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In vitro antagonistic activity of *Trichoderma* spp. isolated from laterite soil against *Sclerotium rolfsii* and *Rhizoctonia solani*

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Abstract
Trichoderma, a widely prevalent fungus classified under the family Hypocreaceae, possesses numerous traits and known for their extreme versatility and adaptability that make it highly suitable as a growth-promoting and biocontrol agent. In the present investigation, *Trichoderma* isolates were derived from rhizospheric soils of weed hosts from different geographical locations of Chhattisgarh. Ten isolates of *Trichoderma* (T_1, T_3, T_8, T_{23}, T_{27}, T_{29}, T_{34}, T_{45}, T_{50}, T_{60}) differing in siderophore production were selected and mycoparasitic ability against two rice pathogens *S. rolfsii* and *R. solani* were screened. Isolate T_{27} (75%) showed maximum inhibition whereas T_{23} (63.8%) showed minimum inhibition towards *S. rolfsii* and isolate T_8 (75%) showed maximum inhibition whereas T_{29} (45%) showed the minimum inhibition towards *R. solani* whereas isolate T_8 was equally effective in inhibiting both the pathogens. *Trichoderma* isolates were more effective against *S. rolfsii* as compared to *R. solani*.

Keywords: *Trichoderma*, siderophore, mycoparasitic activity

Introduction
Soil rhizosphere is the inhabitant of many biologically important microbe which abet in plant growth-promoting activities and vanquishing the diseases caused by phytopathogens (Botelho and Hagler, 2006; Van *et al*., 2008; Hayat *et al*., 2010) [3, 10, 6].
Trichoderma, a fungus belonging to Division Ascomycota, Class Sodariomycetes, Order Hypocreales, and Family Hypocreaceae. It is a ubiquitous filamentous organism found in all types of soils and in the rhizosphere of plants. It offers a promising alternative to chemical fertilizers, pesticides and nutrient additives due to its remarkable ability to promote plant growth and protect them from pathogens. *Trichoderma* acts as an endophyte and enhances plant growth by increasing root density, improving nutrient uptake, solubilizing minerals, and inducing defense responses against both abiotic and biotic stress. This is achieved through effective colonization of the roots in most plant species (Haas & Défago, 2005; Vinale *et al*., 2008) [5, 11], as well as by influencing various factors such as hormone content, phenolic compounds, soluble sugars, amino acids, transpiration rate, photosynthetic rate and water content (Yedidia *et al*., 2003) [13].
The purpose of the present study was to evaluate the isolates of *Trichoderma* spp. collected from different location of Chhattisgarh for their *in vitro* antagonistic activity against two phytopathogens *S. rolfsii* and *R. solani.*

Material and Method
Isolation of the *Trichoderma* spp.
Material under investigation consists of 119 isolates of *Trichoderma* which were isolated from laterite soils collected from the rhizospheric and non-rhizospheric zone from roadsides, bunds, and forests of 7 districts of Chattisgarh. Isolated by adopting serial dilution method in *Trichoderma* Specific Medium (TSM). Pure culture obtained by single spore isolation in water agar (4%) and transferred in slants of PDA for further use.

Quantitative assay for siderophore production
The quantification of siderophore CAS (Chrome Azurol Sulphonate) assay was used as per the protocol of Schwyn and Neiland, 1987. Three to four discs from the edge of actively growing colonies of *Trichoderma* were inoculated in 20 ml Potato Dextrose Broth (PDB) incubated for

7 days at 28±2 ℃. 1ml aliquot then integrated with 1ml CAS solution and 20µl shuttling solution (sulfosalicylic acid). The colour obtained was determined using a spectrophotometer at 630 nm after 20min. Similarly, a reference solution was prepared with PDB, shuttle solution, and chrome azurol sulfonate dye. Uninoculated PDB used as blank and siderophore released in broth are expressed in siderophore units. The amount of siderophore released was expressed in % and was calculated using the following formula:

$$\frac{(Ar - As)}{Ar} \times 100$$

Here,
Ar = Reference solution's optical density,
As = Sample's optical density

In vitro antagonistic activity of *Trichoderma* spp.

