *T. harzianum* or *P. fluorescens* (soil application) followed by the application of hexagonal. The results showed that *Pseudomonas flurorescens* and *T. harzianum* can play an important role in bio control of soil borne diseases of *S. rolfsii.*

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