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RESEARCH ARTICLE

Effect of Trichoderma species on germination and growth of Mungbean (*Vigna radiata* L.) and its antagonistic effect against fungal pathogens

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Abstract

During the present studies, antagonists viz., *Trichoderma viride* and *T. harzianum* were used for antagonize the growth of pathogenic fungi of Mungbean viz. *Fusarium oxysporum* and *Rhizoctonia bataticola*. The inhibition in colony of *F.oxysporum* was found 30.66% by *T.viride* and in the *R.bataticola* was 40%. The *T. harzianum* showed inhibition in *F.oxysporum* 42.54% and in *R. bataticola* 48.14% after 3 days of inoculation. The antagonists viz., *T.harzianum* and *T.viride* were also found to affect the growth response of Mungbean (*Vigna radiata* L.). The germination of Mungbean seeds was found maximum (80%) in *T. harzianum* and *T. viride* (57.77%) in comparison to control (23.33%). The pathogen growth inhibitory ability of different *Trichoderma* strains of same species and among different species varied significantly. Plant growth promotion measured as root and shoot lengths were significantly higher than control.

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INTRODUCTION

Trichoderma is a genus belonging to the filamentous Class Deuteromycetes. The members are generally found in all types of soil. *Trichoderma* Spp. is well known which is capable of controlling many soils borne plant pathogen due to competition of food and space, direct parasitism, production of cellular and various mycolytic enzymes (Karpagavalli and Ramabadan, 2001, Jangid et al., 2004). Biological agent such as fungi and bacteria can offer protection against a number of soil borne pathogens specially *Rhizoctonia* spp., *Sclerotium* spp. and *Fusarium* spp. Plant defense mechanisms have also been manifested by alteration in biochemical responses of the plant which is collectively called as Induced Systemic Resistance (ISR) (Gawande and Sharma, 2003). These species are distinguished on the basis of color and shape of their conidia and colony appearance. *Trichoderma* species are economically important for their production of enzymes (cellulases and hemicellulases), antibiotics and their action as biocontrol agents against plant pathogen fungi based on various mechanisms such as the production of antifungal metabolites, competition for space and nutrients and mycoparasitism (Howell, 2003).

The antifungal enzyme system of *Trichoderma* spp. plays an important role for detection and destroying the host cell wall (Schirmbock et al., 1994). Competitiveness is based on rapid growth and the production of various asexual generated conidia and chlamydo spores (Chet, 1990; Chet et al., 1998). The ability to promote growth and induce resistance in plants is a mechanism which has also been described for members of this genus (Harman, 2006). The experiment was conducted to study the interaction of antagonists viz; *T.harzianum* and *T.viride* against fungal pathogen of mungbean and its effect on growth response.

Methods and Material

Preparation of cultures:

Pure cultures of fungus species were prepared on the PDA medium in different petriplates in the aseptic environment.

Screening of *T. viride* and *T. harzianum* isolates against plant pathogenic fungi using dual culture method:- These isolates were tested for antagonism against broad range of common plant pathogen and further studies were done for selected pathogens by using dual culture techniques as developed by **Morton and Stroube,(1955)**. The mycelia bits of 5 mm diameter of *Trichoderma* spp. and pathogen were placed opposite to each other on petriplates containing sterilized PDA medium. Plates having pathogen served as control. Each treatment was replicated thrice. The plates were incubated at $27\pm 1^{\circ}\text{C}$ for 3 days. The growth of pathogen tested against all the isolates of *Trichoderma* strains. *Trichoderma* spp. were evaluated against pathogenic fungi in vitro for their comparative potential as antagonist by dual culture techniques as described by **Johnson and Curl (1972)**. Percent inhibition over control was calculated by applying the following formula:-

$$I = \frac{C-T}{C} \times 100$$

Where: - I = Inhibition (%), C = Colony diameter in control (cm), T = Colony diameter in treatment (cm)

Plant Growth Promotion:

The experiment was laid out in Randomized Block Design with its three replicates. The Petri dishes containing seeds of *Vigna radiata* L were daily treated with *Trichoderma* species solution. The germination of seed, Root length and Shoot length were recorded at regular intervals i.e. 5th, 10th and 15th DAS. Germination percentage was calculated by following formula

$$\text{Germination \%} = \frac{\text{no. of seed germinated}}{\text{Total no. of seed placed}} \times 100$$

Allelopathic effect of *Trichoderma* in-vitro condition

In- vitro study petridishes containing 15 seeds of Mungbean was sprayed by *Trichoderma* at intervals 3, 5, 7, 9 and 11 days. The treatment without application of *Trichoderma* strain served as control and experiment was conducted in triplicates. Collected data was Tabulated and analyzed statistically to obtain concrete interferences.

