# *IN-VITRO* EVALUATION OF *TRICHODERMA* SPECIES AGAINST SEED BORNE PATHOGENS

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**Abstract:** *Trichoderma* species are omnipresent and very popular as effective means of plant disease management. In present study, two *Trichoderma* species, (*T. viride* and *T. harzianum*) were screened against five seed borne phytopathogens (*Curvularia lunata, Fusarium oxysporum, Alternaria alternata, Colletotrichum gloeosporioides* and *Rhizoctonia solani*) by dual culture technique and the efficacy of volatile and non-volatile metabolites released by them was evaluated by 'inverted plate method' and 'poisoned food technique'. Both antagonists have exerted inhibitory effect on the growth of selected seed borne phytopathogens to a varied extent.
**Key words:** *Trichoderma*, antagonist, seed borne phytopathogen, bio-control.

## INTRODUCTION

Plants play an essential role in the life of mankind by providing food, timber, furniture and raw materials for all kinds of paper products. However, the productivity of plants generally is reduced when they become diseased. This may be due to insect pest attack or by their abnormal physiological process that disrupts the plant's normal structure, growth, function, or other activities. Various approaches are being followed to prevent, control and/or combat plant diseases. In the present study, *in vitro* efficacy of *Trichoderma* species as a bio-control agent has been investigated against five common seed borne fungi. The seed borne pathogenic fungi invade the seedlings in nursery and subsequently results in low productivity and huge economic loss. Therefore, knowledge of seed borne pathogenic fungi in relation to crop quality and quantity bears utmost importance for early identification and isolation of plant pathogenic fungi as well as timely development of suitable management strategies that goes long way in avoiding disease epidemics and crop losses.

It has been reported that incessant use of chemical fungicides may develop resistance in plant pathogenic fungi (Benitez *et al.* 2004; Kim and Hwang, 2007), therefore, alternative method must be followed for an effective disease control. Mohana *et al.* (2011) observed that the toxic effect of synthetic chemicals can be minimized by persistent search for safer pesticides and wider use of eco-friendly and effective management practices. One of such potential non-chemical alternative is the use of microorganisms as biological control agents for eco-friendly and sustainable management of plant disease (Kulkarni *et al.*, 2007). Biological control is a component of an integrated pest management strategy and is defined as the purposeful use of living organisms, their genes, and/or products, such as metabolites that reduces the pest populations. *Trichoderma* spp. are soil-borne fungi and have significant antagonistic potential against a wide range of phytopathogenic fungi (Elad *et al.*, 1982). They are free-living and diverse fungal microbial community known worldwide for their utility as bio-control agents in management of fungal diseases of crop plants. The genus *Trichoderma* was identified long back during the early 17th century but its bio-control ability was revealed only in thirties by Weindling (1932; 1934; 1937) whereby, he described in detail the action and mode of mycoparasitism by *Trichoderma* on *Rhizoctonia* and *Sclerotinia*. Furthermore, most of the *Trichoderma* spp. are known to produce volatile compounds such as acetaldehyde, ethylene, acetone and carbon dioxide (Tamini and Hutchinson, 1975) and few produce antibiotics such as Trichodermin (Godtfredsen and Vangedal, 1964), gliotoxin, viridin (Dennis and Webster, 1971a; Grove *et al.*, 1996; Mujeebur *et al.*, 2004) and ergokonin (Kumeda *et al.*, 1994) accountable for antifungal and antibacterial properties.

The bio-control exercised by *Trichoderma* spp. is due to various mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion (Chet, 1987; Schirmbock *et al*. 1994) that result in enhancement of plant resistance to disease, plant growth and productivity. But the direct mycoparasitic activity of *Trichoderma* spp. is supposed to be one of the major mechanisms responsible for their antagonistic activity (Dennis & Webster, 1971c; Elad *et al.*, 1982; Lynch, 1987). This trait is the basis for the use of different *Trichoderma* strains as an alternative to the hazardous fumigants and fungicides (Chet, 1987; Harman and Bjorkman, 1998). After host recognition, *Trichoderma* spp. attaches to the host hyphae via coiling, and invade the cell wall by secreting cell wall-degrading enzymes like chitinases (Tokimoto, 1982; Elad *et al*., 1983), β -1,3-glucanases (Kubicek, 2001) and proteases (Elad and Kapat, 1999). The combined action of these compounds result in parasitization of the target fungus and dissolution of the cell wall. Moreover, research has revealed that strains having more capacity to produce cell wall degrading enzymes possessed higher bio-control potential (Lima *et al.*, 1997). Bio-control mechanisms

are probably specific for particular antagonists and plant pathogens and several mechanisms can operate independently or synergistically in any microbial interaction.

