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Antagonistic Study of Some Soil Borne Fungi against *Fusarium solani*

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Abstract
Antagonistic activities of some soil borne fungi were studied against *Fusarium solani*, the most frequently occurring soil borne fungal pathogen, attacking a large number of host plants including oilseeds, pulses, vegetables and ornamentals. Bio-control of plant pathogens is an advantage over chemical pesticides as these pesticides frequently causes some phyto-toxicity and are toxic to non-target organisms, as well as cause environmental pollution. *Fusarium solani* was isolated from lady's finger field as the experimental pathogen by serial dilution culture technique. *Trichiderma virens* and *Aspergillus niger* were also isolated from soil through the same technique. Antagonistic potentiality of the two antagonists were studied through dual culture technique and through volatile metabolites effect on colony growth inhibition, on potato dextrose agar medium as the culture medium. In dual culture technique, *Aspergillus niger* showed the 59.49% of bio-control whereas, *Trichoderma virens* showed 39.24%. Volatile metabolites of *Trichoderma virens* were more effective in comparison to *Aspergillus niger* as in volatile effects treatment, there was a 53.33% inhibition of colony growth of *Fusarium solani* by *Trichoderma virens* whereas *Aspergillus niger* showed 47.22% of inhibition. The results indicate that some naturally inhabiting soil borne fungi may be exploited for the bio-control of plant pathogens, either singly or in combination. This paves a way in the direction of eco-friendly, non-toxic control of plant diseases.

Keywords: *Fusarium solani, Trichoderma virens, Aspergillus niger,* antagonists, volatile metabolites

Introduction
Fusarium species are cosmopolitan and are the best known soil borne plant pathogens in term of economic damage in agricultural productions all over the world [1-3]. *Fusarium solani* (Mart.) Sacc, itself attacks a large number of host plants, including oilseeds, pulses, vegetables and ornamental [4-7]. Under *In-vitro* condition, on PDA medium, *Fsarium solani* form white cottony colony and its underside is pale yellow in colour. It produces asexual spores- macroconidia, microconidia and chlamydospores. Macroconidia are slightly curved and have 3, 4 or 5 septa whereas miroconidia are oval and are either aseptate or with 1to 2 septa. Chlamydospores are thick walled and are formed during suboptimal condition. They are the survival structure in the absence of a host plant.
Antagonistic interactions have been recognized as the mechanisms of biological control [8-13]. Recently the control of plant pathogens by adding antagonistic microorganisms to the soil is getting attention as is a potential non-chemical means of plant disease control to overcome the toxic effects of chemical synthetic pesticides. Antagonism involves antibiosis, competition and exploitation. Several studies report on competitive ability among soil inhabiting fungi based on antibiotics or enzyme production or substrate colonization [14]. The success of bio-control of phyto-pathogenic fungi promoted the screening of fungal strains for their antagonistic potentiality. *Trichoderma,* species (soil borne cosmopolitan fungi) are extensively exploited worldwide as biocontrol agentes. They have a unique ability to control the plant pathogens through the mechanism of mycoparasitism, antibiosis, competition, siderophore production, induction of systemic resistance, growth promotion etc. [15-18]. Works are also going on to use *Aspergillus* spp. as bio-control agents. *Aspergillus* species (*A. niger, A. flaves, A. terreus, A. fumigatus*) are used as biological control agents against many fungal pathogens such as *Fusarium oxysporum, Sclerotinia sclerotiorum, Pythum* spp., and are act through mycoparasitism, competition, mycelial lysis and antibiosis via the synthesis of volatile and / or non-volatile metabolites [19-21].

Objectives

Trichoderma spp. are found to be very potential bio-control agents against many pathogens including *Fusarium* spp. [22]. The main of this study was to determine the extent of antagonism of *Trichoderma* virens and *Aspergillus niger* against *Fusarium solani.* This may throw light on selecting appropriate species and isolate combination of fungi to be used against target fungi.

Materials and Methods

Materials

Soil Samples: To isolate pathogen, soil samples were collected from the rhizosphere of the vegetable plants, at the depth of six inches, of local vegetable crop-fields. Soil samples were also collected from moist garden area with healthy vegetation for *Trichoderma* isolation

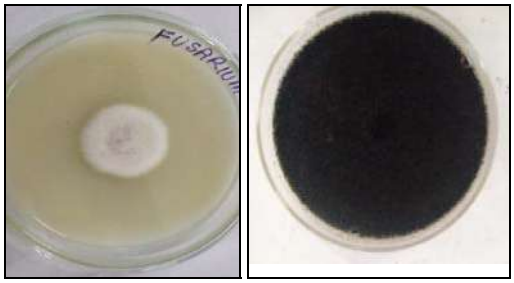
Culture Medium:- Potato Dextrose Agar (PDA) medium was prepared to use as culture medium.

