Antagonistic efficacy of native *Trichoderma* isolates against agricultural plant pathogens in India

ABSTRACT

Biological control agents (BCAs) becoming a key element of sustainable agriculture, which maintain ecological balance and shows antagonism against many major problematic plant diseases of world. It is considered as natural and environmentally acceptable alternative to chemical pesticide (Baker and Paulitz, 1996). Different species of *Trichoderma* used in agricultural fields act against large number of phytopathogens. It also helpful in plant growth–promotion, uptake of nutrition from the soil by mineral solubilization, the secretion of secondary metabolites, and production of phytohormones. Some species play important roles in biofortification, bioremediation, and phytoremediation, as well as contaminants from the environment.

Keywords: *Trichoderma*, Plant diseases, agricultural production, microorganisms

INTRODUCTION

Plant diseases are of paramount importance to humans as they damage the ultimate plant and plant products. In the last 50 years, the world population had increased more than double and soon it is expected to increase to over 9 billion by 2050. So as to satisfy needs of rapidly growing human population, agricultural production must be raised. However, Plant diseases becomes cause of concern and raised difficulty to this challenge (Boyd et al., 2012).

Fungal genera consists of soil borne pathogens are most devasting group responsible for causing root disease complex including root rot, seed decay, crown rot, damping off and wilt like problematic diseases. Lewis and Papavizas (1991) reported that about 90 % of 200 major diseases of 31 principle crops in the United States are results of soil borne microorganisms. In India, according to reports more than 50 % crop losses are due to soil borne microorganisms (Elad *et al*., 1982). Thus, pathogenic soil borne microbes particularly, soil borne fungi like

Fusarium, Sclerotium, Macrophomina and Rhizoctonia species acting significant agents in causing major losses to agricultural resources in developing world [11,.12].

BCAs showed antagonism against plant pathogens in several ways either by mycoparasitism, lytic enzymes, antibiotic mediated suppression, by products production and competition for nutrients and space or by induction of host resistance (Pal and Gardener, 2011). Enormous number of BCAs are now available commercially to directly control diseases or incorporate with reduced amount of synthetic pesticides for managing emerging plant pathogenic microorganisms. BCAs include strains which belongs to fungal genera such as Trichoderma and Gliocladium (Vinale *et al*., 2008; Tarantino *et al*., 2007 and Melnick *et al*., 2008).Among the all BCAs, Trichoderma species are most extensively studied species in fungal genera. Trichoderma species are beneficial opportunistic, a-virulent plant symbiotants inhabiting in soil acts as natural antagonist against predominant soil borne fungal pathogens, including fungal genera like *Fusarium, Sclerotium* , *Machrophomina* and *Rhizoctonia*. The precise use of Trichoderma proven to improve root and plant growth and triggered induction of resistance in plants (Harman *et al*., 2004).

Materials and Method-

Diseased samples of different plants such as sugarcane, Jute, sesame were collected from INS farm during the month of October and brought to the plant pathology laboratory in separate polythene bags.

Table 1. collection of plant disease sample

Crop	**Disease**	**Place of collection**	**Geographical coordinates**	**Date of collection**
Sugarcane	Wilt	INS farm, Bhubaneswar	20°18'29.16"N 85°49'32.11"E	26/10/2021
Sugarcane	Leaf spot	INS farm, Bhubaneswar	20°18'29.16"N 85°49'32.11"E	26/10/2021

Jute	Stem rot	INS farm, Bhubaneswar	20°18'29.16"N 85°49'32.11"E	27/10/2021
Sesame	Root rot	Farmers field, Bargarh	21°20´33.30"N 83°37'27.11" E.	20/12/2021

The leaf sample collected from the field were examined and segregated based on crop and type of symptoms they produce such as leaf spot, black spot, stem rot, wilt, root rot, stem rot. The infected leaf sample were cut into pieces having both healthy and diseased portion. These bits were surface sterilized using 1% sodium hypochlorite for 2-3 min and then washed 3 times with sterilized distilled water. These bits were then aseptically transferred to PDA plates and incubated at 27±1°C. Hyphal tip was located under the microscope and marked with the help of sharp glass marking pencil. The tip was carefully lifted up and transferred by sterilized inoculating needle to a potato dextrose agar slant at room temperature. After 2-3 days, the growth of fungus was observed in culture tube and thus a pure culture of fungus was obtained. The pure culture, it was maintained in PDA medium and sub cultured at an interval of 2 weeks. Aseptic conditions were maintained in the inoculation chamber at the time of inoculation. The inoculated cultures were incubated at 25±1°C for 5-7 days.

List 1 : Collection and isolation of *Trichoderma isolates*

Sl.no	**Name**	**Place of collection**
1	*Trichoderma isolate* 1	Bhawanipatna
2	*Trichoderma isolate* 2	Bhubaneswar

3	*Trichoderma isolate* 3	Bhubaneswar
4	*Trichoderma isolate* 4	Bhubaneswar

Soil samples were collected from different fields of Bhubaneswar and Bhawanipatna and brought to the laboratory.