Ambuse *et al.* (2012) evaluated ten *Trichoderma* species against sensitive and resistant isolates of *Alternaria tenuissima* in dual culture method and recorded up to 80% antagonistic activity by *T. viride, T. koningii* and *T. pseudokoningii*. Choudhary and Reena (2012) screened nineteen isolate of *T. harzianum, T. viride* and *T. koningii* by dual culture technique and liquid culture filtrate assay against *Fusarium oxysporum* f. sp. *lentis;* responsible for wilt of lentil and observed significant growth inhibition of pathogen. Ajith and Lakshmidevi (2010) observed inhibitory effect of volatile and non-volatile compounds released by *T. saturnisporum, T. harzianum, T. viride* and *T. reesei* against *Colletotrichum capsici,* a fungal pathogen responsible for anthracnose disease in bell peppers (*Capsicum frutescence*). Tapwal *et al.* (2011) also found *T. viride* as potential antagonist against species of *Rhizoctonia, Alternaria, Curvularia* and *Fusarium* under laboratory conditions. Rahman *et al.* (2013) evaluated different *Trichoderma* strains against *Colletotrichum capsici* under laboratory conditions by different techniques and found the *T. harzianum* as potential antagonist for inhibition of the mycelial growth, conidial germination, germ tube elongation and disease severity of *C. capsici*. Different *Trichoderma* strains produce more than 100 different metabolites that have known antibiotic activities (Sivasithamparam and Ghisalberti, 1998).

In present investigation, *Trichoderma viride* and *T. harzianum* were evaluated in *in-vitro* conditions against five common seed borne pathogen i.e. *Curvularia lunata, Fusarium oxysporum, Alternaria alternata, Colletotrichum gloeosporioides* and *Rhizoctonia solani.*

## MATERIALS AND METHODS

### Pathogens and antagonists

Seed borne pathogens (*Rhizoctonia solani, Fusarium oxysporum, Alternaria alternata, Curvularia lunata* and *Colletotrichum gloeosporioides* and two fungal antagonists (*Trichoderma harzianum* and *T. viride*) were obtained from Forest Pathology Division, Forest Research Institute, Dehradun.

### Growth inhibition of pathogens in dual culture

5 mm discs of antagonist and pathogen were co-inoculated 4 cm apart on Potato Dextrose Agar (PDA) in Petri plates. In control, only a disc of pathogen was inoculated. The plates were kept in incubator at 25±1°C. Radial growth of the pathogens was measured on the third day of incubation and compared with the growth of pathogen in control (Dennis and Webster, 1971c). Whole of experiment was carried out in triplicates. Percent growth inhibition was determined by the formula:

Percent growth inhibition = $(A_1-A_2)/A_1 x100$

Where $A_1$ is area covered by pathogen in control and $A_2$ is area covered by pathogen in dual culture.

### Growth inhibition of pathogens by non-volatile compounds released by *Trichoderma* species

Poisoned food technique (Nene and Thapliyal, 1993) was followed to evaluate the effect of non-volatile compounds/ metabolites released by the *Trichoderma* spp. on the growth of pathogens. The *Trichoderma* spp. were grown in Potato Dextrose Broth (PDB) assuming that the antagonist will utilize the nutrients from broth and release some non-volatile metabolites the medium, which may affect the growth of pathogen. The *Trichoderma* spp. were incubated for two weeks and harvested at the interval of one week. After the incubation, the broth was collected, filtered through Whatman-I filter paper and later through syringe filter (Ran Disc, PVD 0.45 µm) under aseptic conditions. PDA was amended with culture filtrate (20%) just before pouring and inoculated with pathogen. Colony diameter of pathogen was measured after three days and compared with the growth of pathogen maintained in control Petri plates amended with equal amount of distilled water. The colony diameter of the pathogen was measured on the third day and compared with control.

### Growth inhibition of pathogens by volatile compounds released by *Trichoderma* species

The effect of volatile compounds released by *Trichoderma* species was evaluated by 'inverted plate technique' (Dennis and Webster, 1971b). The antagonists and pathogens were inoculated in the center of Petri plates poured with PDA. The Petri plate inoculated with pathogen was inverted over the Petri plate containing antagonist and two were sealed with the adhesive tape (parafilm) keeping antagonist in lower and pathogen in upper Petri plate. In control, the Petri-plate containing pathogen was inverted over the Petri plate containing medium only and incubated at 25±1°C. The colony diameter of the pathogen was measured on the third day and compared with control.

