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Research Article

TRICHODERMA SP AS A MICROBIAL ANTAGONIST AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

Objective: The antagonistic potential of native *Trichoderma* isolates was investigated *In vitro* with an objective of selecting an efficient native bio-control agent against the most prevalent soil borne pathogen against *R. solani* the causal agent of sheath blight in rice.

Methods: Ten native *Trichoderma* isolates and a commercial formulation of *Trichoderma* were screened by using Dual culture method. Eight isolates have shown effective inhibition of pathogen growth. Four isolates showed maximum growth inhibition of 70 to 76%. Microscopic observation of cultures taken from interaction zone of dual culture plates was done in order to find the mycoparasitic interaction between pathogen and antagonist which showed that the hyphae of *Trichoderma* isolates could grow parallel to the hyphae of *Rhizoctonia solani*, coiled around the hyphae and formed appresoria and hook-like structures. Four *Trichoderma* isolates caused lysis and over growth on *R. solani*.

Results: These native antagonists have the properties of potential bio control agent like *In vitro* antagonism towards the test pathogen, producing fungal cell wall degrading enzymes and mycoparasitic behaviour.

Conclusion: The native isolates proved effective in controlling the pathogen *in-vitro* than the commercial formulation, indicating their superiority in the bio control of phyto pathogens.

Keywords: Bio control agent, Mycoparasitism, *Rhizoctonia solani*, *Trichoderma*.

INTRODUCTION

Soil born, plant pathogenic fungi such as *Sclerotium, Rizoctonia, Pithium, Fusarium* cause diseases in most of the economically important crops. Sheath blight caused by *Rhizoctonia solani* is one of the most important destructive diseases of rice next to rice blast [1]. *R. solani* is a soil borne necrotrophic fungus with wide host range and survives in the soil as hard, resistant sclerotial bodies.[2] Besides rice, it can infect crops of nearly 50 species, including barley, lettuce, tomato, sorghum, and maize which is responsible for damping-off, blackspot and root rot diseases. No effective fungicides are available against Rhizoctonia diseases that damages vegetables and more over chemical control has undesirable effects like phyto toxicity and environmental pollution. [3]

In this regard biological control offers an alternative solution for long term sustainability and effective management of soil borne diseases. *Trichoderma sp* which is a common saprophytic filamentous fungi in almost any soil and rhizosphere microflora, is well recognized as bio control agent against various plant pathogenic fungi. Different mechanisms have been suggested for its bio-control activity, which include competition for space and nutrients, secretion of lytic enzymes, mycoparasitism and production of inhibitory compounds.[4] The objective of this study was to evaluate the use of *Trichoderma* spp. in the biocontrol against *R. solani in vitro*.

MATERIALS AND METHODS

Isolation & Identification of pathogen and antagonistic mycoflora

Test pathogen *Rhizoctonia Solani* was isolated from diseased plants. Diseased portions along with visible mycelia transferred aseptically on to petriplates that contained PDA medium and incubated at 28 ± 1^{0}C. After two days of incubation, hyphal growth was observed, which was further purified by hyphal tip method. The identification of the fungus isolated during the study was done based on morphological characters such as hyphal branching, septal pore type and sclerotial formation. Pathogenicity of the fungus was tested on plants by seed treatment method. Pathogen was reisolated from infected plants and compared with original isolate.

R. solani

- Mycelium was white initially, turned to brown
- Hyphae pale brown, branched with nearly right angled side branches constricted basally, septate.
- Sclerotia brown to dark brown

Isolation of *Trichoderma* spp. from soil samples was done by serial dilution method. By using these pure cultures, identification of species of *Trichoderma* was done based on their cultural and morphological characteristics described by Gams and Bisset [5] For eliminating the bacteria antibiotic was added to the media. One sample was isolated from commercial formulation available in the market which is used by the farmers. Colony radial growth was measured and rated as fast, medium and slow growth.

Finally Ten Trichoderma isolates are selected based on their radial growth from many fungal isolates obtained from different soil samples collected from organic fields, and farmer's fields of natural cropping system of the area (paddy, groundnut and vegetable). These isolates are different from one another by their source, colony colour and radial growth.

Screening of antagonists

Ten isolates of native Tricoderma, and Commercial formulation were tested by dual culture technique. [6]

Twenty ml of autoclaved PDA was poured aseptically in to sterile Petri dishes of 9cm diameter A. 2 mm mycelial disc of *Rhizoctonia solani* and *Trichoderma* were placed opposite to each other near the periphery of the petri plate and incubated at 29± 1^{0}C. *Rhizoctonia solani* alone inoculated plate served as control. Mycelial growth of the pathogen was measured and observations were recorded on formation of inhibition zone, over growth and lysis of pathogen mycelium.

Mycoparasitism

Hyphae from the interaction zone of the dual culture plates was observed under Labomed LX400 compound microscope.

