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Antagonistic Potential of Different Isolates of Trichoderma against *Rhizoctonia solani*

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Abstract

Trichoderma spp. is the most promising and effective bioagents against several soil borne plant pathogenic fungi. In present study, 26 isolates of *Trichoderma* were isolated from soil of Jharkhand and screened for their antagonistic and antibiosis efficacy against *Rhizoctonia solani* by dual culture. Result indicated that the antagonistic potential of 26 isolates of *Trichoderma spp.* against *R. solani* were varied which inhibited *R. solani* ranges 33-54%. Among isolates of *Trichoderma*, seven isolates showed strong antagonistic potential which inhibited >50% mycelial growth of R. solani, viz., RCT1 (53.71%) followed by RCT22 (52.6%), RCT3 (51.85%), RCT7 (51.11%), RCT10 (50.37%), RCT 8 (50%) and RCT14 (50%). Moreover, seventeen (17) isolates were also showed inhibitory but their antagonistic potential <50% of the mycelial growth while two isolates (RCT12 and RCT17) showed <40% mycelial growth. These potential isolates of *Trichoderma* may be further exploited as biocontrol agent against *R. solani* as well as other Soilborne phytopathogenic fungi.

Keywords: Biological control; Trichoderma; Antagonistic potential; Antibiosis; Inhibition

Introduction

Rhizoctonia solani is an important soilborne plant pathogenic fungus which distributed worldwide which has wide host range [1-3]. It causes numerous diseases in crop plants viz. damping-off, black spot and root rot diseases [4]. *R. solani* is a fast growing necrotrophic and sclerotial fungus which survives in the soil as hard, resistant sclerotial bodies [5]. Management of *R. solani* by chemical fungicides was expensive and tedious. No effective fungicides are available in the market. Moreover the negative effect of chemical fungicides causes phytotoxicity and environmental pollution [6]. Intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and environment also in the build-up of resistance of pathogens [7]. In order to tackle these national and global problems, alternatives of chemical control are investigated by the use of antagonistic microbes [8]. In this regard biological control offers an alternative solution for long term sustainability and effective management of soil borne diseases [9,10]. Among the microbes, *Trichoderma spp.* is a common saprophytic filamentous fungi which habitat in soil and rhizospheric soil. It acts as a bio control agent against various plant pathogenic causes several diseases in mono and dicotyledonous crop plants [11-13].

The effectiveness of biocontrol agents depends on several parameters,that include specific pathogen, soil texture, water content, pH, temperature and crop history [14,15], therefore their application should consider the environmental stress that could affect their ability to maintain their biocontrol capacity. It has very wide range in modes of action viz. Plant growth promotion, mycoparasitism, antibiosis, competition for nutrients and space, tolerance to stress through enhanced root and plant development [16-19]. Looking these potential systemic resistances *Trichoderma* against soil borne phytopathogen, the present experiment were designed to explore novel and potential isolates of *Trichoderma spp.* from the soil of Jharkhand in the biocontrol of *R. solani* and other soilborne phytopathogens.

Material and Method

The present experiments were conducted in ICAR, RCER, Research Centre, Plandu, and Ranchi, India.

Collection of soil sample

Soil samples (pH-acidic, N-medium, P-low, K-Sufficient, OC-low) were collected from different localities of Agricultural and vegetable growing areas of Ranchi district of Jharkhand, at the depth of 5-7 cm of soil surface The composite soil samples were collected from a particular field in the polyethylene bag and labeled carefully **(Table 1)**.

Table 1 Collection of soil samples for isolation of Trichoderma from locality of Ranchi, Jharkhand.

