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Screening of native *Trichoderma* isolates against soil borne pathogens of green gram

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Abstract
Use of biocontrol agents against soil borne pathogen is gaining importance in the present situation for eco-friendly management of soil borne diseases. Bioefficacy of native *Trichoderma* spp. has greater impact against soil borne pathogens of green gram. Native *Trichoderma* isolates were collected from soil rhizospheres of green gram from different agroclimatic zones of Odisha. *Fusarium oxysporum* and *Sclerotium rolfsii* were isolated from wilted green gram plants from different green gram plots. Isolate 5 inhibited 71.11% of radial growth of *Fusarium oxysporum* followed by Isolate 7 (60.11%) where as Isolate 5 reduced 60.18% of radial growth of *Sclerotium rolfsii* followed by Isolate 7 (54.31%). All the *Trichoderma* isolates produced toxic volatile metabolites having significant effect in reducing the radial growth of the test pathogens *Fusarium oxysporum* and *Sclerotium rolfsii*. *Trichoderma* isolate-5 inhibited the mycelial growth of test pathogen by 39.62% followed by isolate -7 with 38.60% of inhibition over control. In case of *Sclerotium rolfsii* also *Trichoderma* isolate -5 was found to be most efficient in reducing the mycelial growth of the test pathogen by 44.25%. The significantly maximum zone of inhibition for non-volatile metabolites of *Trichoderma* isolate -5 was found compared to other isolates with 39.64% inhibition against *Fusarium oxysporum* and 36.26% in case of *Sclerotium rolfsii*.

Keywords: *Trichoderma, Fusarium oxysporum, Sclerotium rolfsii*

Introduction
In the human diet, pulses constitute the main source of vegetable protein. A lack of protein in the human diet usually results in protein-energy malnutrition (PEM), which can cause several types of anaemia. Pulses play a significant role in Indian Agriculture as they are accounted for protein rich human diet. India accounts for over one third of the total world area and over 20% of total world production in pulses. The major fungal diseases which infect pulses are Wilt (*Fusarium oxysporum*), Dry root rot (*Rhizoctonia bataticola*), Collar rot (*Sclerotium rolfsii*), Wet root rot (*Rhizoctonia solani*), Ascochyta blight (*Ascochyta rabiei*), Botrytis grey mould (*Botrytis cinerea*), Black root rot (*Fusarium solani*), Seed rot (*Aspergillus flavus*), Stem rot (*Sclerotinia sclerotiorum*), Crown rot (*Sclerotium rolfsii*), Foot rot (*Phacidiopycnis padwickii*) and Sclerotinia wilt (*Sclerotinia sclerotiorum*). Fungal based BCAs have gained wide acceptance next to bacteria (mainly, *Bacillus thuringiensis*), primarily because of their broad spectrum efficacy in terms of disease reduction and yield increase (Copping *et al*., 2000) [6]. In this context, *Trichoderma spp* have been the cynosure of many researchers who have been contributing to biological control pursuit through use of fungi (Ahmad *et al*., 1987 and Aziz *et al*., 1997) [1, 4]. Furthermore, *Trichoderma spp* share almost 50% of the fungal BCAs market, mostly as soil / growth enhancers and this makes them interesting candidates to investigate (Whipps *et al*., 2001) [15]. According to Punja and Utkhede (2003) [12], *Trichoderma spp* are the most widely studied mycoparasitic fungi. In addition to the well-recognized mycoparasitic nature of *Trichoderma* fungus, induction of resistance against pathogens in plants has also been reported by Benhamou (1999) [5]. Hence the present study was carried out to select some local strains of *Trichoderma* effective against soil borne pathogens of greengram.

Materials and Methods
Soil samples from rhizospheres of green gram from different agroclimatic zones of Odisha were collected. For isolation of rhizosphere mycoflora, the dilution plate method proposed by Aneja (2001) [3] was followed. One gram of soil from each sample was taken in a 250 ml conical flask with 100 ml of sterile distilled water. The sample was agitated to prepare a thorough suspension. Serial dilutions of soil suspensions were prepared.

