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Evaluation of Local *Trichoderma* Isolates against Potential Soil Borne Pathogens of Pulses

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A B S T R A C T

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 indigenous *Trichoderma* spp has greater efficacy against soil borne pathogens of pulses. Native *Trichoderma* isolates were collected from soil rhizospheres of paddy, groundnut, brinjal and tomato grown in the Agronomy field and Central farm of Orissa University of Agriculture and Technology, Bhubaneswar and named as Isolate 1, Isolate 2, Isolate 3, Isolate 4, Isolate 5 respectively. Another two isolates were collected from banana fruit (Bhubaneswar) and IIHR Bangalore (*T.harzianum*) and named as Isolate 6 and Isolate 7 (IIHR) respectively. Isolate 2 and Isolate 7 were found to sporulate vigorously and Isolate 5 was fast growing. Highest length of phialide (9.89 µm) was found in Isolate 2 and lowest in Isolate 7 (7.84 µm). Isolate 1 was having the widest phialide (4.82 µm) followed by Isolate 5 with the lowest breadth (2.99 µm). Isolate 7 produced the largest spore of 5.02 µm diameter and Isolate 1 with the lowest i.e 3.6 µm. *Fusarium sp* and *Rhizoctonia sp* were isolated from wilted green gram plants in the fields of OUAT farm. Isolate 5 inhibited 56.40 % radial growth of *Rhizoctonia sp*. followed by Isolate 2 (54.20 %), Isolate 5 reduced 70.1 % radial growth of *Fusarium sp*. followed by Isolate 2.Isolate 2 and 5, through soil application, enhanced 75.97% and 75.12% seedling length in comparison to control respectively against *Rhizoctonia sp*. and enhanced 75.55% and 84.08% seedling length against *Fusarium* sp. soil inoculation.

Introduction

Pulse play a significant role in Indian Agriculture as they are accounted for protein-rich human dietIndia accounts for over one third of the total world area and over 20 per cent of total world production in pulse. 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*), Sclerotinia wilt (*Sclerotinia sclerotiorum*).Fungal based BCAs have gained wide acceptance next to bacteria (mainly,

Bacillus thuringiensis), primarily because of their broad spectrum in terms of disease control and yield increase (Copping *et al.*, 2000). 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). Furthermore, *Trichoderma spp*. share almost 50% of fungal BCAs market, mostly as soil/growth enhancers and this makes them interesting candidates to investigate (Whipps *et al.*, 2001).According to Punja and Utkhede (2003), *Trichoderma* spp. are the most widely studied mycoparasitic fungi. In addition to the well-recognized mycoparasitic nature of *Trichoderma* fungi, induction of resistance against pathogens in plants has also been reported by Benhamou, (1999).

The following studies have been done for the purpose:

In view of the above context local *Trichoderma* isolates were collected from different crop rhizospheres from OUAT fields.

Also two major soil borne pathogens were isolated and identified from wilted green gram plants.

The biocontrol potential of the *Trichoderma* isolates were evaluated both in vitro and in vivo against two soil borne pathogens from green gram.

Materials and Methods

Soil samples from rhizospheres of different crops like groundnut, paddy (Agronomy field OUAT) tomato (Vegetable improvement project, Central farm, OUAT), Brinjal (Trial fields of department of Plant Pathology, College of Agriculture, OUAT). Other isolates were collected from banana fruit and IIHR, Bangalore. The serial dilution plate technique as mentioned below was followed for isolation. Each dilution was transferred aseptically into a sterilized petriplates.

Then different colonies of *Trichoderma sp.*were selected and subcultured in PDA plates by hyphal tip method. Development of *Trichoderma* colonies on PDA were observed from second day till two weeks. *Trichoderma* colonies were observed under the microscope and sub-cultured on PDA at 28±1°C for two weeks. Morphological observations like colony colour, type and growth of colony, macroscopic criteria: mycelium appearance, conidiation colour, pattern, abundance or absence, and coloration of the medium were recorded for the isolates grown on PDA. Entire mycelia and colony growth were observed under Compound Microscope**.** Isolates of T*richoderma* spp. were grouped according to literatures on *Trichoderma* spp. Diseased plants of mungbean showing characteristic symptoms of root rot (*Rhizoctonia sp.)* and wilt (*Fusariumsp* were collected from experimental area of OUAT.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 under research microscopic. Pure culture was prepared following hyphal tip method.