Dual culture interaction

The antagonistic activity of selected 10 isolates of *Trichoderma* was studied *in-vitro* against *Rhizoctonia solani* and *Sclerotium rolfsii*. In sterilized PDA medium the pathogen is grown along with the antagonistic fungi. From the actively growing culture of pathogen and isolates *Trichoderma* 5mm disc was cut with the sterilized cork borer and placed opposite at the periphery of the plates at 180°. Pathogen without *Trichoderma* is kept as control. Plates were incubated at 28±2 ℃.

After 5 days petriplates were observed for antagonistic action. The inhibition percentage of the pathogen by *Trichoderma* was calculated by using the formulae given by Vincent (1947).

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

Where
I%= inhibition percentage
C= Growth of pathogen in control
T= Growth of pathogen in treatment

Result and discussion

Screening for Siderophore Producing Isolates of *Trichoderma* spp.

Quantitative estimation for siderophore production was done by the universal CAS assay by Schwyn & Neilands, 1987. Out of 119 *Trichoderma* isolates, 48 randomly selected isolates were screened for siderophore production by spectrophotometric quantitative assay. Siderophore units produced ranged from 0.86% to 55.25%. The highest siderophore was produced by the isolate T_1 (55.22%) and lowest by T_{34} (0.86%). Isolates were categorized as low (29), medium (11) and high (8) siderophore producers.

Table 1: List of Siderophore Producing 48 isolates *Trichoderma* spp.

Group A	Group B	Group C
Low siderophore producer	Medium siderophore producer	High siderophore producer
(0.86 to 15.98 %)	(18.06 to 30.86 %)	(31.73 to 55.25 %)
T_5, T_7, T_9, T_{10}, T_{11}, T_{13}, T_{14}, T_{20}, T_{23}, T_{34}, T_{39}, T_{42}, T_{49}, T_{58}, T_{64}, T_{72}, T_{74}, T_{75}, T_{77}, T_{81}, T_{83}, T_9, T_{91}, T_{92}, T_{98}, T_{100}, T_{101}, T_{107}, T_{108}	T_8, T_{26}, T_{37}, T_{45}, T_{51}, T_{56}, T_{59}, T_{65}, T_{69}, T_{71}, T_{106}	T_1, T_{27}, T_{29}, T_{50}, T_{60}, T_{63}, T_{79}, T_{82}
29 isolates	11 isolates	8 isolates

Interaction of 10 selected *Trichoderma* against *Sclerotium rolfsii and Rhizoctonia solani*

The antagonistic capabilities of selected *Trichoderma* isolates (T_1, T_3, T_8, T_{23}, T_{27}, T_{29}, T_{34}, T_{45}, T_{50}, T_{60}) against rice fungal plant pathogens, namely *Rhizoctonia solani* and *Sclerotium rolfsii*, were evaluated through a confrontation assay using the dual culture method. The assessment took place after three days of growth, with the dual culture growth being monitored for up to seven days. The results showed significant variations in the isolates' ability to combat both plant pathogens, as shown in (Table 2, Fig.1&2).

Periodical observations were made on the growth of *Trichoderma* isolates and their capacity to inhibit the growth of *S. rolfsii* and *R. solani*. The degree of antagonism displayed by the isolates was assessed using a scale of classes, which was designated by Bell *et al.*, (1982) [2]: 1= *Trichoderma* completely overgrew the pathogen and covered more than 75% of the medium surface, resulting in a high level of inhibition (>75% inhibition); 2= *Trichoderma* overgrew at least 2/3 of the medium surface, leading to 50–75% inhibition; 3= *Trichoderma* and the pathogen each colonized approximately half of the medium surface (more than 1/3 and less than 2/3) and neither organism appeared to dominate the other, resulting in 25–50% inhibition; 4= The pathogen colonized at least 2/3 of the medium surface and seemed to withstand encroachment by *Trichoderma*, resulting in less than 25% inhibition; 5= The pathogen completely overgrew the *Trichoderma* and covered the entire medium surface, leading to no inhibition by *Trichoderma*.