Sl. No	Code	Place of collection (Blocks of Ranchi dist.)	Research centre code	Soil sample detail (Rhizospheric soil)
1.	T1	Namkum	RC T1	Sapota
2.	T2	Namkum	RC T2	Litchi
3.	T3	Mander	RC T3	Mango
4.	T4	Jonha	RC T4	Guava
5.	T5	Nagri	RC T5	Ladyfinger
6.	T6	Bero	RC T6	Tomato
7.	T7	Ratu	RC T7	Onoin
8.	T8	Burmu	RC T8	Pea
9.	T9	French bean	RC T9	Angara
10.	T10	Ormanjhi	RC T10	Pea
11.	T11	Lapung	RC T11	Wheat
12.	T12	Tamar	RC T12	Brinjal
13.	T13	Bero	RC T13	Potato
14.	T14	Chanho	RC T14	Cabbage
15.	T15	Namkum	RC T15	Dioscoria
16.	T16	Namkum	RC T16	Elephant yam
17.	T17	Namkum	RC T17	Tephrosia
18.	T18	Jonha	RC T18	Gram
19.	T19	Namkum	RC T19	Pea
20.	T20	Namkum	RC T20	Mango
21.	T21	Kanke	RC T21	Cabbage
22.	T22	Kanke	RC T22	Beat
23.	T23	Pithoria	RC T23	Potato
24.	T24	Angara	RC T24	Paddy
25.	T25	Silli	RC T25	Mango
26.	T26	Namkum	RC T26	Litchi

Isolation of *Trichoderma* from soil

Isolation of different isolates of *Trichoderma* was made by the collected soil serial dilution technique of the soil sample. One (1) ml of 10-3 dilution was poured on to *Trichoderma* selective Medium ($MgSO_4$: 0.20 g, KH_2PO_4: 0.90 g, NH_4NO_3: 1.0 g, KCl: 0.15 g, Glucose: 3.0 g, PCNB: 20 g, Rose Bengal: 0.15 g, Chloramphenicol: 0.25 g, Agar-agar: 15 g, Metalaxyl: 30 g, Distilled water: 1 L) for selective isolation of *Trichoderma* and after the appearance of the colonies of *Trichoderma* on Petri dishes purified by hyphal tip isolation techniques. *Trichoderma spp.* was identified, picked 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 experimentation **(Table 2)**.

Table2 Periodical growth of different isolates of *Trichoderma* and *Rhizoctonia solani* in dual culture.

Isolates	Periodical growth in (mm/day)							
	24 Hrs		48 Hrs		72 Hrs		96 Hrs	
	T	R	T	R	T	R	T	R