Detection of the *In vitro* activities of protease and cellulase by *Trichoderma* isolates

The antagonistic isolates were evaluated for the *In vitro* activities of protease and cellulase. To determine protease activity indicated by casein degradation, fungal isolates were plated on skimmed milk agar (50 ml of sterilized skim-milk mixed with 50 ml of 4% water agar at 55^{0}C), and the width of each resulting clearing halo was recorded as an indicator of the level of protease activity.[7] The

cellulase activity of antagonists was determined by observing the widths of clearing zones on carboxymethylcellulose (CMC)-agar plates (10 g peptone, 10 g yeast powder, 10 g carboxymethylcellulose-Na, 5 g NaCl, 1 g KH_2PO_4, 18 g agar, 1000 ml distilled water, pH 7.0. [8]

RESULTS AND DISCUSSION

Antagonistic mechanism of *Trichoderma In vitro*

R.solani radial growth in check plate and dual cultured plates T_6, T_8 and T_{11} was on par while in other treatments the growth is inhibited on the 2nd day after inoculation. On the 3rd day T_7 allowed even superior growth than the check. From 4th day onwards growth in check plate significantly surpassed all the treatments.

At the end of 6th day isolate T_5 was significantly effective in inhibiting the radial growth of the pathogen (76%) followed by T_8, T_1 and T_4 recording 72 and 70% inhibition. It is significant to note that T_6 (commercial isolate) was initially very effective but on 6th day pathogen growth was observed. *Trichoderma* isolates T_4, T_5, T_8 and T_9 caused lysis and over growth on *R.solani* and isolates T_1, T_2 and T_{10} caused only over growth and isolates T_3 and T_6 were static.

Table 1: **Radial growth of *R. Solani* dual cultured with native isolates of *Trichoderma***

S. No.	Isolate	Radial growth of *R. solani* (cm)					Percent inhibition
		2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	
1	T_1	0.9	2.0	2.4	2.5	2.4	70
2	T_2	0.8	1.6	2.5	2.5	2.6	68
3	T_3	1.0	1.9	2.6	2.6	3.2	60
4	T_4	1.0	1.6	2.0	2.2	2.4	70
5	T_5	0.9	1.6	1.8	1.9	1.9	76
6	T_6	1.1	1.4	2.0	2.0	3.0	62
7	T_7	1.0	2.7	3.0	3.4	3.9	51
8	T_8	1.1	1.6	2.0	2.0	2.2	72
9	T_9	1.0	1.7	2.2	2.3	2.6	67
10	T_{10}	0.9	1.9	2.0	2.2	3.0	62
11	T_{11}	1.1	2.1	2.8	3.3	3.9	51
12	Check	1.2	2.4	3.9	5.8	8.0	_
CV (%)		11.4	8.3	6.3	6.4	4.9	
SEm +		0.05	0.05	0.05	0.06	0.05	
CD(P=0.01)		0.13	0.14	0.14	0.16	0.15	

DAI Days after inoculation

Each treatment replicated thrice.

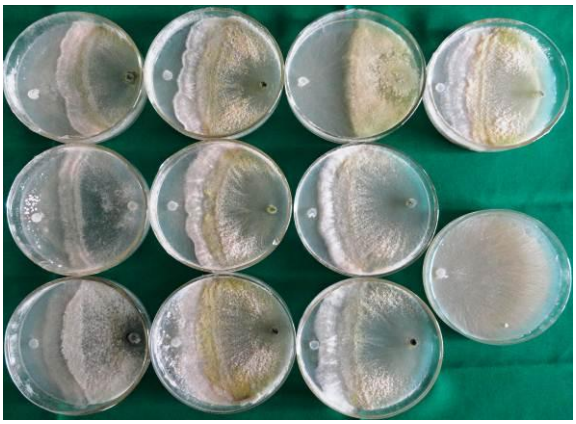


Fig. 1: Interaction of *R.solani* and isolates of *Trichoderma* in dual culture

Prolonged incubation beyond five days resulted in over growth of all the *Trichoderma* isolates over *R.solani* except in case of T_7 and T_{11}. Thus, although initially all the isolates inhibited the pathogen, these could not overcome the pathogen defenses and hence could not overgrow. Jones [9] reported variation in sensitivity to the gliotoxin produced by antagonistic strains of *Trichoderma* and *Gliocladium* for pathogenic *R. solani.*

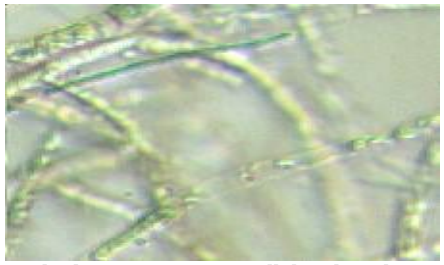
Critical observations on the interaction zone between *Trichoderma* isolates and *R. solani* on the changes in pigmentation, over growth & lysis indicated the effectiveness of the isolates against the pathogen.

i) Zone of inhibition between the two cultures which is an indication of antibiosis by *Trichoderma* against *R. solani*.[10]