The efficacy of *Trichoderma* isolateswere tested against the pathogens by dual culture technique maintaining three replications. The efficacy of *Trichoderma* isolateswere expressed as percentage inhibition of mycelia growth over control. The Percent inhibition over control was calculated according to formula:

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

I= Percent inhibition

C= Radial growth in control
T= Radial growth in treatment

The data obtained *in-vitro* on per cent growth inhibition of test fungus were analysed following Completely Randomized Design (CRD).Good sandy loam to loam type soil was collected from the field and autoclaved twice at 121^0C temperature for 15 lbsp.s.i. for 20 minutes and put in small polypropylene bags according to the number of treatments and replications. Fresh active culture of the pathogens prepared in PDA were mixed with soil in each polybags. They were then left for incubation for one day. Good quality mungbean seeds were collected and presoaked for 4 to 6 hours. After one day of incubation of the polybags the seeds were sown in the polybags @10 seeds/polybag. Two best isolates of *Trichoderma* were taken to test against soil borne pathogens. After the mungbeen seedlings reached two stage the active culture of *Trichoderma* isolates were mixed with the soil in the rhizosphere zone. Three replications for each treatment was maintained.

Results and Discussion

A total seven number of *Trichoderma* isolates were collected from different crop rhizospheres and individual colony characters, mycelial structures, presence of phialides and conidial size were studied vividly in the current study. Dull white fluffy growth of the mycelium was found in Isolate 1 and also it was highly sporulating. Faint greenish sporulation was found in Isolate 2 with white radiating cottony mycelia. Whitish mycelial growth was also found in Isolate 3 with greenish sporulation at the centre. Dense mycelial growth with radial manner and faint greenish colour of the mycelia was found in Isolate 4. Greenish colouration of the petriplates and sporulation with white to dull white nature were found in Isolate 5, Isolate 6 and Isolate 7. On the basis of colony characters, colour and growth behaviour, the isolate were confirmed as *Trichoderma*sp. (Figure-1). Druzhinina and Kubicek (2005) extensively reviewed the species concept of *Trichoderma* fungi and gave the view of difficulty to distinguish morphologically species of *Trichoderma*. Two pathogens i.e *Rhioctonia sp.* and *Fusarium sp.* were isolated from the root region of green gram. In case of *Rhizoctonia sp*. the mycelial growth of the fungus was radiating sparse after 7 days. Red to orange red coloured rounded sclerotia were found towards the periphery of the plate after 7 days.

The mycelium was both intra and intercellular. Hyphae were septate, thick walled. Sclerotia were abundantly found. The results supported the findings of Vijayan and Nair, 1985. In case of *Fusarium sp*. mycelium was extensive, hyphae were septate branched both inter and intracellular. Mycelium became pink to orange coloured at maturity. Conidia were found with slight bend at the middle with rounded ends and mostly two celledwhich is supported by the findings of Burgess *et al.*, 1994.

In Table-1 Figure-2, Highest percentage inhibition (56.04 %) against *Rhizoctonia* sp. was observed by *Trichoderma* Isolate 5 followed by *Trichoderma* Isolate 2 (54.20 %), Isolate 7 was found to be the least effective in controlling *Rhizoctonia* sp.Then same trend was also observed for Isolate 5 inhibiting maximum mycelial growth (70.1%) against *Fusarium* sp followed by Isolate 2 (69.1% inhibition) (Table 1, Figure-2).

The lowest growth inhibition was observed in Isolate 7 (56.5%) against *Fusarium* sp.. Bansode *et al.*, (2011) screened eight isolates of *Trichoderma* spp for antagonistic effect against *Sclerotium roilfsii* and *Rhizoctonia solani.* Kumar *et al.*, (2007) tested three species of *Trichoderma* i.e. *T. virens* and *T. viride* and *T.harzianum* against *F. oxysporum* f.sp *subglutinans* and found all effective.

