RESEARCH PAPER | Botany | Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

# In Vitro Assessment of Antagonistic Activity of Trichoderma Viride and Trichoderma Harzianum Against Pathogenic Fungi

KEYWORDS: Cobb-Douglas Production function, Marginal Productivity of Labour, Marginal Productivity of Capital, Indian Cement Companies.

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**ABSTRACT** *Trichoderma is a promising candidate for the biological control of plant pathogenic fungi. In an attempt to develop biocontrol system for management of different fungal vegetable diseases, Trichoderma viride and Trichoderma harzianum were evaluated for their antagonistic activity against five different pathogens in vitro. Both Trichoderma spp were strongly antagonized with five different pathogenic fungi viz. Colletotrichum lindemuthianum (C.o), Fusarium oxysporum (F.o), Rhizoctonia solani (R.o), Alternaria solani (A.s), Fusarium solani (F.o) in dual culture assay. T. viride gave maximum inhibition of mycelia growth to all pathogenic fungi (67.45%), where as T. harzianum showed (63.89%) inhibition of mycelia growth. Volatile metabolites produced by T. viride exhibited highest growth inhibition rate (37.85%) followed by T. harzianum (37.78%). The Metabolites released from T. harzianum, T.viride were tested in culture medium against all pathogens. Cell free metabolites of T. viride, T. harzianum inhibited the growth of all five pathogens in vitro and appeared to be fungicidal in its activity.*

**INTRODUCTION:**
Agriculture is the backbone of nation's economy, growth and development. Various groups of pathogens are known to cause losses to agricultural yield all over the world including India. Various methods for controlling such diseases have been investigated including the use of resistant varieties (Brisa et al., 2007), chemical control, cultural practices (Punja et al., 1986), plant volatile compounds (El-Mougy et al., 2007), plant extracts (Kumar and Tripathi, 1991). Fungicidal application as seed or soil treatment, however, has been found to be ineffective against these pathogens as the propagules are capriciously distributed in the soil and often beyond the reach of chemicals (Campbell, 1989). Due to environmental concerns there is considerable interest in finding alternatives to chemical pesticides for suppression of soil-borne plant pathogens and plant-parasitic nematodes (Larkin et al., 1998; Raupach and Kloepper, 1998). Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Trichoderma spp. is one of the most important biocontrol agent used for management of different diseases (Harman, 2004). Trichoderma spp. can directly impact other fungi, after sensing a suitable fungal host, Trichoderma spp. responds with the production of antibiotic compounds, formation of specialized structures, and degradation of the host's cell wall, followed by the assimilation of its cellular content, a process known as mycoparasitism (Chet and Chernin, 2002; Steyaert et al., 2003; Benitez et al., 2004). The mechanisms of mycoparasitism, antibiosis and competition afforded by Trichoderma spp. have been widely studied (Howell, 2003; Harman et al., 2004b). The objective of the present investigation was isolation and screening of effective Trichoderma spp. against five fungal pathogens of fruit vegetables.

**MATERIAL AND METHODS**
**Isolation and identification of Trichoderma species:**
Trichoderma species were isolated form soil. One gram of the soil sample was taken and added to 1ml of sterilized distilled water to make a dilution of $10^{-1}$. This suspension was then subjected to serial dilutions and a dilution of $10^{-5}$ was attained. One milliliter of each dilution viz., $10^{-3}$ to $10^{-4}$ was poured on to plate containing fresh PDA and spreaded smoothly by sterile spreader. Then these plates were incubated for 28±2°C for 7 days. After incubation period they were identified by using soil manual of (Jha 2004). The purified and identified cultures of Trichoderma spp. were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further use.

Both the tested bio-control agents, T. viride and T. harzianum were exposed to different pH and temperature, at pH 7, and 30°C temperature both Trichoderma spp showed maximum growth rate after 7 days of incubation period.