Results and Discussion

In the present study two *Trichoderma* species (*Trichoderma viride* and *Trichoderma harzianum*) were selected for screening against fungal pathogen such as *Rhizoctonia bataticola* and *Fusarium oxysporum* of Mungbean

Table 1- Antagonistic activity of two isolates of *Trichoderma* spp. against different fungal plant pathogens of Mungbean (*Vigna radiata*) at 3rd day:

Cultures	Growth of Colony			Inhibition (%)	Cultures	Growth of Colony			Inhibition (%)
	Trichoderma sp.	Pathogenic sp.	Control			Trichoderma sp.	Pathogenic sp.	Control	
T. viride v/s R.bataticola	4.83±0.76	3.5±0.50	5.5±0.25	40	T. harzianum v/s R.bataticola	3.86 ±0.35	2.80±0.25	5.4	48.14
T. viride v/s F.oxysporum	5.01±0.42	2.58±0.14	3.5 ±0.15	30.66	T. harzianum v/s F.oxysporum	3.93±0.60	3.16±0.35	5.5	42.54

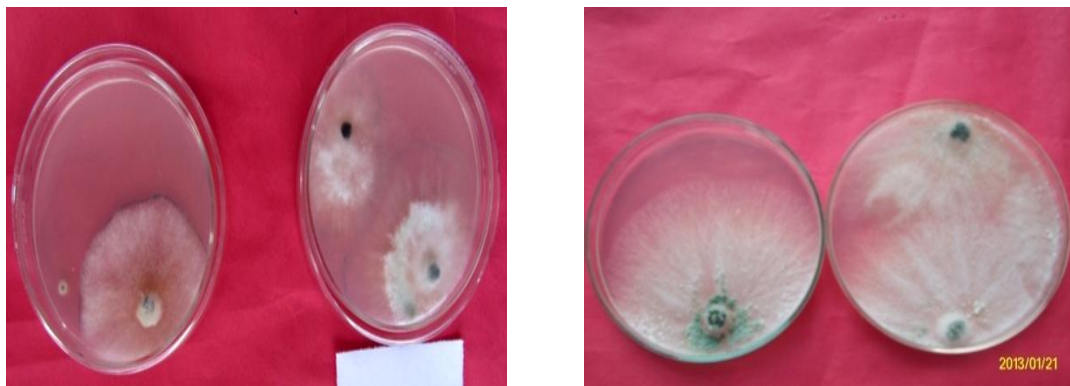


Fig. I- Zone of inhibition against *R. bataticola*

II- Zone of inhibitions against *F. oxysporum*.

However the isolates *T. viride* and *T. harzianum* showed excellent antagonistic activity against fungal plant pathogens causing disease in Mungbean (*Vigna radiata*). In dual culture a clear zone of inhibition was observed exhibiting antibiosis between pathogens and antagonist. Fungal disc (5 mm diameter) of *Trichoderma* was placed at one side and *F. oxysporum* was placed on the opposite side of PDA plates in in vitro. The distance between the two inoculum discs were 7 cm. As same followed in case of other pathogenic fungi *Rhizoctonia bataticola*. It has been observed that 40% inhibition in the growth of *Rhizoctonia bataticola* and in the *Fusarium oxysporum* 36.66% by growth of the colony of *Trichoderma viride* (Fig 1, Table 1). The inhibition in colony of *Rhizoctonia bataticola* was found 48.14% and 42.54% in colony of *Fusarium oxysporum* by *Trichoderma harzianum*. *T. harzianum* was comparatively more effective in reducing the growth of *R. bataticola* & *F. oxysporum* than *T. viride* in vitro condition (Fig 2, Table 1). **Chet et al., (1981)** reported that *Trichoderma* spp. are common inhabitant of rhizosphere and contribute to control of many soil borne plant disease caused by fungi.