RC T1	29.33 ± 0.66	25.00 ± 0.57	48.33 ± 0.88	39.66 ± 0.88	49.00 ± 0.57	40.66 ± 1.45	49.00 ± 0.57	41.66 ± 0.88
RC T2	28.00 ± 1.15	23.00 ± 1.52	41.00 ± 0.57	48.66 ± 0.66	44.66 ± 0.88	49.33 ± 0.66	46.00 ± 0.00	47.33 ± 1.76
RC T3	27.00 ± 0.57	29.66 ± 0.33	44.33 ± 0.33	47.00 ± 0.00	45.33 ± 0.33	48.00 ± 0.00	47.66 ± 0.33	43.33 ± 0.88
RC T4	24.00 ± 1.15	26.66 ± 0.33	45.33 ± 0.33	45.33 ± 0.33	46.33 ± 0.33	47.00 ± 0.57	45.33 ± 1.33	47.00 ± 0.57
RC T5	28.66 0.66	27.33 ± 0.33	48.00 ± 1.52	43.66 ± 0.33	46.66 ± 1.66	46.33 ± 0.66	48.33 ± 1.33	46.33 ± 0.66
RC T6	27.66 ± 0.33	28.00 ± 1.52	43.66 ± 0.88	45.00 ± 0.00	44.66 ± 0.33	46.33 ± 0.66	46.33 ± 0.33	45.33 ± 0.88
RC T7	25.66 ± 0.33	30.33 ± 0.33	44.66 ± 0.33	44.00 ± 0.57	47.33 ± 0.88	42.66 ± 0.88	46.00 ± 0.57	44.00 ± 0.57
RC T8	30.00 ± 0.00	33.00 ± 1.00	46.33 ± 0.66	45.00 ± 0.00	46.66 ± 0.33	48.33 ± 0.66	44.66 ± 0.88	45.00 ± 0.57
RC T9	25.00 ± 1.00	34.00 ± 0.57	42.33 ± 1.33	47.00 ± 1.00	44.66 ± 0.66	49.00 ± 1.52	46.66 ± 0.66	47.33 ± 1.76
RC T10	27.33 ± 1.15	32.66 ± 0.66	45.66 ± 0.33	43.00 ± 1.00	45.66 ± 0.66	47.00 ± 1.00	47.33 ± 3.33	44.66 ± 2.33
RC T11	27.33 ± 0.88	33.33 ± 1.66	41.66 ± 1.20	48.33 ± 0.88	46.00 ± 2.08	49.33 ± 0.33	45.00 ± 2.51	51.33 ± 2.96
RC T12	13.00 ± 1.52	34.00 ± 1.00	23.33 ± 1.76	55.00 ± 2.51	28.33 ± 1.20	64.00 ± 3.05	32.66 ± 1.76	60.33 ± 1.45
RC T13	24.00 ± 1.73	33.33 ± 0.33	41.00 ± 1.20	46.00 ± 0.00	44.66 ± 1.33	48.00 ± 0.00	45.00 ± 2.51	48.00 ± 0.57
RC T14	26.00 ± 0.57	33.00 ± 1.15	44.00 ± 0.57	44.66 ± 1.45	46.00 ± 1.15	47.66 ± 1.45	46.66 ± 1.66	45.00 ± 2.51
RC T15	25.33 ± 0.33	36.33 ± 0.88	47.33 ± 1.20	46.66 ± 0.88	47.66 ± 1.45	46.66 ± 0.33	48.00 ± 2.08	46.33 ± 0.66
RC T16	26.00 ± 2.08	34.66 ± 0.33	44.33 ± 2.08	48.33 ± 0.33	43.66 ± 0.88	47.66 ± 0.33	43.66 ± 0.33	45.66 ± 1.33
RC T17	15.33 ± 1.20	23.33 ± 2.40	25.33 ± 1.33	59.33 ± 1.76	32.00 ± 0.57	59.00 ± 0.57	33.00 ± 0.57	59.66 ± 0.33
RC T18	19.00 ± 1.00	34.33 ± 0.66	33.33 ± 2.18	49.66 ± 0.88	37.00 ± 0.00	53.33 ± 0.88	40.33 ± 0.88	53.00 ± 1.00
RC T19	27.66 ± 0.33	34.33 ± 0.66	43.66 ± 1.33	45.66 ± 1.33	44.33 ± 0.33	47.33 ± 0.66	46.33 ± 0.66	46.00 ± 0.00
RC T20	25.33 ± 0.33	35.00 ± 0.00	46.00 ± 2.00	49.00 ± 0.57	45.00 ± 0.57	49.66 ± 0.33	47.00 ± 1.00	47.66 ± 1.33
RC T21	30.33 ± 0.33	23.66 ± 0.88	44.00 ± 0.57	45.33 ± 0.33	50.33 ± 0.33	46.00 ± 0.57	55.33 ± 0.66	46.33 ± 0.66
RC T22	28.66 ± 0.88	28.33 ± 2.18	45.66 ± 0.33	45.33 ± 0.33	45.66 ± 0.66	47.00 ± 0.57	46.33 ± 1.66	42.66 ± 0.66
RC T23	27.66 ± 0.33	29.33 ± 3.17	46.66 ± 0.33	46.00 ± 0.57	48.00 ± 0.00	47.00 ± 1.52	49.33 ± 0.66	47.33 ± 0.33
RC T24	28.33 ± 0.33	31.00 ± 2.00	44.00 ± 0.00	45.66 ± 0.33	45.00 ± 0.57	47.66 ± 0.33	45.00 ± 0.00	45.66 ± 0.33
RC T25	30.33 ± 0.33	35.00 ± 0.00	45.00 ± 0.00	44.66 ± 0.33	44.33 ± 0.33	46.66 ± 0.66	44.33 ± 0.33	46.66 ± 0.33
RC T26	26.33 ± 0.33	32.33 ± 0.66	43.00 ± 0.57	45.00 ± 0.00	45.66 ± 0.33	45.33 ± 0.33	44.66 ± 0.66	45.66 ± 0.88

± standard error of mean, T=growth of *Trichoderma* isolates, R=growth of *Rhizoctonia solani*

Isolation of sclerotial fungus *Rhizoctonia solani*

Damping off diseased affected plants were collected from vegetable growing area of Ranchi, Jharkhand. Then after the collected sample were surface sterilized by dipping in 0.1% $HgCl_2$ for 5-10 second followed by three subsequent washing with sterilized distilled water and then after they were placed in Petri plates containing Potato Dextrose Agar (PDA) medium and incubated at 25°C ± 2°C for 36-48 h. The culture were purified by hyphal tip isolation and maintained on PDA slants for further experimentation as well as visually appeared sclerotia were collected, wash and inoculated on the petridishes of containing PDA and after 48-72 h colonies appeared on the petridishes **(Figure 1)**.