**Dual culture Technique :**
To determine the effect of Trichoderma spp. on mycelial growth of targeted pathogens, a dual culture method was used. The dual culture of two Trichoderma spp. (T. harzianum, T. viride) and targeted pathogens were studied on Potato dextrose agar (PDA). 20 ml of PDA medium was poured in plates (9 cm) and was allowed to solidify. Discs (5mm diameter) of mycelium cut from the margin of 6 days old culture of each Trichoderma spp. were placed at the edge of each plate., 10 mm from the periphery. Then disc of 5mm diameter of mycelium cut from the growing edge of 7 days old cultures of targeted fungal pathogens were placed on each plate, opposite to the mycelial discs of Trichoderma spp. In control plates, a sterile disc whatman No.1 filter paper of 6mm diameter was placed at opposite side of targeted fungal pathogens in complete aseptic condition. Three replications were maintained for each Trichoderma spp. and targeted fungal pathogens seperataly. All the plates were incubated at 25+1°C for about 7 days after inoculation. The radial growth of all fungi was measured, when the Trichoderma spp. in control plates show complete growth. The colony diameter of both Trichoderma spp. and targeted fungal pathogens were measured at two locations, right angle to each other and the average diameter was calculated. Percent inhibition of mycelial growth of targeted fungal pathogens over control was calculated by following equation given by Vincent (1947):

$$\%\ \text{Inhibition} = \frac{D1 - D2}{D1} \times 100$$

D1 = Colony diameter in the control.

D2 = Colony diameter in treated.

Detection of antifungal activity by Volatile metabolites from antagonistic fungi: (Dennis and Webster., 1971)

Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

The effect of volatile metabolites released by both Trichoderma spp were evaluated against growth of targeted fungal pathogens of vegetables. For antifungal activity of volatile, a petriplate containing PDA medium was inoculated with 5mm diameter plug of Trichoderma isolates growing on PDA. A second petriplate containing PDA was inoculated with a 5mm plug of the targeted pathogens in the center of the plate and inverted over the Trichoderma spp. culture. The two plates were sealed together with nescofilm and incubated at 28°C for 6 days. This ensured that both organisms were growing in the same atmosphere. For control instead of Trichoderma spp. one plug of PDA was placed on Agar surface. All the experiments in vitro were arranged as Randomized complete design with three replications. The surface areas of the colonies of targeted pathogens were recorded compared with controls and the percentage of growth inhibition was calculated by using Vincent (1947).

**Detection of antifungal activity by Non-volatile metabolites from antagonistic fungi:**

To determine the effect of the non-volatile metabolites on mycelia growth of pathogen poisoned food technique was used. For the production of non-volatiles, three discs of mycelial agar plugs (6 mm diameter) obtained from edges of 7 days old culture of Trichoderma spp. were inoculated in 100 ml sterilized potato dextrose broth (PDB) in 250 ml conical flasks and incubated at 25 ± 1°C on a rotary shaker at 100 rpm for 14 days. The control conical flasks were inoculated with sterile PDA plugs respectively. After incubation, the culture was filtered through Millipore filter for removing spores for collecting non-volatile metabolites from Trichoderma spp.

Collect the transparent supernatant containing non volatile metabolites. For poisoned food assay the liquid formed non-volatile was added to molten PDA medium (at 40 ± 5°C) to obtain a final concentration of 10% (v/v). The medium was poured in Petri dishes at 20 ml per plate and inoculated with 5 mm mycelial plugs of the pathogens in the centre of the plates and incubated at 25 ± 2°C for 7 days or until the colony reached the plate edge in control plate. Triplicates were maintained for each treatment and radial growth of the pathogen was recorded by using formula of Vincent (1947).