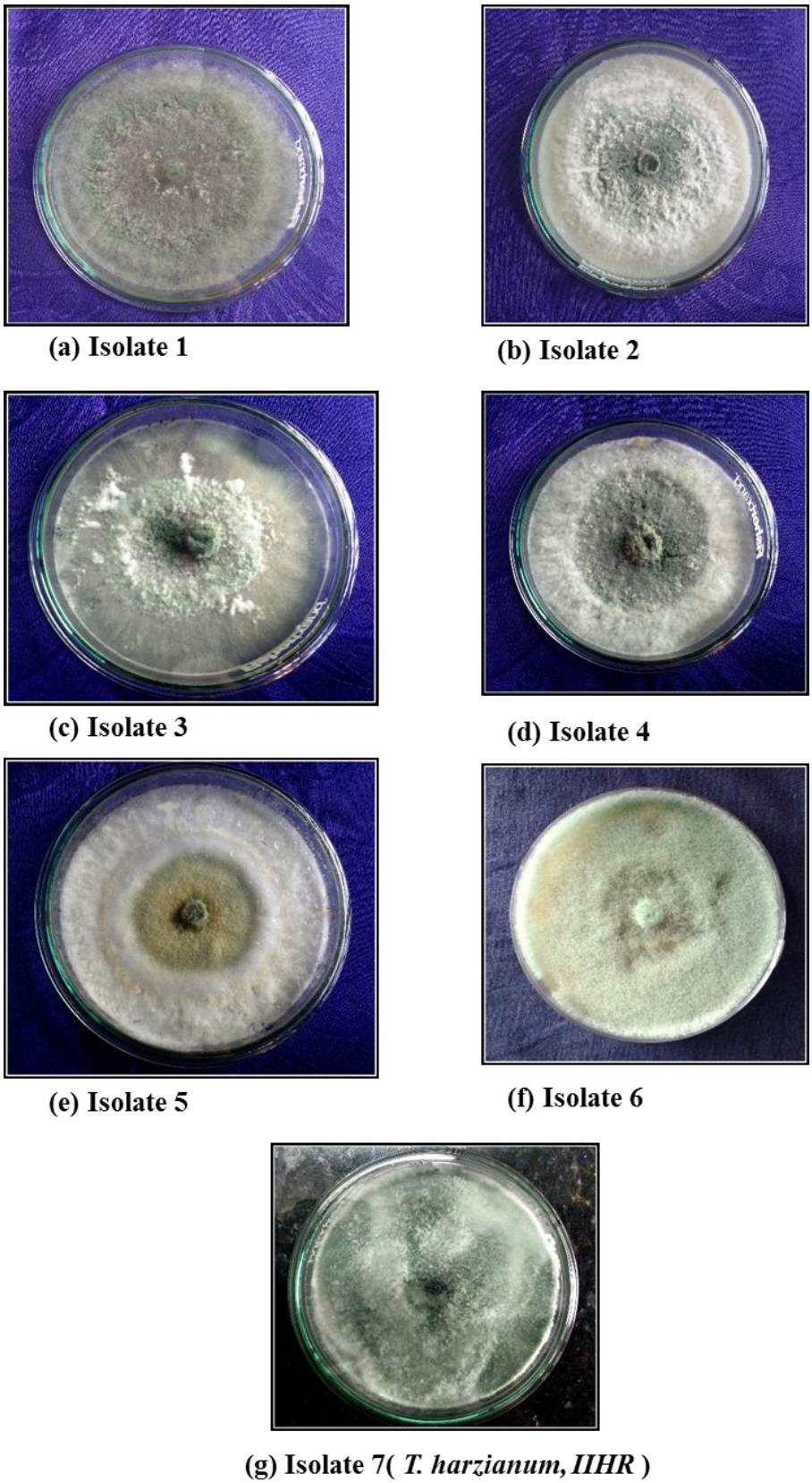
Trichoderma Isolate 5 increased 84.08% seedling length of green gram plants where *Fusarium sp*. was pre-inoculated and Isolate 2 increased 75.9% seedling length where *Rhizoctonia sp*. was pre-inoculated (Table-2). It indicated greater efficacy of Tr. Isolate 5 against *Fusarium sp.* when applied as soil inoculation method than Isolate 2. Deshmukh and Raut (1992), Hussain *et al.*, (1990) reported efficacy of *T. harzianum* against *R.solani* of mungbean.