Methods

Isolation of Fungi

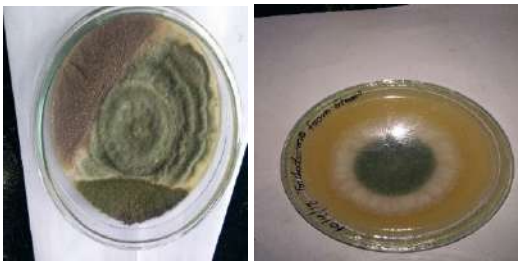
Collected soil samples were cultured through serial dilution culture technique on PDA medium and incubated at 25±2° c. After 3-4 days, a number of fungal colonies were appear to grow in the petri-plates. A pure culture of each colony type, growing in petri plates, was obtained and maintained by sub-culturing. Cultures were identified on the basis of macro and microscopic characteristics, reverse surface coloration of colonies, conidial morphology and slide culture technique [23-27]. Thus pure cultures of *Fusarium solani*, *Aspergillus niger* and *Trichoderma virens* were isolated and maintained.

Fig 1: A number of fungi colonies growing in soil culture



Isolated pure *Fusarium* colony | Isolated pure *Aspergillus* colony

Fig 2



Trichoderma in soil culture plate with others colonies | Isolated *Trichoderma virens*

Fig 3

Determination of antifungal activities of the antagonists against Fusarium solani –

Two methods were used to study the antagonistic effects against Fusarium solani.

a) Dual culture technique

b) Volatile metabolites effects of antagonists

a) Dual culture technique.

In dual culture technique [28], five days old cultures of each, antagonists as well as pathogen were used for further experiments. A five mm disc of Fusarium solani culture was cut with the help of a sterilized cork-borer and placed in a 90mm petri plate, containing PDA-medium, approximately 25mm apart from periphery. The same was repeated to prepare required number of plates for treatment with antagonists and control. Then with the help of sterilized cork-borer, 5mm disc of Trichoderma sp. was cut and placed nearly 40mm apart and opposite to Fusarium disc. The same was repeated with Aspergillus niger. Controls for each antagonist were also cultured. All the combinations were in triplicates and incubated at 25±2°c.

Fig 4: Dual culture showing antagonistic effects

Colony growths of both, antagonists and test pathogen were measured periodically and data was recorded. The percentage inhibition of growth was calculated as follows:-

Percentage inhibition of growth = $(r\text{-}r^1/r) \times 100$, where

r = growth of the fungus, measured from the centre of the colony towards the centre of the plate in the abscence of antagonistic fungus (control).

r^1 = growth of the fungus, measured from the center of the colony towards the antagonistic fungus.

The colony interaction between the test pathogen and the antagonists soil fungi were assessed [29-30]. Five type of interaction grade [31] have been used.

1. Mutual intermingling without any macroscopic sights of interaction. – Grade1.
2. Mutual intermingling growth, where the growth of fungus is ceased and being over growth by the opposed fungus. – Grade2.
3. Intermingling growth, where the fungus under observation is growing on the opposed fungus either above or below. –Grade3.
4. Sight inhibition of both the interacting fungi with narrow demarcation line. – Grade4.
5. Mutual inhibition of growth at a distance of >2mm. – Grade5.

b) **Volatile effects of antagonists**

For the study of volatile effect of *Trichoderma virens* and *Aspergillus niger* on *Fusarium solani*, re-culture of each were done on PDA medium, from the maintained stock. On the third day of culture, under sterilized condition, lids of petriplates containing cultures of *Fusarium solani* and *Trichoderma virens* were removed and the plate of *Fusarium solani* was kept inverted on the plate of *Trichoderma virens*, then the two plates were bounded by cello-tape. The same was done for *Fsarium solani* and *Aspergillus niger*. All the combination were in triplicate and incubated at 25±2°c. Control plates for each were also maintained. Measurements of the colony growth of *Fusarium solani* in each combination were taken periodically till the day when the test fungi showed the full petriplate growth in control. The percentage inhibition due to volatile effect was calculated using the formula:-

% inhibition = $(d-d^1/d) \times 100$, where
d = diameter of the colony of the test fungi in control
d^1 = diameter of the colony of the test fungi in treatment

Results and discussion

Table 1: Inhibition of Fusarium solani colony in dual culture.