**RESULTS:**

When all the pathogenic fungi were tested in combination with T. viride and T. harzianum among them Rhizoctonia solani was found to be most susceptible and revealed highest percent of inhibition of mycelia growth of 85.32%. While Fusarium oxysporum was most resistant and revealed lowest percent inhibition of mycelial growth as 38.27% in combination with T. viride and 41.65% in combination with T. harzianum (Table.1). The biological agent has the ability to produce volatile metabolites which were evaluated against different fungal pathogens of vegetables by adopting the method (Dennis and Webster, 1971). In this screening, when T. viride was tested, Fusarium oxysporum was found to be more susceptible and showed 44.88%, where as Alternaria solani showed least percent inhibition of mycelia growth as 25.26% over control. The antifungal activity of volatile metabolites produced by T. harzianum, showed 46.04% maximum inhibition of mycelia growth of Colletotrichum lindemuthianum. On contrary Fusarium solani was found to be most resistant and showed 25.27% (Table.2) inhibition of mycelia growth. As the Biological agents has the ability to produce Non-volatile metabolites, Antifungal activity of non-volatile metabolites were tested against all tested fungal pathogens by Agar diffusion method. Among all the fungal pathogens of Fusarium oxysporum was found to be more susceptible to non-volatile metabolites of T. viride and showed 81.62% inhibition of mycelia growth over control. Whereas Alternaria solani showed minimum percent inhibition of mycelia growth as 76.42%. . But in case of T. harzianum, the maximum percent inhibition was shown by Colletotrichum lindemuthianum as 69.36% (Table.3)

| Bioagents | Pathogens | Radial Growth of Bioagents(cm) | Growth of Pathogens (cm) | % of Inhibition |
|---|---|---|---|---|
| Trichoderma viride | Fusarium oxysporum | 5.6±0.02 | 3.6±0.09 | 38.27% |
| | Fusarium solani | 7.7±0.04 | 1.3±0.04 | 78.54% |
| | Alternaria solani | 7.3±0.04 | 1.7±0.04 | 76.17% |
| | Rhizoctonia solani | 8.2±0.05 | 0.8±0.13 | 85.32% |
| | Colletotrichum lindemuthianum | 5.7±0.09 | 3.3±0.09 | 41.16% |
| Trichoderma harzianum | Fusarium oxysporum | 5.6±0.11 | 3.4±0.13 | 41.65% |
| | Fusarium solani | 7.5±0.09 | 1.5±0.09 | 75.32% |
| | Alternaria solani | 8.2±0.09 | 1.7±0.04 | 76.17% |
| | Rhizoctonia solani | 8.2±0.09 | 0.8±0.05 | 85.32% |
| | Colletotrichum lindemuthianum | 6.7±0.17 | 2.3±0.13 | 58.83% |
| Control (pathogens) | | - | 9.0 | - |

Each value is an average of 3 replicate samples, + Standard error.

**Table 1. Dual culture technique.**

**Table 2. Volatile compounds produced by Trichoderma spp. against five pathogens.**

| Treatments | Radial Growth of Pathogens (cm) | % inhibition |
|---|---|---|
| T.v+Fusarium oxysporum | 3.93±0.08 | 44.88% |
| T.v+ Fusarium solani | 3.73±0.16 | 41.07% |
| T.v+ Alternaria solani | 2.84±0.04 | 25.26% |
| T.v+ Rhizoctonia solani | 4.06±0.08 | 42.49% |
| T.v+Colletotrichum lindemuthianum | 3.26±0.24 | 35.57% |
| Control | 9.0 | - |
| T.h+ Fusarium oxysporum | 4.46±0.26 | 37.44% |
| T.h+ Fusarium solani | 4.73±0.08 | 25.27% |
| T.h+ Alternaria solani | 2.26±0.08 | 40.52% |
| T.h+ Rhizoctonia solani | 4.26±0.16 | 39.66% |
| T.h+ Colletotrichum lindemuthianum | 2.73±0.12 | 46.04% |
| Control | 9.0 | - |

Each value is an average of 3 replicate samples + Standard error.