The inhibition levels varied between 63.89±2.778% to 75.00±1.667% for *S. rolfsii* and 45.00±2.78% to 75.00±2.78% for *R. solani*. *Trichoderma* isolate T_{27} exhibited the highest inhibition, while T_{23} displayed the lowest inhibition against *S. rolfsii*. Similarly, T_8 isolate displayed the highest inhibition, while T_{29} showed the lowest inhibition against *R. solani*. All the isolates belonged to antagonism class 2 when confronting *S. rolfsii*. However, during antagonism against *R. solani*, six out of ten isolates were categorized as class 2 and the remaining four isolates fell into class 3. *Trichoderma* isolates demonstrated greater effectiveness in inhibiting *S. rolfsii*, as all the isolates exhibited inhibition percentages above $_{60}$%. Nevertheless, due to *R. solani* rapid growth, the growth inhibition after three days of the confrontation assay resulted in a limited response (with inhibition percentages ranging from 45% to 57% for most *Trichoderma* isolates). However, after seven days, almost all *Trichoderma* isolates completely overgrew both phytopathogens, particularly *R. solani*.

Notably, even *Trichoderma* isolates with low siderophore production, such as T_3 and T_{34}, demonstrated competitive abilities in restricting and inhibiting the growth of phytopathogens (Table 2). In this study, the antagonistic response of *Trichoderma* isolates proved to be more effective against *S. rolfsii* than *R. solani* on agar plates. The degree of antagonism varied for specific *Trichoderma* isolates against

each phytopathogen. For instance, T_{27} isolate exhibited 75% apparent inhibition against *S. rolfsii* but showed poorer inhibition against *R. solani* (only 48.89% inhibition). However, one isolate, T_8, demonstrated equal effectiveness in inhibiting both pathogens (73.33% against *S. rolfsii* and 75% against *R. solani*).

This study revealed that different *Trichoderma* isolates possess varying capacities as biological weapons in inhibiting pathogens. Several *Trichoderma* species have been extensively documented as mycoparasites and have been successfully used against specific pathogenic fungi for years (Bell 1982; Chet *et al*., 1998; Vannacci and Gullino 2000; Agrawal & Kotasthane 2010, Kotasthane *et al*., 2015) [2, 4, 9, 1, 7]. The susceptibility of pathogens to *Trichoderma* is typically measured through *in-vitro* screening using an arbitrary rating system, which represents a simplistic approach to understanding a small sector of the biological system in disease control. For broader applications against a greater number of phytopathogens, it might be more advisable to assess blends of antagonist isolates. Further screening of antagonistic isolates against as many isolates of *S. rolfsii* and *R. solani* as possible would enhance the identification of the most effective *Trichoderma* isolate. Additionally, conducting synchronous *in-vivo* inhibition assays would provide valuable insights. Evaluating blends of antagonist isolates could be more prudent for wider applications against a greater variety of phytopathogens.

Table 2: Siderophore production of 10 selected *Trichoderma* isolates and its interaction with *R. solani* & *S. rolfsii* (percent inhibition)

Serial no.	Isolate no.	Percent of Siderophore Units	Confrontation against *S. rolfsii*		Confrontation against *R. solani*	
			Percent inhibition	Antagonism class	Percent inhibition	Antagonism class
1	T_1	$55.24^{g}\pm0.027$	$65.56^{b}\pm1.11$	2	$51.67^{bcde}\pm1.66$	2
2	T_3	$1.53^{a}\pm0.007$	$69.44^{ab}\pm1.67$	2	$57.78^{b}\pm2.22$	2
3	T_8	$22.97^{c}\pm0.016$	$73.33^{a}\pm1.11$	2	$75.00^{a}\pm2.78$	2
4	T_{23}	$15.69^{b}\pm0.064$	$63.89^{b}\pm2.78$	2	$52.78^{bcd}\pm2.78$	2
5	T_{27}	$32.01^{d}\pm0.066$	$75.00^{a}\pm1.67$	2	$48.89^{cde}\pm3.38$	3
6	T_{29}	$39.44^{e}\pm0.011$	$66.67^{b}\pm1.11$	2	$45.00^{e}\pm2.78$	3
7	T_{34}	$0.87^{a}\pm0.018$	$69.44^{ab}\pm0.56$	2	$46.67^{de}\pm2.23$	3
8	T_{45}	$23.52^{c}\pm0.041$	$67.22^{b}\pm0.56$	2	$51.67^{bcde}\pm1.66$	2
9	T_{50}	$44.03^{f}\pm0.043$	$69.44^{ab}\pm2.78$	2	$46.11^{de}\pm 1.67$	3
10	T_{60}	$44.03^{f}\pm0.049$	$73.89^{a}\pm 2.78$	2	$54.44^{bc}\pm1.12$	2
	C.D.	0.128	5.973		7.467	
	SE(m)	0.04	1.841		2.302	
	SE(d)	0.057	2.604		3.255	
	C.V.	2.265	3.752		6.141	