## RESULTS AND DISCUSSION

### Growth inhibition of pathogens by *Trichoderma* spp. in dual culture

It has been observed that both *Trichoderma* spp. restricted the growth of selected pathogens and an inhibition zone was observed at the point of contact. Since *Trichoderma* spp. are fast growing, they overgrew the pathogens with increase in the incubation period. The results revealed that on third day of incubation *T. harzianum* recorded maximum growth inhibition (34.20%) against *A. alternata* (Table-1) followed by *F. oxysporum* (27.04%), *C. lunata* (25.64%), *C. gloeosporioides* (15.00%) and minimum for *R. solani* (5.10%), while in case of *T. viride* highest growth inhibition was observed against *C. lunata* (46.79%) followed by *A. alternata* (34.27%), *F. oxysporum* (15.80%), *C. gloeosporioides* (12.50%) and lowest for *R. solani* (1.45%). The inhibition in radial growth of two interacting organisms in dual culture is attributed to inhibitory substance released by one or both organisms, competition and also mechanical

obstruction and hyperparasitism (Dennis and Webster, 1971c; Barnett and Binder, 1973). In the present study, selected species of *Trichoderma* showed varied degree of inhibition against five selected seed borne phytopathogens. This may be due to mycoparasitism or secretion of antibiotics in agar plates. Similarly, Tapwal *et al.* (2011) and Prasad and Kumar (2011) reported the *Trichoderma* as potential antagonist against the phytopathogen like *R. solani, A. alternata, C. lunata* and *F. oxysporum* in dual culture technique. Ambuse *et al.* (2012) reported 80% antagonistic activity by three *Trichoderma* species *i.e. T. viride, T. koningii* and *T. pseudokoningii* against sensitive and resistant isolates of *Alternaria tenuissima* in dual culture experiments.

**Table-1. Growth inhibition (%) of seed borne pathogens by *Trichoderma* species on third day of incubation in dual culture**

| S. No. | Seed borne pathogen | Growth inhibition (%) | |
|---|---|---|---|
| | | *T. viride* | *T. harzianum* |
| 1 | *Rhizoctonia solani* | 1.45 | 05.10 |
| 2 | *Curvularia lunata* | 46.79 | 25.64 |
| 3 | *Alternaria alternata* | 34.27 | 34.20 |
| 4 | *Fusarium oxysporum* | 15.80 | 27.04 |
| 5 | *Colletotrichum gloeosporioides* | 12.50 | 15.00 |
| | CD (rows = 2.87; columns = 3.70) p=0.05 | | |

**Effect of non-volatile compounds released by *Trichoderma* species on the growth of pathogens**

The percent growth inhibition of seed borne pathogens by culture filtrate of *Trichoderma* spp. of different ages is presented in Table-2. The data revealed a significant growth inhibition of all pathogens in comparison to respective control. The growth inhibition was increased with the age of incubation period of *Trichoderma* spp. in PDB. After one week incubation of *T. viride*, the culture filtrate exhibited maximum growth against *A. alternata* (28.57%) followed by *C. lunata* (15.78%), *C. gloeosporioides* (13.33%), *R. solani* (11.11%) and minimum for *F. oxysporum* (10.52%). While in case of *T. harzianum*, it was highest against *R. solani* (28.50%), followed by *C. gloeosporioides* (25.00%), *F. oxysporum* (22.38%), *C. lunata* (12.72%), and minimum (11.11%) for *A. alternata.* Whereas the culture filtrate obtained after two weeks of incubation, the culture filtrate of *T. viride* exhibited maximum growth inhibition of *A. alternata* (36.00%) followed by *C. lunata* (16.56%), *F. oxysporum* (15.00%), *C. gloeosporioides* (13.33%) and minimum (12.72%) for *R. solani.* Likewise, the culture filtrate of *T. harzianum* showed highest growth inhibition was recorded against *R. solani* (35.00%) followed by *C. gloeosporioides* (33.33%), *F. oxysporum* (25.00%), *C. lunata* (24.54%), and lowest (11.76%) against *A. alternata*. In similar experiments, Mishra *et al.* (2011) observed that the non-volatile compounds of *Trichoderma* spp. inhibited the growth of *Rhizoctonia, Fusarium, Alternaria, Colletotrichum* etc. They recorded low growth inhibition at 10% culture filtrate amendment in comparison to 20% amendment. Choudhary and Reena (2012) also reported significant inhibitory activity of *T. harzianum, T. viride* and *T. koningii* by liquid culture filtrate assay against *Fusarium oxysporum* f.sp. *lentis*; causal agent for wilt of lentil.