Identification of *Trichoderma* species

Fungal hyphae of *Trichoderma* species are septet, hyaline and smooth-walled which produces numerous conidiophores are highly branched. Normally, the branches will form at or near 90° with respect to the main branch. The typical conidiophore terminates with one or a few phialides that usually arising directly from the axis near the tip. Phialides, also known as conidiogenous cells, are typically enlarged in the middle like a flask-shape, and may be cylindrical or nearly sub-globose. Conidia are one-celled, and either ellipsoidal (3-5 × 2-4 µm, L/W ≥ 1.3) or globose (L/W<1.3). They are typically light to dark green, or sometimes colourless, greyish or brownish which typically smooth surface.

Some strains are also produces chlamydospores which play important role in survival. They are normally found as thick-walled, enlarged vegetative cells with condensed cytoplasm

which are unicellular, globose to subglobose chlamydospores are either formed within hyphae or at the hyphal tips [20-22].

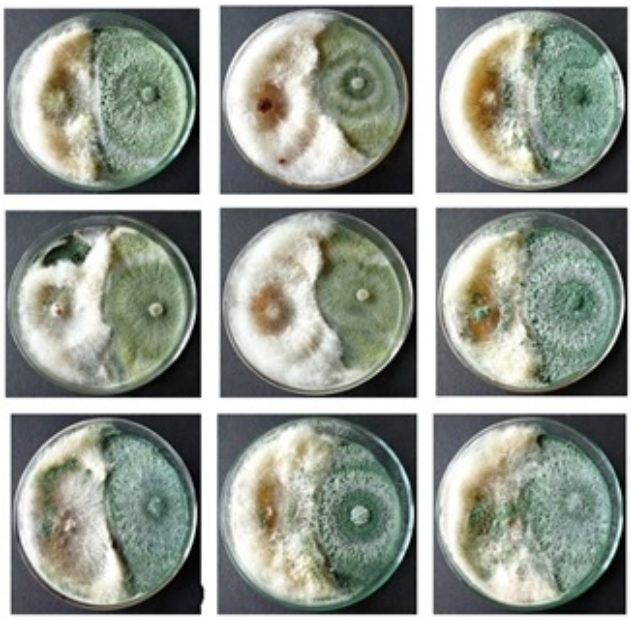

Figure 1 Antagonistic (mycoparasitism) potential of different isolates of *Trichoderma* against *R. Solani* (a) RCT1 (b) RCT2 (c) RCT3 (d) RCT4 (e) RCT5 (f) RCT6 (g) RCT7 (h) RCT8 (i) RCT9.

Typically, they are colourless, pale yellowish or greenish. Morphological characters was compared with morphologically and molecularly identified strains *T. asperellum* (NAIMCC-F-03167) **(Table 3)**.

Table 3 Inhibition of *Rhizoctonia solani* by different isolates of *Trichoderma* in dual culture (in mm).

Strains	Growth of *R. solani* in treatment	Growth of *R. solani* in control	Inhibition (%)
RC T1	41.66 ± 0.88		53.71
RC T2	47.33 ± 1.76		47.41
RC T3	43.33 ± 0.88		51.85
RC T4	47.00 ± 0.57		47.77
RC T5	46.33 ± 0.66		48.52
RC T6	45.33 ± 0.88		49.63
RC T7	44.00 ± 0.57		51.11
RC T8	45.00 ± 0.57		50
RC T9	47.33 ± 1.76		47.41
RC T10	44.66 ± 2.33		50.37
RC T11	51.33 ± 2.96		42.96
RC T12	60.33 ± 1.45		32.96
RC T13	48.00 ± 0.57		46.66
RC T14	45.00 ± 2.51		50
RC T15	46.33 ± 0.66		48.52
RC T16	45.66 ± 1.33		49.26
RC T17	59.66 ± 0.33		33.71
RC T18	53.00 ± 1.00	90.00 ± 0.00	41.11
RC T19	46.00 ± 0.00		48.88
RC T20	47.66 ± 1.33		47.04
RC T21	46.33 ± 0.66		48.52
RC T22	42.66 ± 0.66		52.6
RC T23	47.33 ± 0.33		47.41
RC T24	45.66 ± 0.33		49.26
RC T25	46.66 ± 0.33		48.15
RC T26	45.66 ± 0.88		49.26
RCT12,RCT17 b) RCT11,RCT18 c) RCT20 d)RCT23,RCT25			