Collection of *Trichoderma isolates*

Different strains of *Trichoderma* isolated in laboratory using serial dilution technique and are maintained in potato dextrose agar media.

Isolation, Identification and storage of Trichoderma

Trichoderma isolates were identified by standard review of literatrure and further confirmed by Indian Type Cultures Collection (ITCC), New Delhi were maintained in slant and stored in refrigerator at 4°C. It was further sent to Heredity biosciences LLP, Bhubaneswar, Odisha for molecular identification using sanger dideoxy sequencing.

Evaluation of *Trichoderma* isolates against pathogens *in vitro*

All the *Trichoderma* strains were evaluated *in vitro* against the test pathogens following standard dual culture method. Twenty mililitres of sterilized potato dextrose agar was poured into sterile Petri dishes and allowed to solidify. Trichoderma was evaluated by inoculating the antagonist at one side of the Petri plate and the test pathogen at exactly opposite side of same plate by leaving 3-4cm gap. Each treatment was replicated four times. Radial growth of pathogen was measured at different intervals of time. All the plates are incubated at room temperature 28±2°C till the control plate was fully covered. After required period of incubation, the radial growth of pathogen was measured. Antagonistic potential was determined by using parameters viz. degree of inhibition or intermingled zone between both the colonies.

One treatment without Trichoderma inoculation was maintained, which served as control. The radial growth of Trichoderma strains and were study at regular time interval.

Percent inhibition over control: The growth inhibition percentage was expressed in terms of inhibition percentage of radial growth of the pathogen by comparing with control plates without antagonist. It was calculated employing Vincent formula(1947).

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

Where I=percent inhibition of mycelium

C= growth of mycelium in control

T=growth of mycelium in treatment

RESULT AND DISCUSSION

Morphological characteristics of Trichoderma isolates

The details of morphological study on different Trichoderma were expressed below. Mycelium varied from watery white to whitish green in colour and the reverse side of petri-plate showed slightly green colour in TA1.The colony had smooth surface, and arial mycelium. Conidiophores were highly branched and formed loose tufts. TA2 had white to dark green colour with dull blackish green shades granules,having green colour at the reverse side of plate. Conidiophores were branched irregularly near the apex . TA3 showed a smooth hairy greenish yellow pattern having a dull yellow reverse colony colour and conidiophore were highly branched in compact form. TA4 showed a nice green and whitish alternate band, mycelium having smooth surface with a light green reverse colony colour. Conidiophores were highly branched. The edge of the colony were smooth in TA1 and TA2 where wavy margin appeared in case of TA3 and TA4.

Similar findings were reported earlier. Bisset, (1991) and Rifai, (1969) observed that the colony grows as fluffy white tufts which later change into greenish

colour due to production of conidia and characterized by presence of concentric rings on the agar plate. The reverse of the colony is whitish yellow or light tan to yellow or pale orange. Rifai, (1969) recognized isolates of Trichoderma based on conidiophores (branching), phialides (shape and size) and some macroscopic characters including rapid growth in culture, sparse aerial mycelium, and production of distinctive, white or green, conidiogenous pustules.

Isolation of different plant pathogenic fungi

Four different plant pathogenic fungi were isolated from different crops and were purified and brought to fresh culture for further investigation.

Confrontation assay

A confrontation assay was performed to evaluate the antagonistic potential of each isolate of Trichoderma against four plant pathogenic fungi the results were described in below with respect to each plant pathogenic fungus.

Root rot of sesame (*Macrophomina phaseolina*)

All the four isolates of Trichoderma were significantly inhibited the test plant pathogen *M phaseolina* ranging from 41.82% to 83.63% after 96 hours of incubation. However, per cent inhibition ranged from 47.5% to 85.2% after prolonged incubation of 168 hours. After 96 hours of incubation period TA2 recorded maximum inhibition of 83.63% followed by TA3(57.27%) and TA1 (50%). Least inhibition was recorded in TA4 (41.81%). However, in 168hr maximum mycelial inhibition was recorded in TA2 (85.24%) followed by TA3 (61.48%) and TA1 (54.91%) and least inhibition resistered in TA4(47.54%)

List 2 : Bio – efficacy of Trichoderma isolates against root rot of sesame

Isolate	**Radial diameter of pathogen after 96hr**	**Per cent of inhibition over control**	**Radial diameter of pathogen after 168hr**	**Per cent of inhibition over control**
TA1	1.38*	50	1.6*	54.91
TA2	0.45	83.63	0.52	85.24
TA3	1.18	57.27	1.35	61.48
TA4	1.60	41.81	1.67	47.54
Control	2.50		2.47	
SE(m)±	0.16		0.10	
CD(0.05)	0.48		0.30	