	***Fusarium solani* in control**	***F. solani* in treatment with *Trichoderma virens***	***F. solani in treatment with* Aspergillus niger**
Colony growth in mm	39.5	24	16
% inhibition		39.24	59.49

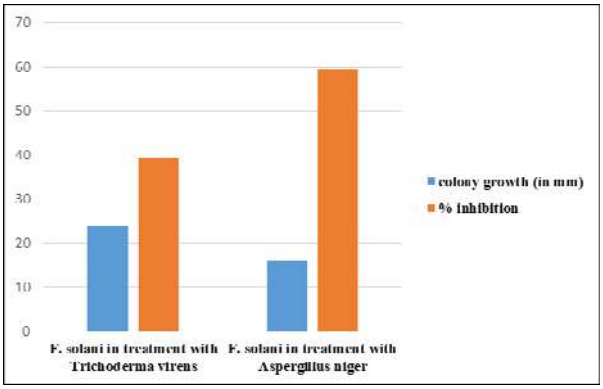


Fig 6: Graphical representation of Table-1

Interaction Grade between the test pathogen and the antagonists in dual culture.

Fusarium solani in treatment with *Trichoderma virens*	Grade 2
Fusarium solani in treatment with *Aspergollus niger*	Grade 4

In dual culture, *F. solani* with *T. virens* treatment had Grade 2 type of interaction, with a overlapping zone of 4-5mm and there was a 39.24% inhibition of colony growth of *Fusarium* whereas *F.solani* with *A.niger* treatment had Grade 4 type of interaction with a clear zone on both side of the *Fusarium* colony but there was a marginal contact of the two fungal colonies in-front. It showed 59.49% inhibition of *Fusarium* colony (Table-1).

Table 2: Inhibitory effects of volatile metabolites of *T. virens* and *A. niger* on *F. solani.*

	Fusarium solani in control	*Fusarium solani* in treatment with *Trichoderma*	*Fusarium solani* in treatment with *Aspergillus*
On the day of treatment	38.5	38.5	38.5
After the 6th day of treatment	90	42	47.5
% Growth	133.77	9.09	23.38
% Inhibition	0	53.3	47.22

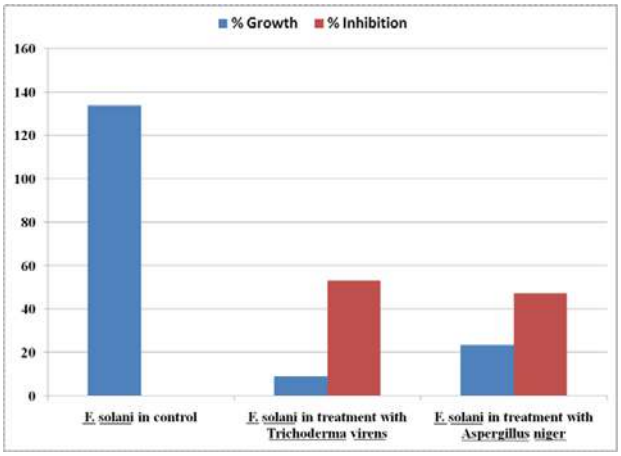


Fig 6: Graphical representation of Table-2

In the treatment for volatile metabolites effect, *Trichoderma virens* was more effective and inhibited 53.3% of colony growth of *Fusarium solani* in comparison to that of *Aspergillus niger*, showing 47.22% of inhibition.

Data of the two tables also indicate that volatile metabolites of *Trichoderma virens* are more effective in pathogen control (53.3% of inhibition) in comparison to non-volatile metabolites, as in dual culture there is only 39.24% inhibition of pathogen colony. In dual culture, inhibition of *Fusarium* colony is may be due to parasitism.

Fusarium control by *Aspergillus niger* is more in dual culture (59.49%) in comparison to volatile metabolites effect (47.22%). This interfers that non-volatile metabolites of *Aspergillus niger* are more effective in controlling pathogen than the volatile metabolites and control may be due to antibiosis.

Conclusion

The above results clearly suggest that *Trichoderma virens* as well as *Aspergillus niger* are promising and suitable for

application as bio-control agents against soil borne pathogen," *Fusarium solani*" and may also be exploited to control other pathogens causing diseases in different crops. To make their use, as bio-control agents more meaningful and environmentally safe, research efforts are needed to find out the toxic compounds present in them and their mode of action.

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