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STUDIES ON ANTAGONISTIC ACTIVITY OF TRICHODERMA AGAINST FUNGI

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Abstract

During the in vitro assay of antagonism of *Trichoderma atroviride* and *Trichoderma herzanium* were screened against the five dominant soil fungi viz. *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata* and *Alternaria alternata*werestudied by percent growth of inhibition of *Trichoderma atroviride* result were shows the highest growth of inhibition was seen against the *Alternaria alternata* (66.66%) after that *Curvularia lunata*(62.82%) and Aspergillus flavus (59.61%) the lowest percent of growth inhibition was seen *Colletotrichum falcatum* (53.84)and *Aspergillus niger* (56.25%)and *Trichoderma harzianum*shows highest potency against the *Curvularia lunata* (68.57%),next to that *Aspergillus flavus* (63.46%) and *Alternaria alternata* (60.00%), the lowest inhibition of *Aspergillus niger*(52.08%) *Colletotrichum falcatum*(58.97%).

Keywords : Soil isolates, Trichoderma, Antagonistic

Introduction:

India is the second most populated country in the world. Food, Shelter and Cloths are the necessary things to survive for every human being. Limited land is used in agricultural sector to fulfill the basic food need of the human beings. To overcome this problem, researchers started the new techniques and new findings to increase the yield andnutrition production of the crops. To increase the production and nutrition in the crops peoples use many chemical and synthetic fertilizers which are very hazardous to soil ecosystem, and long time use of such fertilizers are affects the soil fertility as well as crops. Due to such use of chemicals, fertilizers loss of soil health as well as microorganisms and mycobiota extinct from the ecosystem. Now days to overcome this problem various non chemical and eco friendly fertilizers have been searched by the

scientist for better and healthy crops. Many bacteria, algae, fungi used as bio control agent play a significant role in the field of plant disease control, thereby increasing plant productivity.

Some soil borne fungi survive for several years in soil because of their conserved structures then germinate and develop under favorable environmental conditions. In fact, management of diseases caused by soil borne pathogens is difficult. Synthetic fungicides and fumigants, though still used, are not considered a potential solution to sustainable plants production because of their non-specific targets application and negative impact on human health and environment (Howard et al., 1994).

The genus Trichoderma and its associated various fungal species occurs commonly in all types of soils and can be easily isolated and rapid growth occur on different culture medium. *Trichoderma* is a versatile fungus, used commercially in a number of ways. It is used for There are several studies evaluating the antagonistic potential of *Trichoderma* against plant pathogens (Doley et al. 2014; El-Gali 2015; Kumar et al. 2015). However, there is a gap of information about plant biochemical response when using these fungi as a biocontrol for soil-borne pathogen (Kipngeno et al. 2015; Srivastava et al. 2014; Tapwal et al. 2015), particularly in cassava (Sobowale et al. 2010). The efficiency of *Trichoderma* against several pathogens was demonstrated for a number of crops such *T. viride* against root rot and white mold diseases in bean plants (Mohamed et al. 2010), *Trichoderma* spp. against *Pythium* isolated from *Lycopersiconesculentum* –Mill root rot infection, (Patil et al. 2012), *T. harzianum* for controlling peanut crown rot (Abdel-Kader et al. 2013) and *T. harzianum* in melons (Galletti et al. 2015).Biljana et al. (2018) evaluate that the in vitro antagonistic activity of *Trichoderma asperellum* and *T. harzianum* against 18 genetically diverse *B. cinerea* isolates. The results showed considerable antagonistic abilities of both *Trichoderma* species against all tested *B. cinerea*isolates. Both antagonists significantly ($p < 0.01$) inhibited the mycelial growth (*T. asperellum* from 74.24% to 96.91% and *T. harzianum*from 71.072% to 95.88%) and conidial germination (*T. asperellum* from 76.932% to 95.107% and *T. harzianum* from 76.93% to 93.65%) of *B. cinerea* isolates.Considering all these fact a studies on antagonistic activity of Trichoderma spp. Ere studied against soil fungal isolates Antagonistic activity of *Trichoderma atroviride* and *Trichoderma herzanium* were screened against the five dominant soil fungi viz. *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata* and *Alternaria alternata.*

Material and Method

Isolation of fungi from agricultural field:

Fungal isolation was carried out with dilution plate technique from soil samples. 01 gm weight of soil sample was diluted in 10 ml of distilled water and shakes the mixture carefully. One ml. of diluted sample was diluted in10 ml of distilled water then diluted sample was poured and spread on sterilized petriplates containing sterilized Potato Dextrose Agar medium, 1 percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. All this processes are carried out in sterilized Laminar Air Flow. The inoculated plates were stored in incubator at room temperature for 3 Days. Microscopic study was also done. Characters observed like types of hyphae their branching pattern, presence and absence of septa, types of spores, sporophore were observed during the study.

