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In vitro evaluation of different biocontrol agents against soil borne pathogens

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Abstract
Numerous fungi and bacteria, including existing biocontrol strains with known activity against soil borne fungal pathogens as well as isolates collected from the roots and rhizosphere of plants growing in the field, were tested for their efficacy in controlling soil borne pathogens. The *Chaetomium globosum* and other biocontrol agents *viz*., *Pseudomonas* isolate 1 and 2, *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma kuhinji* were tested under *in vitro* condition by dual culture method for their efficacy against the three soil borne fungal plant pathogens. *Chaetomium globosum* showed maximum antagonistic effect to *Fusarium* (72.22%) followed by *Sclerotium* (46.66%) while *Trichoderma harzianum* inhibited the growth of *Rhizoctonia* (67.77%) more as compared to other bioagents.

Keywords: Bio-control agents, antagonism, soil borne pathogens

Introduction

Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resources, *i.e*. certain rhizosphere organisms, common components of ecosystems, known to develop antagonistic activities against harmful organisms (bacteria, fungi, nematodes *etc*.). Bio-control involves harnessing disease-suppressive microorganisms to improve plant health. Disease suppression by bio-control agents is the sustained manifestation of interactions among the plant, the pathogen, the bio-control agent, the microbial community on and around the plant, and the physical environment. Therefore, despite its potential in agricultural applications, bio-control is one of the most poorly understood areas of plant-microbe interactions (Handelsman and Eric V. Stabb 1996) [3].

Soil borne disease organisms are widely found in soil. As a group, they can affect a wide range of plants, including fruits and vegetables, ornamental plants, trees, and shrubs. Common names for plant disease often reflect the visual damage to the plant but do not necessarily indicate the pathogen responsible for the disease. For example, seedling damping-off, the condition when seedlings die or fall over can occur in most vegetables and can be caused by *Pythium, Phytophthora, Rhizoctonia, Fusarium, Sclerotium*, or any combination of these. Identification of symptoms of root diseases include wilting, dieback, browning or rotting of tissues, and cankering.

Growers need to know which treatments are most effective for their specific problems and growing conditions in order to prevent needless expenses, ineffective treatments, and crop losses. In 2009, soil borne pathogens were responsible for an estimated 10% of losses in vegetable crops. Because fungicide use is not consistently effective, economical, ecologically desirable due to environmental and worker exposure concerns or commercially desirable while production of pesticide-free or organic crops can increase crop value by 30%, biological control and plant growth promoting agents should be considered key management components (Anonymous 2012) [1].

In turn, this research will help to reduce our reliance on chemical fungicides and increase the sustainability of agriculture.

Materials and Methods

Materials used

Collection of soil samples

The soil samples were collected from post graduate research field of M.P.K.V., Rahuri for isolation of bio-control agents *Chaetomium* spp.

Bioagents
Bioagents *viz*., *Pseudomonas* isolate 1, *Pseudomonas* isolate 2, *Trichoderma harzianum*, *Trichoderma viride*, and *Trichoderma kuhinji* were collected from Microbial culture collection library of department of plant pathology, MPKV, Rahuri for *in vitro* testing.

Methods
Isolation of *Chaetomium globosum* from soil
The fresh rhizosphere soil samples were obtained from various places from the field of M.P.K.V. Rahuri. The soil samples were air dried, grinded and sieved through 0.50 mm sieve so as to obtain fine sized particles. The 10.0 g of rhizosphere soil samples were weighed on electronic balance and used for isolation of *Chaetomium globosum* by serial dilution and pour plate method.

Sterilization of glassware and media
The glassware required for isolation of bio-control agents and soil borne pathogens were sterilized in hot air oven at 160^0C for two hours. The culture media for isolation of organisms were steam sterilized at 121.6 ^{0}C (*i.e.* 15 1bs pressure / inch^2) for 15 minutes.

Preparation of media for isolation of *Chaetomium globosum* fungus and pathogens
Potato dextrose agar medium were prepared in conical flasks of 250 ml capacity separately and poured in petri dish for isolation of *Chaetomium* by serial dilution method and pour plate method.

Purification and maintenance of pure culture
The fungus growth obtained from the soil were purified by hyphal tip method and maintained on fresh potato dextrose agar medium slant under aseptic condition for further studies.

Identification
The fungus isolated from soil sample and diseased specimen and established in pure form was identified on the basis of colony and morphological characters and microscopic observations.

