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IN VITRO ANTAGONISTIC ACTIVITY OF *TRICHODERMA HARZIANUM* AGAINST *CERCOSPORA ARACHIDICOLA* AND *ASPERGILLUS FLAVUS*



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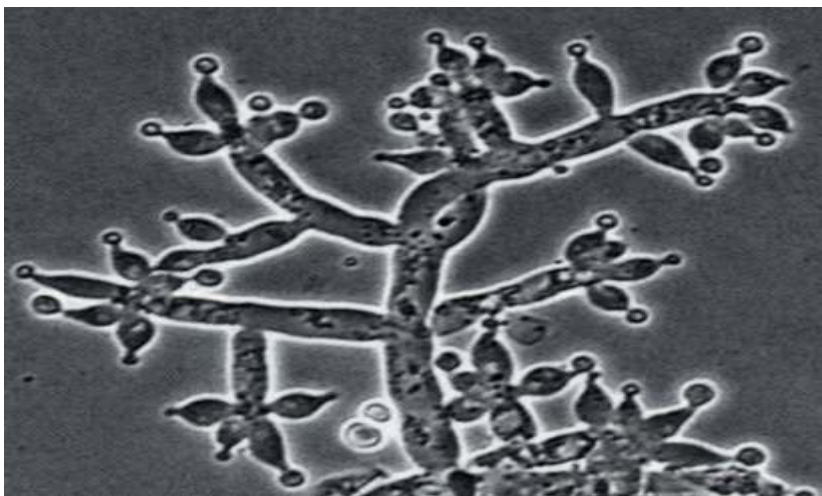
Short Profile

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ABSTRACT:

The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. *Trichoderma* strains exert biocontrol against fungal phytopathogens either directly, by competing for nutrients and space, modifying the environmental condition, or promoting plant growth and plant defensive mechanisms and

antibiosis or directly by mechanisms such as mycoparasitism. In the present investigation in vitro antagonistic potential of the *Trichoderma harzianum* against *Cercospora arachidicola* and *Aspergillus flavus* were tested by dual culture method.

KEYWORDS

Antagonism, mycoparasitism, Biocontrol agent, *Trichoderma harzianum*.

INTRODUCTION :

Trichoderma, a genus under Deuteromycotina, Hyphomycetes, Phialasporace, Hyphales, Dematiaceae has gained immense importance since last few decades due to its biological control ability against several plant pathogens (Kubicek and Harman, 1998). Researchers are interested in this genus because of its novel biological properties and biotechnological applications. *T.harzianum* is one efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Knowledge concerning the behavior of these fungi as antagonists is essential for their effective use since they can act against target organisms in several ways. Strains of Trichoderma can produce extracellular enzymes and antifungal antibiotics but they may also be competitors to fungal pathogens, promote plant growth and induce resistance in plants. The commercial use of Trichoderma biocontrol agents must be preceded by precise identification, adequate formulation, and studies about the synergistic effects of their mechanism of biocontrol (Grondona,1997). The present study deals with invitro antagonistic potential of the *Trichoderma harzianum* against *Cercospora arachidicola* and *Aspergillus flavus* were tested by dual culture method.

MATERIALS AND METHODS

Dual plating and study of Mycoparasitism

The invitro antagonistic potential of the *Trichoderma harzianum* against *Cercospora arachidicola* and *Aspergillus flavus* were tested by dual culture method (Dennis and Webster,1971a) on PDA medium.

(a) Isolation and identification of *Trichoderma harzianum*

Four soil samples of 500 grams each were collected randomly from top six-inch layer of soil from each agro climatic zone of Karnataka and packed in polyethylene bag. Each soil sample was sieved through a 1000 μ mesh to remove the bigger soil particles and debris. The sieved soil samples were used for the isolation of the organism by standard plate count method (Malloch,1997). One ml of dilutions was used for plating on Martin's Rose Bengal agar (MRBA) medium and was incubated at 30 $^{\circ}$ C for 4 days. Based on the colony morphology, the mold colonies were selected and cultured separately to obtain pure culture. Microscopic observation was carried out in order to confirm the isolates (Gilman,1961)

(b) Invitro antagonistic activity by Dual culture method

The fungal plant pathogens *Cercospora arachidicola* and *Aspergillus flavus* were obtained from Department of pathology (UAS, GKV, Bangalore). In order to get fresh active culture, each host fungus as well as *Trichoderma harzianum* were grown on potato dextrose agar (PDA) petridishes for a week at room temperature (28 $^{\circ}$ -30 $^{\circ}$ C). Approximately 20 ml PDA was poured into each 90cm diameter sterilized petridishes. Following solidification, 5mm disc of fungal growth was cut with a sterile cork borer and placed near the periphery of the Plate and *Trichoderma harzianum* was placed on the other side. Plates

with no antagonistic plate was served control for the pathogen. The petridishes were incubated at room temperature and provided alternate light and darkness for seven days. Growth into petridishes was observed periodically. Each treatment was replicated thrice.

