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ANTAGONISTIC EFFECT OF RHIZOSPHERIC TRICHODERMA SPECIES AGAINST SOIL BORNE PATHOGENS

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ABSTRACT

Seven strains of Trichoderma viz. *T. viride, T. harzianum, T. atroviride, T. longibrachiatum, T. koningii, T. asperllum* and *T. virens* have been isolated from rhizosphere soil by serial dilutuion plate technique. In-vitro they were tested for antagonistic capacity against soil borne pathogens such as *Pythium aphanidermatum* and *Sclerotium rolfsii,* by dual culture method. Among the seven species of *Trichoderma harzianum* (Th. azad) and *Trichoderma viride* (01PP) are known to be effective and potential antagonists of tested plant pathogens.

Key Words : ***Trichoderma sp, soil borne pathogens.***

After green revolution people have been used chemical fertilizers and fungicides and chemical pesticides for better crop health but in recent year we have began to understand the widespread and repeated use of chemical biocide to control the host of organisms such as insect's weeds and fungi that threaten human interest. The global consensus to reduce inputs to chemical pesticides which are perceived as being hazardous by some consumer has provided opportunities for the development of novel sustainable crop protection strategies. There is a need to develop alternative control systems in the new future that is biocontrol and these must be implied.

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (1). Trichoderma species have been known since the 1930s to show antifungal activity and there have been extensive efforts to use them for plant disease control since then (2). They have been used as biological control agents and their isolates have become commercially available however, the production of effective Trichoderma based product. In large scale to fulfillment the requirement is not yet feasible because of several regions. The quality of a microbial bio-protectant is dependent on the propagule density in the biomass and its ability to survive in nature. Production of adequate quantities of good quality inoculum is an essential component of the biocontrol programmed. Development of simple and reliable production system for the production of spore, which having long longevity period and very effective for fungal plant pathogen can be produce in bulk is needed. Therefore the indigenous potent Trichoderma sp. Were isolated from soil through soil plate dilution technique.

MATERIALS AND METHODS

Isolation and Identification of *Trichoderma* species

Trichoderma sp. were isolated using soil dilution plate technique (3) and soil washing methods (4) on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czepex, Dox Agar (CZA). Streptomycin 50 mg/L added in culture media for avoiding bacterial growth in petriplate and plates were incubated at room temperature 25±2 for five days were also recorded by (5). The colonies were isolated and purified on potato dextrose agar (PDA).The pure culture were identified up to species level with the help of standard monographs and reference book (6). The isolates were preserved in sterile soil, mineral oils and stored at 4°C.

Screening of Isolates for their Antagonistic Activities

The species of *Trichoderma* were screened against soil borne fungi Such as Pythium aphanidermatum and Sclerotium rolfsii, by dual culture plate technique on PDA Medium. Ten days old cultures of respected pathogens were inoculated onto the PDA medium, 2mm away from center Inoculated plates were incubated at 25ºC for four days. After the end of four days the same plates were inoculated with Trichoderma sp. 5mm away from previous inoculums

Table-1: Antagonistic activity of Trichoderma species against soil borne pathogens in dual culture.

Bio agents	Source	Accession No.			*P. aphanidermatum*		*S. rolfsii*	
		ITCC	NBAIM	GenBank	Mycelial growth (mm)	Percent inhibition	Mycelial growth (mm)	Percent inhibition
T. viride 01PP	Hardoi	8315	F-03110	JX119211	11.0	59.25	10.0	56.52
T. harzianum Th azad	CSA Kanpur Nagar	6796	F-03109	KC800922	10.3	57.08	9.0	60.86
T. asperellum Tasp/CSAU	CSA Kanpur Nagar	8940	F-03108	KC800921	11.0	54.16	12.0	55.5
T. koningii TK (CSAU)	CSA Kanpur Nagar	5201	F-03112	KC800923	13.9	48.51	12.6	45.21
T. atroviride 71 L	Hardoi	7445	F-03107	KC 008065	14.2	40.83	14.0	39.13
T. longibrachiatum 21 PP	Kaushambi	7437	F-03111	JX978542	16.0	33.33	14.3	37.82
T. virens T.vi (CSAU)	CSA Kanpur Nagar	4177	F-03106	KC800924	17.0	29.16	17.9	33.70
Control					90	-	90	-

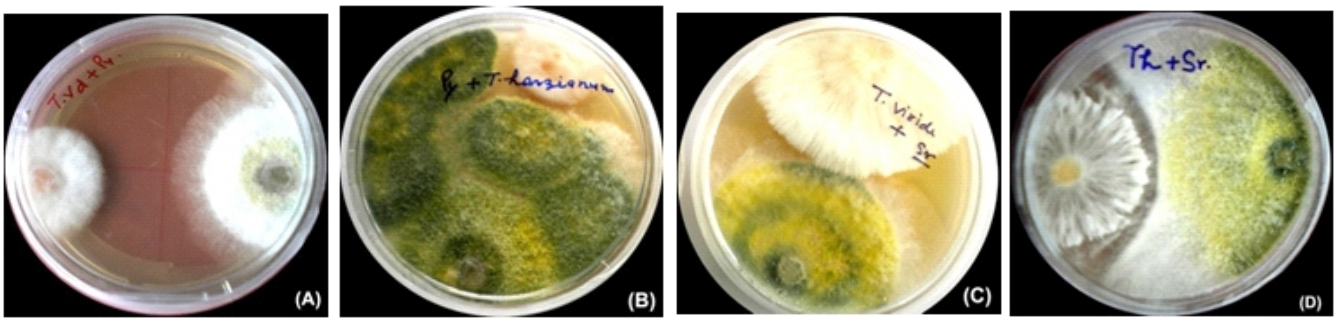

Fig.-1 (A-D) Antagonistic activity of *Trichoderma viride* (01PP) and *Trichoderma harzianum* (Th. azad) with *Pythium aphanidermatum* and *Sclerotium rolfsii*