Identification of fungi

All the selected pathogenic fungi like *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata*and*Alternaria alternata*and two fungal speciesof*Trichoderma atroviride*and*Trichoderma herzanium* were microscopically identified and analyzed by with help of books and manual and most updated keys viz. Raper and Thom (1949), Raper et al., (1965), Nagamani A, Kumar IK and Manoharachary C. (2006), Eills (1976), Mukadam (2006) and Barnett and Hunter, (1998).

Antagonistic activity of *Trichoderma*

Seven days pure cultures of five different soil isolates like *Aspergillus niger, Aspergillus flavus, Colletotrichum falcatum, Curvularia lunata*and*Alternaria alternata* were inoculated on PDA medium containing at the centre of the petriplate and same culture of fungi were inoculate at four different places in the different petriplate with Trichoderma culture in the centre of the four samples containing petriplates. These inoculated petriplates kept for incubation at 280 C for four days. The antagonistic activity of *Trichoderma sp.* was observed. Inhibition of mycelial growth of pathogenic fungi was calculated by

% Inhibition of radial mycelial growth=	$\frac{C-T}{C}$	X 100

C = radial mycelia growth of the pathogen in control
T = radial mycelia growth of the pathogen in the presence of *Trichoderma*

Results and Discussion:

Antagonistic activity of *Trichoderma atroviride* and *Trichoderma herzanium* were screened against the five dominant soil fungi viz. *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata* and *Alternaria alternata.* During the in vitro assay of antagonism was calculated by percent growth of inhibition of *Trichoderma atroviride* result were shows in (Table 1 and Figure 1.) that the highest growth of inhibition was seen against the Alternaria alternata (66.66%) after that *Curvularia lunata*(62.82%) and *Aspergillus flavus* (59.61%) the lowest percent of growth inhibition was seen *Colletotrichum falcatum* (53.84)and *Aspergillus niger* (56.25%). José Aldo et. al. (2017) Two Trichoderma strains showed a significant (P≤0.05) potential antagonism activity against *S. Lignicola* (CMM 1098) mycelial growth. All two Trichoderma strains inhibited the mycelia growth of *S. lignicola* (CMM 1098), which evidenced a direct antagonist action. The most efficient was T. Harzianum (URM 3086), with 80.78% of mycelial growth inhibition followed by T. aureoviride(5158) with 69.38% of mycelial growth inhibition Reddy et al. (2014) tested *Trichoderma viride, T. harzianum, T. reesei, T. atroviride, T. pseudokoningii, T. koningii* and *T. virens* against *Fusarium oxysporum f. sp. lycopersici,Alternaria solani, Aspergillus niger* and *Macrophominaphaseolina* and found that all antagonists inhibited the growth of pathogens, suggesting an inhibition mechanism such as mycoparasitism and the production of volatile and non-volatile metabolites

Table 1. Antagonistic effect of *Trichoderma atroviride*

Percentage of growth Inhibition			
Fungi	**C**	**T**	**Percentage inhibition**
Alternaria alternata	30	10	66.66
Aspergillus flavus	52	21	59.61
Aspergillus niger	48	21	56.25
Colletotrichum falcatum	39	18	53.84
Curvularia lunata	35	13	62.82

Table 2 . Antagonistic effect of *Trichoderma harzianum*

	C	T	Percentage inhibition
Alternaria alternata	30	12	60.00
Aspergillus flavus	52	19	63.46
Aspergillus niger	48	23	52.08
Colletotrichum falcatum	39	16	58.97
Curvularia lunata	35	11	68.57