***mean radial diameter of 4 replications**

Stem rot of jute (Macrophomina phaseolina)

All the isolates of Trichoderma significantly reduced the growth of *M phaseolina* in confrontation assay. Maximum inhibition of 67.07% was recorded by

Trichoderma isolate T2 after 96 hours of incubation followed by TA1 (40.24%) and TA3(31.70%) . Minimum inhibition of 18.29% in growth of *M phaseolina* was recorded by Trichoderma isolate T4 after 96 hours of incubation. However, upon prolonged incubation, increased value of per cent inhibition was recorded. After 168 hours of incubation period, maximum inhibition in growth of *M phaseolina* was recorded by same Trichoderma isolate T2 (82.44%) while Trichoderma isolate T3 (75.53%) minimum inhibited the growth of *M phaseolina*.

Table 2: Efficacy of Trichoderma iasolates against stem rot of jute

Isolate	**Radial diameter of pathogen after 96hr**	**Percent of inhibition over control**	**Radial diameter of pathogen after 168 hr**	**Percent of inhibition over control**
TA1	1.22*	40.24	1.05*	77.65
TA2	0.67	67.07	0.82	82.44
TA3	1.4	31.70	1.15	75.53
TA4	1.67	18.29	1.12	76.06
Control	2.05		4.7	
SE(m)±	0.13		0.22	
CD(0.05)	0.41		0.67	

***mean radial diameter of 4 replications**

Wilt of sugarcane (*Fusarium sacchari*)

All the four isolates of Trichoderma were effective in inhibiting the growth of *Fusarium sacchari*. However, Per cent inhibition was ranging from 32.55% to 54.65% after 96 hours of incubation. However, after prolonged incubation period of 168 hours per cent inhibition ranged from 46% to 68%. Maximum inhibition was recorded by isolate T2 after 96 hours (54.65%) and 168 hours (68%) . After 168 hours of incubation Trichoderma starts covering the growth of *Fusarium sacchari*. Lowest mycelium inhibition observed in TA4 36%.

Table 3: Efficacy of Trichoderma isolates against wilt of sugarcane

Isolate	**Radial diameter of pathogen after 96hr**	**Percent of inhibition over control**	**Radial diameter of pathogen after 168hr**	**Percent of inhibition over control**
TA1	1.35*	37.20	1.35*	46.00
TA2	0.975	54.65	0.8	68.00
TA3	1.425	33.72	1.52	39.00
TA4	1.45	32.55	1.6	36.00
Control	2.15		2.5	
SE(m)±	0.15		0.15	
CD(0.05)	0.33		0.47	

***mean radial diameter of 4 replications**

Leaf spot of sugarcane (*Curvularia lunata)*

All the isolates of Trichoderma significantly reduced the growth of *Curvularia lunata* in dual culture test. However, inhibition percentage ranged from 32.89% to 71.05% after 96 hours of incubation period and from 45.16% to 77.41% after 168 hours of incubation period. Maximum inhibition percentage of 71.05% in growth of *Curvularia lunata* was recorded by Trichoderma isolate T2 after 96 hours of incubation. However, minimum inhibition percentage of 25% was recorded in T1 isolate of Trichoderma. After prolonged incubation period of 168 hours, inhibition percentage was calculated and it was observed that Trichoderma isolate TA2 (77.41%) inhibited maximum to the growth of *Curvularia lunata* followed by TA3 (32.89%) and TA4 (27.63%). Minimum inhibition percentage of 45.16% in growth of *Curvularia lunata* was recorded by Trichoderma isolate T1 after 168 hours of incubation period.

List 3: Potentiality of Trichoderma isolates against *curvularia lunata* causing leaf spot of sugarcane

Isolate	**Average colony of growth of pathogen(96hr)**	**Percent of inhibition over control**	**Average colony growth of pathogen(168hr)**	**Percent of inhibition over control**
TA1	1.42*	25	1.27*	45.16
TA2	0.55	71.05	0.52	77.41
TA3	1.27	32.89	1.55	33.33
TA4	1.37	27.63	1.57	32.25
Control	1.9		2.32	
SE(m)±	0.09		0.15	
CD(0.05)	0.29		0.45	

***mean radial diameter of 4 replication**

Conclusion

Several reports have been made by researcher on the application of trichoderma as biocontrol agent for managing a wide range of soil borne diseases. But it has been observed from the above findings that native isolates of Trichoderma were effective against the plant diseases. In this case, TA2 showed the highest inhibition percentage (85.24%) in root rot of sesame,(82.44%) in stem rot of jute, (68%) in wilt of sugarcane,(77.41%) in leaf spot of sugarcane at 168hr after inoculation. The best Trichoderma Isolate (TA2) was identified as *Trichoderma asperellum* from morphological characteristics and further confirmed by ITCC with id number TA2(11667.22). From the molecular characterization also, it was confirmed as *Trichoderma asperellum.*

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