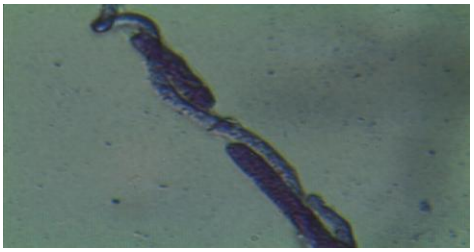
ii) Change in pigmentation on the underside of petri plate with *R. solani* indicated the movement of secondary metabolites of *Trichoderma* unchallenged by *R. solani.* Further, at the interaction zone dark brown coloration appeared beneath the *R. solani* colony. Such zone signifies lysis and death of the pathogen hyphae. [11]

iii) In interactions involving T_2 T_4 T_8 and T_9, growth of *R. solani* at the interaction zone was only suppressed but not stopped. However, in the case of T_1, T_5 interactions growth of *R. solani* came to stand still. This further indicated that these two are more effective than the other isolates.

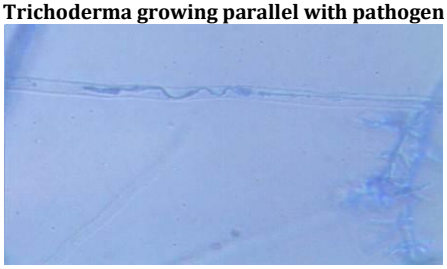
In the present study microscopic observations revealed coiling of *Trichoderma* hyphae around *R. solani* hyphae. It grew parallel to the pathogen hyphae and attached itself to its mycelium by forming hooks/appresoria and penetration of hyphae of *R. solani* and chlamydospores production by *Trichoderma* inside *R. solani* hyphae. As a result pathogen hyphae was ruptured and shrunk in size than the normal hyphae. Coagulation and granulation of *R. solani* cytoplasm indicated the lysis. Similar results were reported by Fausto [12] on the hyperparasitization of *R. solani* by *Trichoderma* sp and stated that coiling, parallel growth, penetration of host hyphae resulted in the Vacuolation, lysis and shrinkage of host hyphae. Microscopic observations in the present study clearly revealed the formation of pores formed on *R. solani* hyphae, indicating the penetration sites of *Trichoderma* by digesting the cell wall.



Trichoderma growing parallel with pathogen



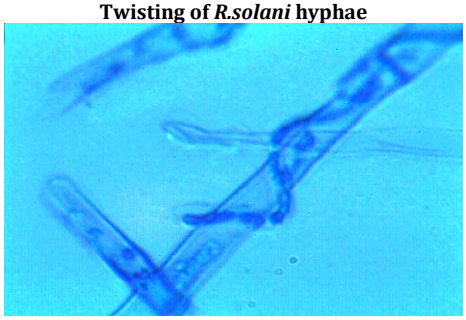
Coiling Trichoderma on *R.solani*



Trichoderma inside the pathogen

Twisting of *R.solani* hyphae

Vacuolation in the hyphae



emptying the pathogen hyphae

Fig 2: Mycoparasitic signs of *Trichoderma*

Cell wall degrading enzymes

All the isolates were tested for their activities on fungal cell wall degrading enzymes i.e. protease and cellulase by observing the clearing zones on milk agar and carboxymethylcellulose plates. All the isolates have shown protease and cellulase activity.

Cellulase (β- 1, 4 glucanase), chitinases and proteases were reported to play a role in myco parasitism. [13] Proteases were responsible for further lysis of the cell wall and subsequent utilization of the disintegrated pathogen as a protein source. Cellulases play a role in disintegration of cell walls of pathogenic hyphae for subsequent utilization as carbon source by Trichoderma. Present investigation supports the view that the Trichoderma involves the use of enzymes in the process of hyphal dissolution. Several works [14] supports the possible role of enzymes in the process of bio control of the pathogen.

Many microorganisms have potential to control fungal plant pathogens but are not successful in all the cases due to different reasons. Bio prospecting to find novel, highly chitinolytic microorganisms which help in developing potential biocontrol agent is the need of the hour and the present investigation could throw light on this aspects as these native antagonists have the properties of potential BCAs— *In vitro* antagonism towards the test pathogen, producing fungal cell wall degrading enzymes and mycoparasitic behaviour.

Previous studies [15, 16] reported 40 to 56% inhibition of *R.solani* by *Trichoderma* isolates in dual culture and rated them for strong antagonism. In the present investigation four native isolates have shown maximum growth inhibition of 70, 70, 72, and 76% against *R.solani* in dual culture studies. Hence these native isolates are far more superior in their antagonistic effect against *R.solani* compared to the results obtained by earlier researchers.

CONCLUSION

The native *Trichoderma* isolates are more effective and showed excellent control of *R.solani*. These isolates could be exploited on a large scale for field application in local conditions which will be promising bio control agents against diseases caused by *R.solani* in field crops.

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