

# Biocontrol Potentials of *Trichoderma* against Pathogenic Fungi from the Rhizosphere soils of Green gram

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**Abstract:** *Trichoderma* species are well known bio control agents against pathogenic fungi. Hence, an attempt was intended to corroborate the positive relatedness of molecular and morphological characters with antagonistic ability. Nine *Trichoderma* species were isolated from Green gram rhizosphere soil from Khammam dist. (Telangana) using *Trichoderma* selective medium and characterized. The isolates were screened for antagonistic activity against three pathogenic fungi i.e. *Fusarium oxysporum*, *Rhizoctonia solani* and *Colletotrichum capsicii* in dual culture plate technique. Among the 9 isolates *T.Reeseii* (T7) & *T.Pseudokonigii*(T6) showing potential antagonism and inhibited the *Fusarium oxysporum*, *Rhizoctonia solani* and *Colletotrichum capsicii*.

**Keywords:** *Trichoderma*, Green gram, Antagonism, Pathogenic fungi.

## 1.Introduction

Imperfect *Trichoderma* fungi has application as an antagonist of phytopathogenic fungi to control plant diseases (Monte, 2001), few strains have the ability to kill the plant pathogen under variety of environmental conditions. Fungi showing biocontrol activity under the genus *Trichoderma* has developed surprising ability to interact parasitically and symbiotically (Harman and Kubick, 1998).

*Trichoderma*, a soil borne mycoparasitic fungus has been shown effective against many soil borne phytopathogens (Papavizas, 1985; Herman et al., 1998; Herman, 2000; Pan et al., 2001; Jash et al., 2004; Herman, 2006; Maurya et al., 2008, Rajkonda et al. 2011, Dolatabadi et al. 2012). Biological control of soil borne phytopathogens has been the subject of extensive research in the last few decades. However, with the increasing interest in biological control, owing to environmental and economic concerns, thousands of research experiments are going on for searching novel, potential, safe and have ability to inhibit wide range of soil borne phytopathogens. *Trichoderma spp.* is well documented as effective biological control agents of soil borne diseases which inhibit the pathogens by direct antagonism or by secreting several cell wall degrading enzymes, antibiotics (Sivan et al., 1984 and Coley-Smith et al., 1991 and Ashwin G.Lunge et al (2012) ).

## 2. Materials & Methods

### 2.1 Collection of Soil Samples

The rhizosphere soil samples were collected at Khammam district and Botanical garden of Hyderabad by random sampling method. The rhizosphere soil samples of the green gram during the seedling stage, vegetative stage and flowering stage were collected from three agricultural fields in triplicates in the winter season. The green gram plants were uprooted from the agricultural fields and the

rhizosphere soil was pooled together and immediately processed for the isolation of *Trichoderma* .

### 2.2 Isolation of *Trichoderma*

The present investigation was conducted in Applied plant pathology laboratory, Dept. of Botany, Osmania University, Telangana, India. In the present work *Trichoderma* was isolated from the collected soil samples by the serial dilution Technique of soil sample. One ml of 10 dilution was poured on to *Trichoderma* selective Medium (MgSO<sub>4</sub>: 0.20g, KH<sub>2</sub>PO<sub>4</sub>: 0.90g, NH<sub>4</sub>NO<sub>3</sub>: 1.0g, KCl: 0.15g, Glucose: 3.0g, PCNB: 20g, Rose Bengal: 0.15g, Chloremphanicol: 0.25g, Agar-agar: 15g, Metalaxyl: 30g, Distilled water: 1L) for isolation of *Trichoderma* and after the appearance of the colonies of *Trichoderma*, purified by hyphal tip isolation techniques. They were identified on the basis of their morphological and microscopic characteristics. The purified and identified cultures of *Trichoderma spp.* were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further use.

### 2.3 Identification of *Trichoderma*

The genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or postulate repetitively branched conidiophores with lageniform phialides and hyaline or green conidia born in slimy heads. The primary branches of conidiophores produce smaller secondary branches that also may produce tertiary branch, and so on. Conidia are hyaline usually green, smooth – walled or roughened. Phialides are ampliform to lageniform, usually constricted at the base, more or less swollen near the middle, and abruptly near the apex into short sub-cylindrical neck. Identification is based on standard identification keys (Rifai 1969; Bisset 1991a, b; Samuels (1996); Nagamani et al., 2006).