Screening of antagonistic potential of *Trichoderma* with sclerotial fungi

Screening of antagonistic potential of different isolates of *Trichoderma* against *R. solani* was assessed by dual Culture in Petri dishes [23]. A mycelial bits of 5 mm diameter obtained from the margin of 3 day old actively growing colony of test fungus (*R. solani*) was place on a fresh PDA plate 2 cm from the centre of petriplate and antagonist (*Trichoderma* spp.) were placed opposite to test fungus at 4 cm apart in petriplate in triplicate. *R. solani* alone inoculated in PDA plate served as control. The radial growth of the pathogens in dual culture and control plates was measured periodically which incubated at 24°C ± 2°C in BOD [24]. The percent inhibition of mycelia growth over control was calculated by following equation [25].

$$I\% = C\text{-}T/C \times 100$$

Where, I=Percent inhibition of mycelial growth, C=Growth of mycelium in control.

T=Growth of mycelium in treatment in *R. solani*.

Statistical analysis

Data were analyzed using statistical package SPSS version 20.

Result and Discussion

Native strains of *Trichoderma* were isolated from 26 different rhizospheric soils from sites selecting from different blocks of Ranchi district of Jharkhand and coded **(Table 1)** and individual culture of every isolate was maintained for further experimentation. The isolates were studied for species identification and the same revealed that they belong to *Trichoderma* Isolates. The isolates of *Trichoderma* were cultured in Petri plates individually and also in dual culture with *R. solani* and growth data has been recorded **(Table 2)**. Further, the percent inhibition by all the 26 *Trichoderma* isolates against *R. solani* has been evaluated **(Table 3)**. Among isolates of *Trichoderma*, seven isolates showed strong antagonistic

potential which inhibited >50% mycelial growth of R. solani, viz., RCT1 (53.71%) followed by RCT22 (52.6%), RCT3 (51.85%), RCT7 (51.11%), RCT10 (50.37%), RCT 8 (50%) and RCT14 (50%). Moreover, seventeen (17) isolates were also showed inhibitory but their antagonistic potential <50% of the mycelial growth while two isolates (RCT12 and RCT17) showed <40% mycelial growth. *Trichoderma* species was found to be an effective biological control agent for protecting a number of crop plants from damaged induced by *S. rolfsii* and *R. solani* under both greenhouse and field conditions in the study conducted by [26,27]. Various agro products is used for biomass production and applied as a biocontrol agent. Several mechanisms may explain the biocontrol activity of these strains [28,29]. Hyperparasitism and volatile metabolites may be involved in the inhibition of *R. solani* [30]. Cell wall degrading enzymes (CWDEs) such as chitinase, glucanase and proteases are thought to be closely related to the mycoparasitism of *Trichoderma* strains [17-32]. Inhibitory volatile substances such as alkylpyrons may also contribute to the biocontrol activity of some *Trichoderma* strains [33-35]. Harman et al. reported that the *Trichoderma* have ability to antagonized soilborne phytopathogens as well as it also induced plant growth promotion and protect plants form biotic and abiotic stresses [36,37]. Thus, it can be concluded that *Trichoderma* isolates proves to be effective biocontrol agent and native isolates of it may be further explored as biocontrol agent against *R. solani*.

Conclusion

Twenty six (26) native strains of *Trichoderma* were isolated from acid soil of Jharkhand and were characterized on the basis of their morphological features, antagonistic potential and compared with known strains of T. asperellum. Seven (7) isolates of *Trichoderma* showed strong antagonistic potential which inhibited >50% mycelial growth and seventeen (17) isolates were also showed inhibitory which antagonized <50% of the mycelial growth of *R. solani* while two (2) isolates only showed <40% antagonistic potential. As per result, seven potential isolates of *Trichoderma* may be further exploited as biocontrol agent against *R. solani* as well as other Soilborne phytopathogenic fungi.

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