From that required dilution of 10^{-3} was poured in sterilized petriplates containing suitable media (potato dextrose agar media) and the petriplates were incubated at room temperature (28 + 1 ºC). Five replications were maintained for each dilution tested. Observations on number of colonies per gram of rhizosphere soil, and the number of days taken for appearance of each fungal colony on the plates were recorded. Entire mycelia and colony growth were observed under Compound Microscope. Isolates of *Trichoderma* spp. were grouped according to literatures on *Trichoderma spp.* Diseased plants of Mung Bean showing characteristic symptoms of collar rot (*Sclerotium* sp.) and wilt (*Fusarium* sp. were collected from green gram plots. The samples were cut into small pieces and surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) for 30 seconds followed by repeated washing with sterilized water before keeping them on Petri plate containing PDA. Growing colony was identified by observation under research microscope. Pure culture was prepared following single hyphal tip method. The efficacy of *Trichoderma* isolates was tested against the pathogens by dual culture technique (Mortan and Straube, 1955) [9] maintaining three replications. The efficacy of *Trichoderma* isolates was expressed as percentage inhibition of mycelial growth over control. Percent inhibition of the pathogen over control was calculated by adopting the formula from Nene and Thapliyal (1983) [10] as given below.

I (%) = (C-T) / C x 100

I = Percent growth inhibition, C= Growth in control (monoculture),
T= Growth in treatment (dual culture).

Growth inhibition of pathogens by volatile compounds released by *Trichoderma* sp. was evaluated by 'inverted plate technique' (Dennis and Webster, 1971b) [7]. The antagonists and pathogens were inoculated in the centre 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.

Growth inhibition of pathogens by non-volatile compounds released by *Trichoderma species* was done by poisoned food technique (Nene and Thapliyal, 1993) [10]. The *Trichoderma* sp. was grown in Potato Dextrose Broth (PDB) assuming that the antagonist will utilize the nutrients from broth and release some non-volatile metabolites in the medium, which may affect the growth of pathogen. The *Trichoderma* sp. was incubated for two weeks and harvested at the interval of one week. After the incubation, the broth was collected, filtered through Whatman no 1 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.

Results and Discussion

In vitro evaluation of local *Trichoderma* isolates revealed that highest percentage of inhibition (71.11%) in case of *Fusarium oxysporum* was observed by *Trichoderma* Isolate 5 followed by Isolate 7 (60.11%). Isolate 10 was found to be the least effective in growth inhibition of *Fusarium oxysporum* (Table-1). Similar trend was also observed for Isolate 5 inhibiting maximum mycelial growth (60.18%) of *Sclerotium rolfsii* followed by Isolate 7 (with 54.31% inhibition). The lowest growth inhibition was observed by Isolate 2 (8.28%) against *Sclerotium rolfsii*. Kumar *et al.* (2007) [8] tested three species of *Trichoderma* i.e. *T. virens* and *T. viride* and *T. harzianum* against *F. Oxysporum f. sp. subglutinans* and had found that all were effective against the pathogens. Faheem Amin (2010) [2] tested six isolates of *Trichoderma* sp for their ability to inhibit soil borne pathogens of different vegetables *viz*., *Rhizoctonia solani* (isolates from tomato), *Sclerotium rolfsii* (causing collar rot of tomato) and *Sclerotinia sclerotiorum* (causing web blight of beans) under *in vitro* conditions. These earlier reported findings are confirmed inthe present investigation.

The volatile compounds released by *Trichoderma* isolates also showed inhibitory effect on the growth of the test pathogens i.e., *Fusarium oxysporum* and *Sclerotium rolfsii*. *Trichoderma* isolate-5 inhibited the mycelial growth of *Fusarium oxysporum* by 39.62% followed by isolate -7 with 38.60% inhibition over control. In case of *Sclerotium rolfsii* also *Trichoderma* isolate -5 was found most efficient in reducing the mycelial growth of the test pathogen by 44.25%. Species of *Trichoderma* have been demonstrated *in-vitro* to act against fungal plant pathogens by producing diffusible volatile antibiotics by several earlier workers like Stoppacher *et al.* (2010) [13], pan *et al.* (2013) [11] and Waseem *et al.* (2013) [14] which are confirmed in the present study.