### 2.4 Isolation of Pathogenic fungi:

Test pathogens were isolated from different naturally infected host plants parts viz., roots, stems, pods, seeds,

leaves and soils. The infected plant parts were surface sterilized with formaldehyde and washed several times with sterile distilled water (dH<sub>2</sub>O) to free formaldehyde, blotted dry on whatman No. 1 filter paper. The samples were transferred to Petri plates (3-5 pieces/plate) containing PDA supplemented with streptomycin (to suppress bacterial growth) and incubated at 25±2<sup>0</sup>C for 3-5 days. Aseptically, bits of mycelia from the margins of the colonies were transferred to the PDA slants and stored at 4<sup>0</sup>C until further use. Pathogenicity test was conducted on the host plant variety sown in pots containing soil mixed with inoculums of each isolate multiplied on sand-sorghum meal medium in the ratio of 95:5 w/w. The pathogen reisolated from the inoculated plants that caused disease was shown to be the same pathogen as the original, thus proving the Koch's postulates. The virulent strain thus screened by pathogenicity test was multiplied by using sand and sorghum meal for further experimental purpose (Menge *et al.*, 1977 and . Ritesh kumar *et al.*, (2012) ) and employed for pot and field experimental trials.

**2.5 Antagonistic characteristics of Trichoderma against pathogenic fungi in dual culture plate method**

The antagonism experiments were done as described by Dennis and Webster (1971) and Goes et al. (2002) with certain modifications. The fungal isolates were cultivated in Petri dishes with PDA for seven days. Discs of 5mm diameter were cut and removed from the growing borders of the colonies and transferred to another Petri dish with PDA. In each plate two discs, one with *Trichoderma* spp. mycelium and another with plant pathogen (*Colletotrichum capsicii*, *Rhizoctonia solani* and *Fusarium oxysporum*)mycelium were placed opposite to each other, in time intervals according to the growth speed of the organisms. The plates were incubated at 28°C. The experiment was conducted with three replications and observed at 12 h interval for 12 days.

**3. Results and Discussion**

A total of 50 *Trichoderma* spp. were isolated from various varieties of green gram rhizosphere soils from Khammam district during 2011-2013. These isolates were evaluated for their antagonistic activity& plant growth promotion traits and finally selected 9 best potential *Trichoderma* spp.identified by using standard identification keys (Rifai 1969; Bisset 1991a, b; Samuels (1996); Nagamani *et al.*, 2006).(Table:1, Fig:1)

**Table 1:** Growth patterns of *Trichoderma* isolates on Trichoderma selective media after 3 days of incubation

S.NO	Isolate Name	Diameter of the colony(mm)	Colour of the colony
1	<i>Trichoderma harzianum</i> (OUT1)	7.0	Dark-green
2	<i>Trichoderma viride</i> (OUT2)	5.2	Dark-bluish green
3	<i>Trichoderma atroviride</i> (OUT3)	6.0	Green
4	<i>Trichoderma virens</i> (OUT4)	7.0	Bluish-green

5	<i>Trichoderma konigii</i> (OUT5)	7.5	Dark-green
6	<i>Trichoderma pseudokonigii</i> (OUT6)	8.0	Pale-yellow
7	<i>Trichoderma reeseii</i> (OUT7)	7.0	Grey-green
8	<i>Trichoderma hamatum</i> (OUT8)	5.5	Grayish-green
9	<i>Trichoderma longibracheatum</i> (OUT9)	6.0	Yellowish-green

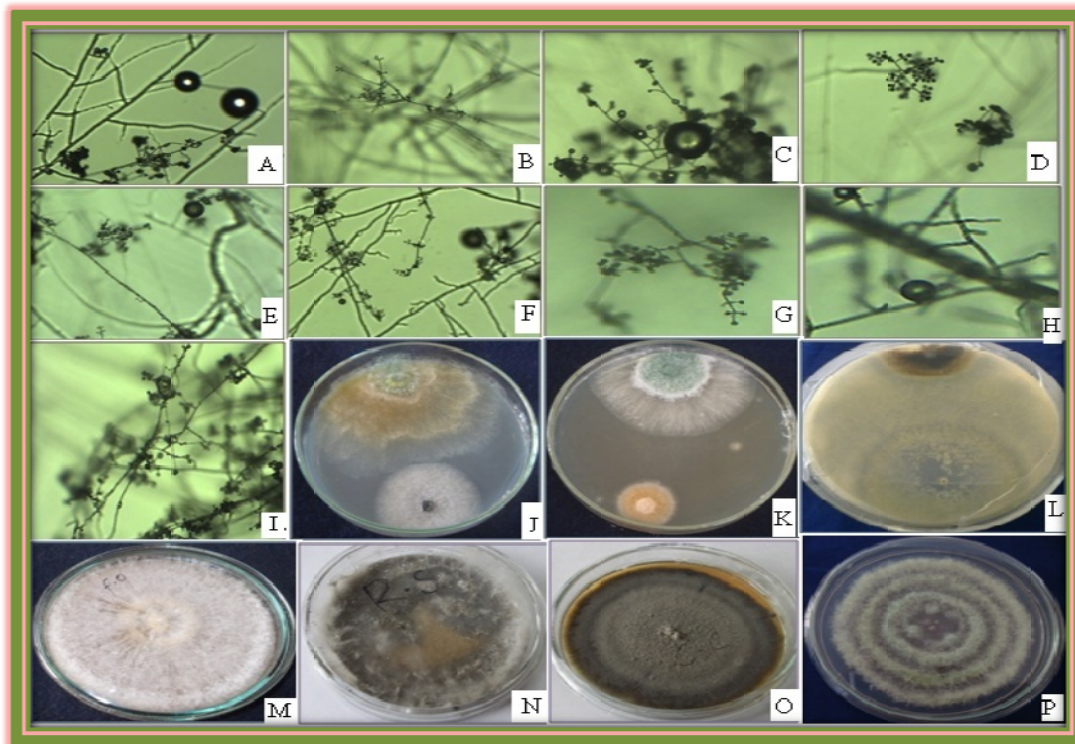
*Trichoderma* species were screened for antifungal activity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Colletotrichum capsicii* and zone of inhibition was taken as an indicator of antifungal property in the dual culture method. The inhibition percentage was calculated using the formula described by Idris *et al.* (2007) which is (R - r) / R × 100 (r: radial growth of the fungal colony opposite to the pathogen colony, R: the radial growth of the pathogen in control. Every 24h after inoculation, radial growth was recorded and it was observed that both pathogenic fungi and *Trichoderma* is fast growers and it covered 50% area of Petri plates of 90mm diameter within 48h and it covers full on fourth day, i.e., 90h of inoculation.