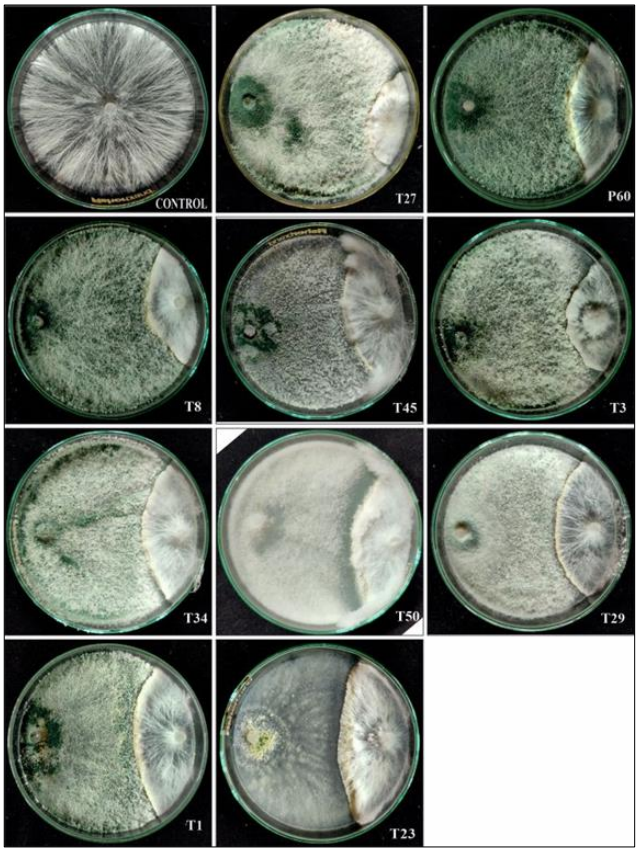

Fig 1: Interaction of *Trichoderma* spp. against *Sclerotium rolfsii* by dual culture method

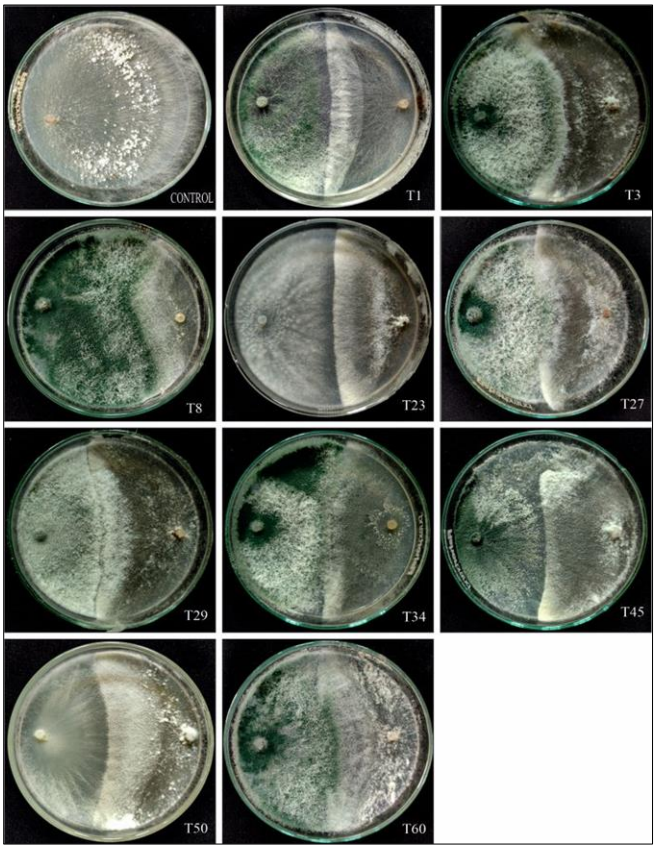

Fig 2: Interaction of *Trichoderma* spp. against *Rhizoctonia solani* by dual culture method

Conclusions

Among the *Trichoderma* isolates derived from the water stressed lateritic soil some potential isolates identified were capable of the production of strong siderophores and were identified to have strong antagonistic activity against phytopathogens *R. solani* and *S. rolfsii.* Significant high siderophore producer isolate can contribute as a candidate agent for both bio-control and PGPR applications.

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