Evaluation of different bio-control agents against different soil borne plant pathogens *in vitro*
The antagonistic effect of bio-control agents against the fungal pathogen *Fusarium*, *Sclerotium* and *Rhizoctonia* were studied by adopting dual culture technique. The pure culture of *Chaetomium globosum, Pseudomonas* isolate 1 and 2, *Trichoderma harzianum, Trichoderma viride and Trichoderma kuhinji* was grown separately on PDA plates for seven days. The discs of 5 mm diameter were cut with sterilised cork borer and transferred aseptically in PDA contains petriplates. The disc of the test fungus was placed exactly opposite to the disc of biocontrol agent. The disc was placed in such a manner that both the test fungus and bio-agent would get an equal opportunity for their growth. The experiment was conducted with seven replications. The petriplate inoculated with discs of *Fusarium, Sclerotium* and *Rhizoctonia* alone was measured to assess the antagonistic potential of biocontrol agents against pathogen. The percent growth inhibition of pathogen colonies was calculated by using formula given by Arora and Upadhyay (1978).

$$\text{Percent growth inhibition} = \frac{D1-D2}{D1} X\ 100$$

Where, D_1 = Diameter of pathogen colony in control
D_2 = Diameter of pathogen colony in treatment

Result and Discussion
***In vitro* evaluation of different biocontrol agents against soil borne pathogens**
Biocontrol agents obtained from the soil samples and department were tested for their efficacy against soil borne plant pathogens *viz. Fusarium, Sclerotium* and *Rhizoctonia* by dual culture method. The data on the colony diameter and the % inhibition are calculated and given in the Table 1.
Result depicted in Table no.1 revealed that growth of soil borne pathogens influenced due to different bioagents. In case of soil borne pathogen *Fusarium* maximum inhibition (72.22%) was observed in treatment no.1 where *Chaetomium* was used. It was followed by treatment no.4 (68.80%). Minimum inhibition of *Fusarium* (55.00%) was observed in treatment no.6 where *Trichoderma kuhinji* was used.
In case of *Rhizoctonia* spp. maximum inhibition (67.77%) was observed in treatment no. 4 where *Trichoderma harzianum* was used. It was followed by treatment no.1 (66.66%). Minimum inhibition of *Rhizoctonia* (30.95%) was observed in treatment no.6 where *Trichoderma viride* was used.
In case of *Sclerotium* spp. maximum inhibition (46.66%) was observed in treatment no.1 where *Chaetomium* was used. It was followed by treatment no.6 (45.55%). Minimum inhibition of *Sclerotium* (11.11%) was observed in treatment no.5 where *Trichoderma viride* was used. While *Pseudomonas* isolate1 and 2 did not show any effect on *Sclerotium* spp. Study indicated that among bioagents *Chaetomium* is better antagonist to minimise soil borne pathogens like *Fusarium* spp. Otherwise soil borne *Sclerotium* spp. is rather difficult to manage with the antagonist cent-percent.
Similar results were reported by Singh *et al.* (2002) [4], who reported the integrated management of pigeonpea wilt by biotic agents and biopesticides. Two isolates of *Trichoderma viride*- l and 2 and one each of *Trichoderma harzianum, Gliocladium virens, Chaetomium globosum* and *Bacillus subtilis* were found antagonistic to *Fusarium udum* by dual culture method *in vitro.*
Guang *et al*. (1991) [2] evaluated antagonism of *Chaetomium* ND35 against six plant pathogens (*Macrophoma kuwatsukai*, *Valsa mali*, *Cytospora chrysosperma* (*Valsa sordida), Dothiorella gregaria, Rhizoctonia solani* and *Sclerotium rolfsii (Corticium rolfsii) in vitro* and *in vivo* and found effective.
Manjula *et al*. (2004), tested isolates of *Trichoderma viride* and *Pseudomonas fluorescens* for their antagonistic activity against *S. rolfsii*, causing stem rot of groundnut. The disease was suppressed upto 58.00% and 70.00%, respectively by both the isolates under controlled conditions.

Table 1: Efficacy of different bio-agents against soil borne pathogens

Sr. No.	Treatments	Antagonism against soil borne pathogens					
		***Fusarium* spp.**		***Sclerotium* spp.**		***Rhizoctonia* spp.**	
		Mean growth (mm)	% Inhibition	Mean growth (mm)	% Inhibition	Mean growth (mm)	% Inhibition
1	*Chaetomium globosum*	25	72.22	48	46.66	30	66.66
2	*Pseudomonas* isolate 1	31.79	64.67	90	00	45	50
3	*Pseudomonas* isolate 2	30	66.66	90	00	40	55.55
4	*Trichoderma harzianum*	28	68.80	72.14	19.8	29	67.77
5	*Trichoderma viride*	35	61.11	80	11.11	62.14	30.95
6	*Trichoderma kuhinji*	40	55.55	49	45.55	48	46.66
7	Control	90	00	90	00	90	00
8	SE (±)	0.55	00	0.42	00	0.53	00
9	CD @ 5%	1.58	00	1.20	00	1.52	00