All the screened isolates of *Trichoderma* spp. showed diverse antagonistic efficacy in a dual culture against all the pathogenic fungi but their antagonistic potentials varied isolate to isolate. Among all the screened isolates, the isolates (OUT6 and OUT7) showed strong antagonistic potentials followed by the isolates OUT9, OUT3 and OUT8. It was found that OUT6, OUT7 isolates have strong antagonistic potential on the fifth day of inoculation and overlapped the colonies of *R. solani* and *F.oxysporum*, *C. Capsicii*.(Table:2, Fig:1).

**Table 2:** *Invitro* antagonisms of *Trichoderma* isolates against *Rhizoctonia solani*, *Colletotrichum capsicii*, *Fusarium oxysporum*.

Isolate names	Time taken to contact(days)			Time taken to overlapping(days)			Pigmentation at the point of contact (days)			Bell's Ranking (R1-R4)		
	R.S	C.C	F.O	R.S	C.C	F.O	R.S	C.C	F.O	R.S	C.C	F.O
OUT1	2	3	3	5	5	5	-	-	-	R <sub>1</sub>	R <sub>3</sub>	R <sub>3</sub>
OUT2	3	4	4	5	4	4	-	-	-	R <sub>3</sub>	R <sub>2</sub>	R <sub>2</sub>
OUT3	3	3	3	5	5	5	-	-	-	R <sub>3</sub>	R <sub>3</sub>	R <sub>3</sub>
OUT4	2	4	3	3	3	4	-	-	-	R <sub>2</sub>	R <sub>1</sub>	R <sub>3</sub>
OUT5	2	3	3	3	4	4	-	-	-	R <sub>1</sub>	R <sub>1</sub>	R <sub>1</sub>
OUT6	4	4	4	5	5	5	yellow w	yellow w	orange	R <sub>4</sub>	R <sub>4</sub>	R <sub>4</sub>
OUT7	4	4	4	4	5	5	grey	grey	grey	R <sub>4</sub>	R <sub>4</sub>	R <sub>4</sub>
OUT8	3	3	4	4	5	5	-	blue	-	R <sub>2</sub>	R <sub>3</sub>	R <sub>3</sub>
OUT9	4	4	5	5	5	5	green	-	yellow w	R <sub>3</sub>	R <sub>3</sub>	R <sub>4</sub>

R<sub>1</sub>=Complete over growth; R<sub>2</sub>=75% over growth; R<sub>3</sub>=50% over growth; R<sub>4</sub>=Locked at the point of contact; R.S=*Rhizoctonia solani*; C.C= *Colletotrichum capsicii*; F.O= *Fusarium oxysporum*.



**Figure 1:** Morphology and Antagonism of *Trichoderma* isolates against pathogenic fungi

A. *Trichoderma Atroviridae*; B. *Trichoderma Harzianum*; C. *Trichoderma Hamatum*; D. *Trichoderma Konigii*; E. *Trichoderma Longibracheatum*; F. *Trichoderma pseudokonigii*; G. *Trichoderma reesei*; H. *Trichoderma Viridae*; I. *Trichoderma Virens*. **Antifungal activity** J. *Colletotrichum Capsici*; K. *Fusarium oxysporum*; L. *Rhizoctonia solani*; **Control pathogens** M. *Fusarium oxysporum*; N. *Rhizoctonia solani*; O. *Colletotrichum Capsici*; **Control** P. *Trichoderma atroviride*

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#### 5. Future Scope

The isolates tested positive for antifungal activity will be further explored in for pot and field experiments to study plant growth, yield, and bio-control ability against (pathogen) on green gram.

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