Table.1 In vitro efficacy of *Trichoderma* isolates against *Rhizoctonia sp*. and *Fusarium sp.*

Serial No.	Isolates	Soil Borne Pathogen			
		Rhizoctonia sp.		***Fusarium sp.***	
		Mean radial growth(mm)	**% Inhibition of mycelial growth**	**Mean radial growth(mm)**	**% Inhibition of mycelial growth**
1.	Isolate 1	48.00	32.60	17.80	59.3
2.	Isolate 2	32.70	54.20	13.60	69.1
3.	Isolate 3	47.30	33.70	16.60	62.2
4.	Isolate 4	39.40	44.70	14.10	67.9
5.	Isolate 5	31.10	56.40	13.10	70.1
6.	Isolate 6	33.30	53.30	18.10	58.7
7.	Isolate 7	51.60	27.60	19.10	56.5
8.	Control	71.30		43.90	
SE(m)+/-		0.76		0.84	
C.D. (5%)		2.30		2.55	

Table.2 Effect of soil application with *Trichoderma* isolates on seedling length of green gram plant inoculated with different soil borne pathogen

Serial No.	Isolates	***Rhizoctonia sp.***		***Fusarium sp.***	
		Mean seedling length (mm)	**%increase over control**	**Mean seedling length (mm)**	**%increase over control**
1.	*Trichoderma* Isolate 2	14.43	75.97	13.57	75.55
2.	*Trichoderma* Isolate 5	14.36	75.12	14.23	84.08
3.	Control	8.20		7.73	



(a) Isolate 1

(b) Isolate 2

(c) Isolate 3

(d) Isolate 4

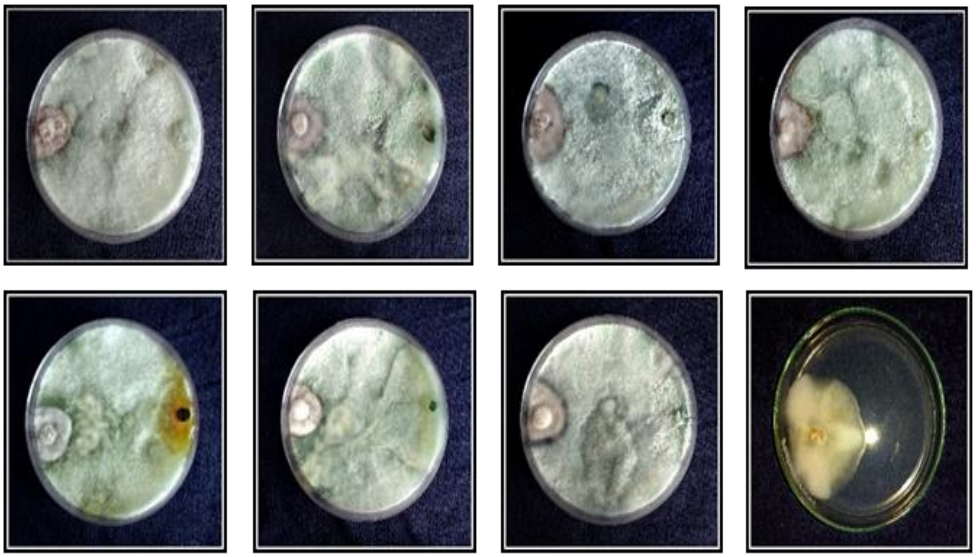
(e) Isolate 5

(f) Isolate 6

(g) Isolate 7(*T. harzianum, IIHR*)

Figure 1. Pure cultures of different *Trichoderma* isolates in PDA.

(a) *Rhizoctonia sp.*



(a) *Fusarium sp*

T1: Isolate 1
T2: Isolate 2
T3: Isolate 3
T4: Isolate 4
T5: Isolate 5
T6: Isolate 6
T7: Isolate 7
T8: Control

Figure 2. Efficacy of *Trichoderma* isolates against *Rhizoctonia sp.* & *Fusarium sp. in vitro*

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