**Table-2. *In-vitro* growth inhibition of seed borne pathogens by non-volatile metabolites released by *Trichoderma* spp.**

| S. No. | Pathogens | Growth inhibition (%) by *T. viride* | | Growth inhibition (%) by *T. harzianum* | |
|---|---|---|---|---|---|
| | | 1 week incubation | 2 week incubation | 1 week incubation | 2 week incubation |
| 1 | *R. solani* | 11.11 | 12.72 | 28.50 | 35.00 |
| 2 | *C. lunata* | 15.78 | 16.56 | 12.72 | 24.54 |
| 3 | *A. alternata* | 28.57 | 36.00 | 11.11 | 11.76 |
| 4 | *F. oxysporum* | 10.52 | 15.00 | 22.38 | 25.00 |
| 5 | *C. gloeosporioides* | 13.33 | 13.33 | 25.00 | 33.33 |
| | | CD (rows = 3.09; columns = 3.98) p=0.05 | | CD (rows = 4.31; columns = 5.57) p=0.05 | |

**Effect of volatile compounds released by *Trichoderma* spp. on the growth of seed borne pathogens**

The volatile compounds released by *Trichoderma* spp. have also exerted inhibitory effect on the growth of the selected pathogens (Table-3). *T. viride* has recorded maximum growth inhibition for *C. gloeosporioides* (34.37%) followed by *A. alternata* (20.00%), *F. oxysporum* (15.21%), *R. solani* (12.00%) and minimum for *C. lunata* (13.63%) and while in case of *T. harzianum* the highest growth inhibition by was observed for *C. gloeosporioides* (35.00%) followed by *F. oxysporum* (14.80%), *A. alternata* (11.11%), *C. lunata* (9.52%) and lowest for *R. solani* (4.54%). Dennis and Webster (1971b, c) reported the influence of volatile and non-volatile antibiotics of *T. harzianum* Rafai on *Rhizoctonia solani* Kuhen and other fungi. The

major advantage of antibiosis in case of volatile metabolites is that the toxic substances produced by the antagonists may diffuse through air filled pores in soil and help in checking the root rot pathogen without establishing actual physical contact with pathogen. Amin *et al.* (2010) too studied the volatile effect of *Trichoderma* species on fungal pathogens like *F. oxysporum. R. solani, Alternaria* etc. The result showed the *T. viride* and *T. harzianum* are effective in reducing the mycelium growth. Gveroska and Ziberoski (2011) observed inhibitory activity of volatile compounds of *Trichoderma* species on the growth of *Alternaria alternata.*

**Table-3. Effect of volatile compounds released by *Trichoderma* species on the growth of seed borne fungi**

| S. No. | Seed borne pathogen | Growth inhibition (%) | |
|---|---|---|---|
| | | *T. viride* | *T. harzianum* |
| 1 | *R. solani* | 12.00 | 4.54 |
| 2 | *C. lunata* | 13.63 | 9.52 |
| 3 | *A. alternata* | 20.00 | 11.11 |
| 4 | *F. oxysporum* | 15.21 | 14.80 |
| 5 | *C. gloeosporioides* | 34.37 | 35.00 |
| | CD (rows = 2.70; columns = 3.48) p=0.05 | | |

Numerous studies have covered the status of *Trichoderma* spp. as a successful biological control agent. Inhibition of plant pathogenic fungi by different species of *Trichoderma* was studied under *in vitro* conditions by many researchers (Buragohai *et al.*, 2000; Singh and Islam, 2010; Sharma, 2011; Tapwal *et al.*, 2004, 2014; Shaikh and Sahera, 2013; Enespa and Dwivedi, 2014). *Trichoderma* is useful to cultivated plants in several ways like bio-control of plant diseases, induction of systemic resistance, promotion of plant growth (bio-fertilization), increase in plant nutrient availability and degradation of xenobiotic pesticides (Harman, 2006). For the said reasons, it has gained a stature as one of the most practical and promising means eco-friendly management soil borne pathogens in agricultural crops. Therefore, they are being used in reasonably large quantities, both for disease control and augmentation of yield. *Trichoderma* spp. are found to be effective across a range of plant crops, for example, lettuce, onion, cotton, grapes, peas, apples, tomato, carrots and others to control various pathogens like *Phytophthora, Pythium, Sclerotinia, Rhizoctonia, Colletotricum* and *Fusarium* etc., (Benitez *et al*., 2004; Nirupama Devi *et al.*, 2013). Application of *T. viride* and *T. harzianum* have proved to be very effective in managing *Fusarium oxysporum* in different crops like tomato, cotton in terms of time taken to parasitize (Sharma, 2011) and inhibit mycelia growth as well.

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