Allelopathic Effect of *Trichoderma*:

The increase in cumulative root length, root surface area and the number of root tips following *Trichoderma* treatment in both environments suggested a common role of *T. harzianum* in improving the plant root system. The rate of plant growth was higher following treatment with *T. harzianum*, in both systems. **Harman (2000)** suggested that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones (**Baker, 1989; Kleifield and Chet, 1992**). *T. harzianum* reduced root rot of sugar beets (**Ciccarese et al., 1992**), stem rot of ground nut (**Cilliers et al., 2000**), damping-off and stem rot of cowpea (**Adandonon, 2000**), root rot of beans and tomatoes (**Elad et al., 1980**).

As such the above experiments we also performed the same experiment on Mungbean (*Vigna radiata*) and observe that *Trichoderma harzianum* significantly enhance the germination of mungbean seeds and also enhance the growth of shoot length and root length in early days after the inoculation of the mungbean seeds in vitro conditions. In this experiment two strains of *Trichoderma* (*Trichoderma viride* and *Trichoderma harzianum*) and consider the allelopathic effect on Mungbean (*Vigna radiata*) and was observed the effect of *Trichoderma* on the germination of Mungbean seeds.

The maximum percentage germination with *Trichoderma harzianum* i.e. 80% followed by with *T. viride* 57.77% and minimum percentage of germination was 23.33% in control. On 5th day maximum shoot length was found in *T. harzianum* (5.73 cm) & root length was also maximum in *T. harzianum* (3.54 cm) than *T. viride* and control (Fig 2, Table 2). The same trend was found on 10th and 15th day. Effect of *Trichoderma harzianum* on shoot length and root length of Mungbean (*Vigna radiata*) was more positively significant

Table: 2- Effect of *Trichoderma* isolates on seed germination, shoot length and root length:-

Strains	Percentage	5 th Day	10 th Day	15 th Day
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	Germination (%)	Shoot length (Mean±SD)	Root length (Mean±SD)	Shoot length (Mean±SD)	Root length (Mean±SD)	Shoot length (Mean±SD)	Root length (Mean±SD)
T. viride	57.77	4.91±0.66	3.28±0.29	11.35±0.81	5.36±0.48	14.66±0.32	4.95±0.17
T.harzianum	80	5.73±0.38	3.54±1.06	11.92±0.26	5.47±1.35	15.29±1.71	4.99±1.74
Control	23.33	4.54±0.25	3.19±0.72	11.31±0.72	4.62±0.53	12.87±0.33	3.33±0.38

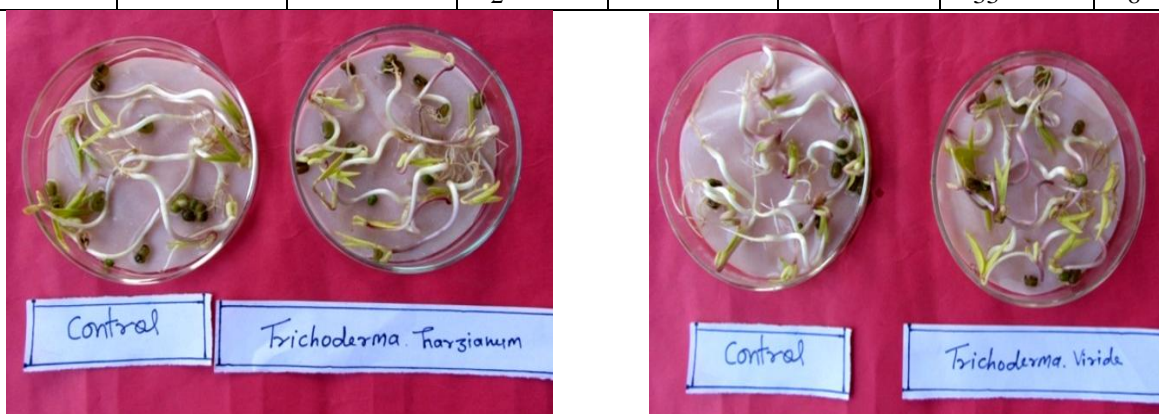


Fig-2: Effect of *T. harzianum* and *T. viride* on germination of Mungbean on 5th DAS.

Conclusion:

Antagonistic interactions of *Trichoderma* strains showed excellent activity against various plant pathogens causing disease in Mungbean (*Vigna radiata*) thus the *Trichoderma* strains could be further exploited for commercial scale up under localized climatic condition. Effect of *Trichoderma harzianum* on shoot length and root length of Mungbean (*Vigna radiata*) was positively significant. Thus it is concluded that *Trichoderma* is the beneficial fungi for the growth of the crops and other plants by inhibiting the growth of pathogenic microorganisms.

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