Fungi *Percentage of growth Inhibition*

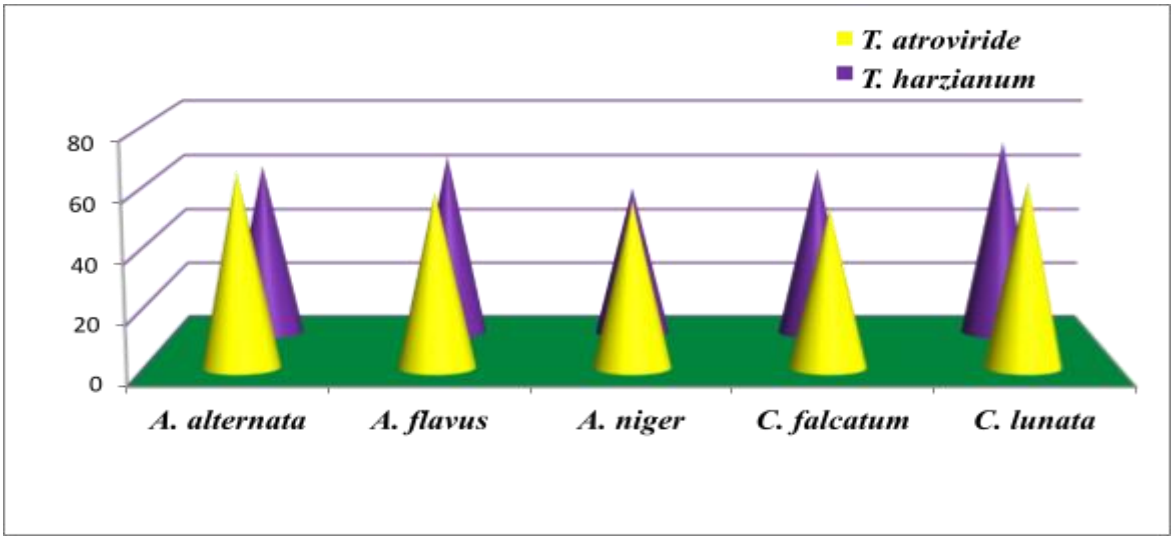

Figure 1. Percent Inhibition of *Trichodermasp*. on different fungi

It was clear from the Table 1 and Figure 1 the *Trichodermaharzianum* showed a high antagonistic activity on PDA, and it led to the highest percentage of inhibition of *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata* and *Alternaria alternata*.*Trichoderma harzianum*shows highest potency against the *Curvularia lunata* (68.57%), next to that *Aspergillus flavus* (63.46%) and *Alternaria alternata* (60.00%), the lowest inhibition of *Aspergillus niger*(52.08%) *Colletotrichum falcatum*(58.97%). Dennis and Webster (1971) and Jinantara (1995). In this study, *T. harzianum*(IMI-

392432) displayed the best performance using the poison agar method at different concentrations of metabolites and on

different days. To know whether the antibiotic action of secondary metabolites of Trichoderma were diffusible as well as antifungal, the modified bilayer agar experiment was carried out. The inhibition of radial growth of *C. paradoxa*was very pronounced compared to the growth of the uninoculated control bilayers. It is clear that the presence or absence of Trichoderma a metabolites can have a significant role on the outcome of *C. paradoxa*mycelia. This experiment confirmed that the metabolites produced by *T. harzianum* are diffusible and can prevent, inhibit, or sup-press the growth of *C. paradoxa* in culture. Therefore, *Trichoderma* has a large potential as a biocontrol agent against *C. paradoxa.* In previous studies, Schoeman et al. (1996) reported that metabolites of *T. harzianum* could influence the outcome of the decay caused by Basidiomycetes in freshly-felled pine.

Conclusion

Trichoderma atroviride and *Trichodermaharzianum* have potential antagonism against different fungi during the analysis of antagonistic activity on soil fungi like *Aspergillus niger, Colletotrichum falcatum, Curvularia lunata* and *Alternaria alternata* on mycelia growth and sporulation. *Trichoderma atroviride* and *Trichodermaharzianum* shows the antigonastic activity considered as promising biological control agents for control of soil fungi as wel soil borne diseases. These findings can be useful to develop locally customized and innovative approaches to address major threats facing soil borne diseases.

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