All the *Trichoderma* isolates significantly inhibited the test pathogens by production of non-volatile inhibitors at 20%. The maximum zone of inhibition for non-volatile metabolites of *Trichoderma* isolate -5 was found over other isolates with 39.64% inhibition against *Fusarium oxysporum* where as in case of *Sclerotium rolfsii* also *Trichoderma* isolate -5 was found to be most efficacious in reducing the mycelial growth of the test pathogen by 36.26%.

Table 1: *In vitro* efficacy of native *Trichoderma* isolates against *Fusarium oxysporum* and *Sclerotium rolfsii*

Serial no	Isolates	Soil borne Pathogens			
		Fusarium oxysporum		*Sclerotium rolfsii*	
		Mean radial growth (mm)	Percent growth inhibition	Mean radial growth (mm)	Percent growth inhibition
1	Isolate- 1	18.00	38.78	37.00	13.08
2	Isolate- 2	26.00	25.04	39.00	8.28
3	Isolate- 3	24.00	37.20	36.33	9.35
4	Isolate- 4	19.00	34.20	30.67	27.64
5	Isolate- 5	7.67	71.11	17.00	60.18

6	Isolate- 6	12.33	53.06	24.67	42.01
7	Isolate- 7	9.67	60.11	19.33	54.31
8	Isolate- 8	20.67	27.15	35.00	17.56
9	Isolate- 9	14.00	50.86	27.67	34.80
10	Isolate- 10	29.67	23.11	32.33	23.64
S.E(m) +		31.00	-	42.67	-
CD (0.05)		2.07	-	1.28	-

Table 2: Effect of Volatile compounds of native *Trichoderma* isolates against *Fusarium oxysporum* and *Sclerotium rolfsii*

		Effect of Volatile Metabolites			
		Fusarium oxysporum		***Sclerotium rolfsii***	
Serial no	**Isolates**	**Mean radial growth (mm)**	**Percent growth inhibition**	**Mean radial growth (mm)**	**Percent growth inhibition**
1	Isolate- 1	20.56	31.35	23.20	25.44
2	Isolate- 2	20.83	30.54	23.36	24.89
3	Isolate- 3	20.46	31.71	21.56	30.70
4	Isolate- 4	19.80	33.90	21.00	32.50
5	Isolate- 5	18.10	39.62	17.33	44.25
6	Isolate- 6	19.06	36.34	20.13	35.22
7	Isolate- 7	18.40	38.60	9.10	38.57
8	Isolate- 8	19.80	33.93	21.40	31.22
9	Isolate- 9	19.36	35.35	20.56	33.89
10	Isolate- 10	19.96	33.38	22.80	27.04
SE(m) +		30.03	-	31.13	-
CD (0.05)		0.65	-	0.61	-

Table 3: Effect of Non-Volatile compounds of native *Trichoderma* isolates against *Fusarium oxysporum* and *Sclerotium rolfsii*

		Effect of Non-Volatile Metabolites			
		Fusarium oxysporum		***Sclerotium rolfsii***	
Serial no	**Isolates**	**Mean radial growth (mm)**	**Percent growth inhibition**	**Mean radial growth (mm)**	**Percent growth inhibition**
1	Isolate- 1	22.50	27.64	21.16	29.43
2	Isolate- 2	23.00	26.03	22.33	25.43
3	Isolate- 3	22.20	28.60	20.70	30.88
4	Isolate- 4	21.06	32.24	20.26	32.37
5	Isolate- 5	18.76	39.64	19.10	36.26
6	Isolate- 6	20.03	35.57	19.63	34.48
7	Isolate- 7	19.26	38.04	19.40	35.26
8	Isolate- 8	21.56	30.80	20.66	31.02
9	Isolate- 9	20.56	33.86	19.93	33.48
10	Isolate- 10	21.86	29.68	21.43	28.14
SE(m) +		31.10	-	30.00	-
CD (0.05)		0.28	-	0.40	-

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