(soil borne fungi *Pythium aphanidermatum and Sclerotium rolfsii*, and again keep it for incubation at 28°C for four days. The antagonistic activity of *Trichoderma* sp. were observed and calculated. Percent inhibition of mycelial growth of targeted fungal pathogens over control was calculated by following equation given by (7) :

$$\% \text{ inhibition} = \frac{D_1 - D_2}{D_1} \times 100$$

D_1 = Colony diameter in the control.

D_2 = Colony diameter in treated.

RESULTS AND DISCUSSION

Evaluation for in vitro antagonistic potential and effective strains against two very serious soil borne pathogens viz. fungi *Pythium aphanidermatum* and *Sclerotium rolfsii*, by dual culture plate techniques was done. Trichoderma viride (01PP) and *Trichoderma harzianum* (Th. azad) inhibited mycelia growth of both pathogens which were well stabilized in plate (Fig.1 (A-D). In dual culture plates *Trichoderma viride* (01PP) and *Trichoderma harzianum* (Th. azad) completely colonized *Pythium aphanidermatum* and *Sclerotium rolfsii.* The dual culture plates of both antagonistic fungi against *Pythium aphanidermatum* and *Sclerotium rolfsii* showed completely restricted the mycellial growth of pathogen in plates. Among the two test phytopathogens and antagonistic activity of 01PP isolate of *Trichoderma viride* was found highly effective (59.25 and 56.52) against *Pythium aphanidermatum* and *Sclerotium rolfsii,* similarly Th. azad isolate *Trichoderma harzianum* was also found highly effective (57.08 and 60.86) against *Pythium aphanidermatum* and *Sclerotium rolfsii,* followed by other species of *Trichoderma* (Table-1). Similar result were also

predicted by (8). It might be secretion of some secondary metabolites which diffused in the culture medium and inhibited the growth of pathogen. It is thus evident from result that *Trichoderma viride* (01PP) and *Trichoderma harzianum* (Th. azad) can effectively colonize *Pythium aphinedermatum* and can therefore be used as a biocontrol agent effectively against these six soil borne pathogens pathogens. Both Trichoderma viride (01PP) and Trichoderma harzianum (Th. azad) is well known biocontrol agents and used for managing various plant diseases (9). For effective management there is needed to be screened potent strains of these antagonistic.

CONCLUSION

The antifungal activities Trichoderma species play an important role in controlling soil-borne fungal pathogens pythium aphanedermatum and sclerotium rolfsii. The *Trichoderma harzianum* (Th. azad) were the best antagonists followed by *Trichoderma viride* (01PP) for controlling the wilt of leguminous crops. The use of these bio-agents are not only safe for the farmers and consumers, but also eco-friendly, cost effective, easy to produce and easy to apply the formulations.

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REFERENCES

1. Eziashi, E.I.; Omamor, I.B. and Odigie, E.E. (2007). Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on Ceratocystis paradoxa. *African Journal of Biotechnology 6 :* 388-392.
2. Hjeljord, L.G. and Tronsmo, A. (1998). Trichoderma and Gliocladium in biocontrol: an overview. In: *Trichoderma* and *Gliocladium*, edited by Kubicek CP, Harman GE Taylor and Francis (London, United Kingdom) 135-151.
3. Johnson, L.E.; Bond, C.J. and Fribourg, H. (1959). Methods for studying soil micro flora-plant disease relationships. Minneapolis : *Burgess Publishing Company.*
4. Gams, W.; Domsch, K.H. and Anderson, T.H. (1980). Compendium of soil fungi. *Academic press* (London) 85.
5. Kumar Vipul, Shahid, M.; Srivastava, Mukesh; Bansal Anuj (2013). Influence and Effect of Temperature pH and media for growth and sporulation of *Trichoderma koningii* and its shelf life study in different carrier based formulation, *Progressive Research 8 : (special) :* 452-454.
6. Samuels, G.J.; Chaverri, P.; Farr, D.F. and McCray, E.B. (2004). USDA, Beltsville, USA. Trichoderma online systematic. Botany and Mycology Laboratory ARS (USDA).
7. Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature, 150 :* 850-853.
8. Singh, Anuradha; Shahid, M.; Srivastava, Mukesh; Kumar Vipul and Bansal Anuj (2013). Antagonistic Activity of *Trichoderma viride* isolate against different pathogen of Fusarium oxysporum isolated from legume crop of U.P. *Progressive Research, 8 (1) :* 47-50.
9. Rini, C.R. and Sulochana, K.K. (2007). Usefulness of Trichoderma and Pseudomonas against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *Journal of Tropical Agriculture 45 :* 21-28.