Experimental Results

Trichoderma harzianum were tested for in vitro antagonistic activity against fungal pathogens, *Aspergillus flavus* and *Cercospora arachidicola* by dual plating method. It is clear from the results that *Trichoderma harzianum* showed its significant overlapping growth on *Aspergillus flavus* and *Cercospora arachidicola*, thus indicating a strong antagonistic activity against fungal pathogens. Initially, host fungi grew towards *Trichoderma harzianum*, however the growing edge of *Cercospora arachidicola* (plate 1) ceased before it could make contact with growing colony of *Trichoderma harzianum* showing visible inhibition zone (2 mm) which indicated antibiosis through the production of some diffusible antifungal substance by antagonist. Later, antagonist gradually overgrew the *Cercospora arachidicola* and inhibited the growth. The growing edge of, *Aspergillus flavus* (plate 2) met the growing edge of *Trichoderma harzianum*, at which time the growth of *Aspergillus flavus* ceased. *Trichoderma harzianum* continued its growth resulting into overgrowth to host fungus gradually. The present study indicated the promising antagonistic property of *Trichoderma harzianum*.

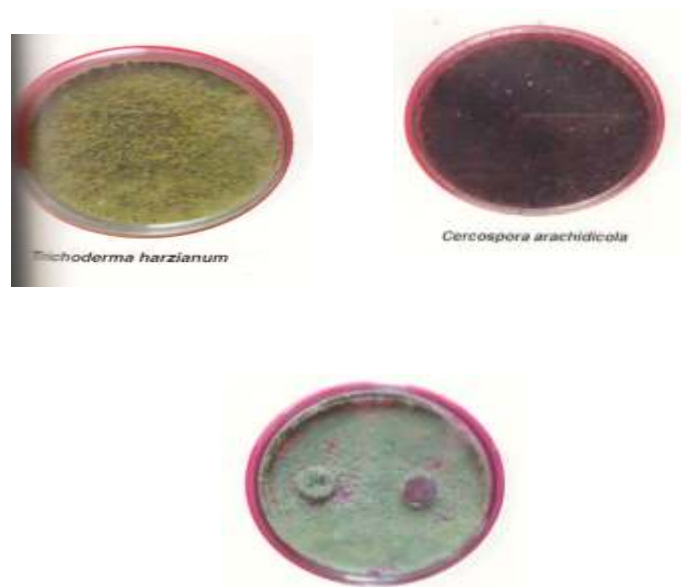


Plate -1 *Trichoderma harzianum* Vs *Cercospora arachidicola*
Antagonistic Effect of *Trichoderma harzianum* on *Cercospora arachidicola*

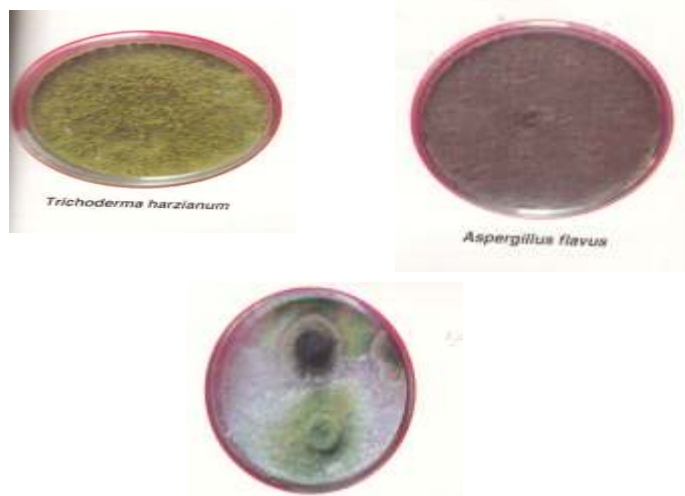


Plate -2 *Trichoderma harzianum* Vs *Aspergillus flavus*
Antagonistic Effect of *Trichoderma harzianum* on *Aspergillus flavus*

DISCUSSION

One of the key elements of sustainable agriculture is the ecological approach to solving the problems with plant pathogens, by the application of biocontrol agents. The genus *Trichoderma* is most important in achieving that and, at the same time, sustaining a favorable environment, instead of using chemicals.

Fungi of the genus *Trichoderma* have long been recognized for their ability to act as biocontrol agents against plant pathogens. (Harman, 2006)

Antibiosis, mycorparasitism and food competition are the main mechanisms in biological control (Ghildyal and Pandery, 2008; Umamaheswari, 2009, Ranasingh, 2006). (Singh, 1997) also reported that *Trichoderma harzianum* overgrew the colony of *F.oxysporium f.sp.ciceri* within 7 days of inoculation and showed parasitism. (Elad,1983)observed that *Trichoderma harzianum* entered *R.solani kuchn* and *S.rolfsii* by dissolving holes in the hyphae,while (Henis,1983)found that it destroyed sclerotia from *S.rolfsii*.

Most of the workers have reported that *Trichoderma* treatment has been successfully for the control of soil borne diseases in crops. (Bijana , 2012) found the strong reducing effect of *Trichoderma harzianum* towards *Alternaria alternate*, can be applied in the biological control of this pathogen, causing agent of the brown spot disease on tobacco. (Seema, 2012) Studied the invitro evaluation of biological control agents against *Rhizoctonia solani* revealed *Trichoderma harzianum* as a potential antagonists capable of controlling the pathogenicity of *R. solani* on tobacco seedlings.

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