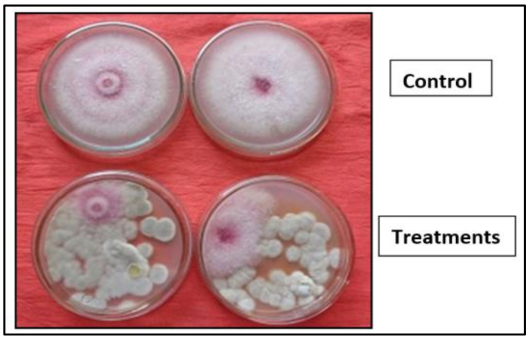

Plate 1a: Effect of *Chaetomium globosum* on growth of *Fusarium* spp. *in vitro*

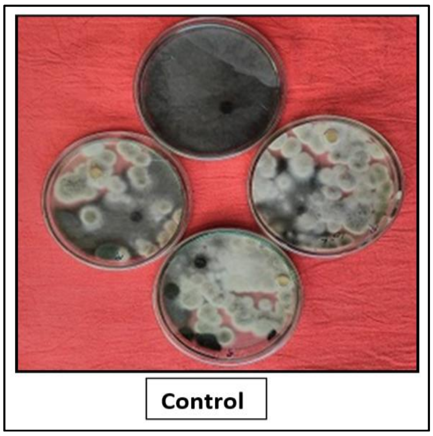

Plate 1b: Effect of *Chaetomium globosum on growth Rhizoctonia* spp. *in vitro.*

Plate 1c: Effect of *Chaetomium globosum* on growth *Sclerotium* spp. *in vitro*.

Plate 1: Bioefficacy of *Chaetomium globosum* against soil borne pathogens *i.e. Fusarium* spp., *Rhizoctonia* spp. And *Sclerotium* spp.

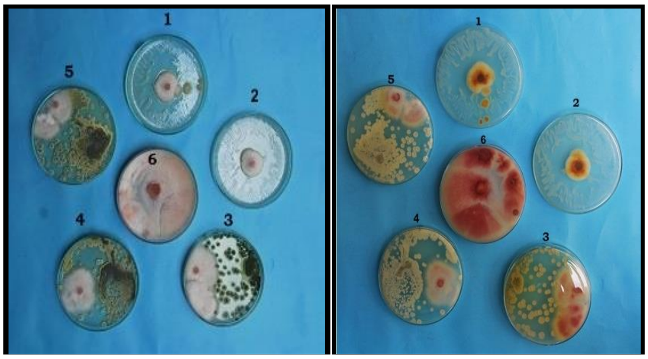

Plate 2a: Performance of different biocontrol agents against *Fusarium* spp.

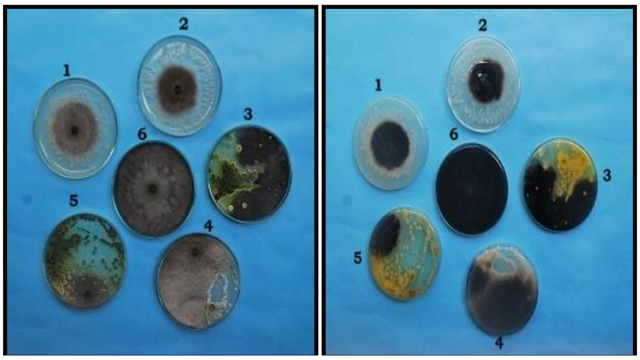

Plate 2b: Performance of different biocontrol agents against *Rhizoctonia* spp.

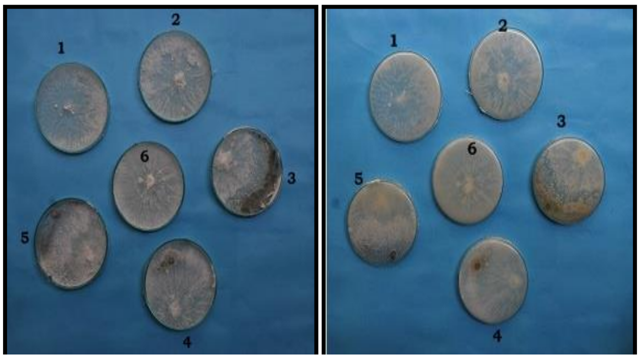

Plate 2c: Performance of different biocontrol agents against *Sclerotium* spp.

1) *Pseudomonas* isolate 1
2) *Pseudomonas* isolate 2
3) *Trichoderma harzianum*
4) *Trichoderma viride*
5) *Trichoderma kuhinji*
6) Control

Plate 2: Bioefficacy of biocontol agent against soil borne pathogens.

Conclusion

1. Antagonist *Chaetomium globosum* isolated from soil samples was more effective against *Fusarium* than *Rhizoctonia* and *Sclerotium*.

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