**Table 3. Effect of non-volatile compounds produced by antagonist(s) on the radial growth of five pathogens.**

| Treatments | Radial Growth of Pathogens (cm) | % inhibition |
|---|---|---|
| T.v+Fusarium oxysporum | 0.93±0.09 | 81.62% |
| T.v+ Fusarium solani | 0.93±0.04 | 77.58% |
| T.v+ Alternaria solani | 0.66±0.09 | 76.42% |
| T.v+ Rhizoctonia solani | 0.93±0.04 | 79.47% |
| T.v+Colletotrichum lindemuthianum | 0.66±0.09 | 80.92% |
| Control | 9.0 | - |
| T.h+ Fusarium oxysporum | 1.66±0.16 | 67.19% |
| T.h+ Fusarium solani | 2.46±0.09 | 46.52% |
| T.h+ Alternaria solani | 1.46±0.12 | 47.85% |
| T.h+ Rhizoctonia solani | 2.23±0.20 | 51.43% |
| T.h+ Colletotrichum lindemuthianum | 1.06±0.24 | 69.36% |
| Control | 9.0 | - |

Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

Each value is an average of 3 replicate samples + Standard error.

**DISCUSSION:**

Our results revealed that both *T. harzianum* and *T. viride,* which obtained from the rhizosphere, have been reported as the best antagonists for controlling the vegetable diseases caused by five fungal pathogens under laboratory conditions. Both *Trichoderma* spp. treatments reduced the mycelial growth of five pathogenic fungi. It is very important, especially the chemical methods are not economical in the long run, because they cause pollution in the atmosphere, damage the environment, leave harmful residues, and can lead to the development of resistant strains among the target organisms with repeated use. Biological control is a good alternative for sustainable agriculture to overcome the problems of public concern associated with pesticides and pathogens resistant to chemical pesticides and to become eco-friendly (Akhtar and Siddiqui, 2008). In the present study *T. viride* was found superior to *T.harzianum. T. viride* was also found to be superior, reported by Amin et al. (2010) as an important antagonist with the highest percent inhibition against soil borne pathogens of different vegetables viz., *Rhizoctonia solani, Sclerotium rolfsii* and *Sclerotinia sclerotiorum* under in vitro conditions. As dual culture method is widely used in antagonistic studies, the present investigation, was carried out to screen two *Trichoderma* spp. against five fungal pathogens by this method. The degree of inhibition varied from one pathogen to another. Maximum inhibition of the pathogens averagely (67.45%) was observed with *T. viride*. Different mechanism are said to be involved i.e. competition, production of antibiotics inhibiting fungal growth by producing volatile and non- volatile compounds as reported by (Michrina et al., 1995; Calistru, 1997). Growth inhibition of the pathogens by the *Trichoderma* metabolites has been reported by several workers (Dennis and Webester, 1971a, 1971b; Howell and Stipanovic, 1983; Sivan et al., 1984; Claydon et al., 1987; Ghisalberti and Sivasithamparam, 1991; Howell, 1998). In the present study, we tested *Trichoderma* species for their production of volatile compounds that inhibit the growth of five fungal pathogens in-vitro and it was found that *T. viride* inhibited the maximum mycelial growth of pathogens (37.85%). The earlier studies also revealed that antimicrobial metabolites produced by *Trichoderma* is effective against a wide range of fungal phytopathogens eg., *Fusarium oxysporum, Rshizoctonia solani, Curvularia lunata, Bipolaris sorokiniana* and *Colletotrichum lagenarium,Colletotrichum acutatum, Colletotrichum gloeosporioides* (yan et al 2006, Svetlana et al 2010). The non-volatile secondary metabolites from *Trichoderma* species were found more effective in suppressing the mycelial growth of all fungal pathogens when compared to volatile compounds. The culture filtrates of *T. viride* were found to inhibit the growth of pathogens averagely (37.85%). The effect of culture filtrate of *Trichoderma* on the pathogen might be due to the production of antibiotics (Upadhyay and Rai, 1987).

In conclusion, the *T. viride* is found to be effective against all five pathogenic fungi. If biocontrol potential of this strain is enhanced by generating mutants, it will become a promising biocontrol agent. Volatile and Non-volatile compounds produced by *Trichoderma viride* drastically reduced the mycelia growth and conidial production of test pathogens which is helpful in disease reduction by checking the survival and spread by pathogen.

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