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RESEARCH PAPER

Screening of fungal biocontrol agents against *Sclerotium oryzae* the causal agent of stem rot of rice

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Abstract : *Scleortium oryzae* causes rice stem rot disease which is an important disease of rice. In an effort to develop eco-friendly measures for the control of this disease different *Trichoderma harzianum* and *T. virens* isolates were screened. Variability in all the 15 isolates of *Trichoderma* spp. *was* tested against *Sclerotium oryzae* through monoculture. Results of monoculture showed variability amongst the isolates tested. All the isolates of *Trichoderma* spp. produced volatile compounds and maximum inhibition of the mycelial growth of *Sclerotium oryzae* (28.88%) was recorded by *Trichoderma* sp. (isolate no. 4). Cultural filtrate of all the four isolates of *Trichoderma* spp. resulted in the maximum inhibition of (94.44%) mycelial growth of *Sclerotium oryzae* when used at 50 per cent concentration. At 25 per cent concentration of cultural filtrate, maximum inhibition of mycelial growth (94.44) was observed in case of *Trichoderma* isolate 1 and 2. Glass house experiments showed that all the Trichoderma isolates tested were helpful in disease control and plant growth promotion both.

Key Words : T*richoderma* spp., *Sclerotium oryzae*, Monoculture, Dual culture

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INTRODUCTION

Stem rot caused by *Sclerotium oryzae* Catt. is one of the most serious diseases of rice causing significant reduction in yield. Management of stem rot of rice is a problem, since commercial varieties resistance to this disease is not available. There are few effective chemicals are available but their use is limited due to rice has been used as alternative disease management strategies. There are few reports indicating effectivity of bioagents against stem rot pathogen. However, no systemic studies have so far been made to explore the possibility of developing biological management of stem rot. *Trichoderma harzianum* has been used as an effective biocontrol agent for damping off disease of peanut caused by *S. rolfsii* and *Rhizoctonia solani* (Chet *et al*., 1979; Elad *et al.* 1980). The present investigation was carried out on the screening of different isolates of *Trichoderma* spp. against *S. oryzae* to find out the most effective strains for their further evaluation.

MATERIALS AND METHODS

Sclerotium oryzae was isolated from diseased plants showing characteristic symptoms and maintained on Potato dextrose agar (PDA) medium. Isolates of *Trichoderma* spp., *T. harzianum* isolates, *T. hamatum and T. virens* used in present investigation were obtained from Rice Pathology and Bio-control laboratory, Pantnagar. Few isolates of *Trichoderma* spp. were also isolated from rice leaves collected from different location of the district U. S. Nagar. Variability amongst different isolates of *Trichoderma* spp. was evaluated by using mono culture method. Twenty ml of sterilized melted PDA was aseptically poured in sterilized Petri plates and allowed to solidify. 5 mm mycelial discs of fungal isolates (biocontrol agents) cut from actively growing culture plates, were placed at a centre of Petri plate. These plates were incubated at 28 ± 1^0C. Periodic observations on the growth of fungal isolates were recorded. Fungal isolates (biocontrol agents) were

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screened for their antagonistic potential against the pathogen following dual culture technique (Morton and Stroube, 1955). Fungal biocontrol agents were grown on Petri plates containing PDA for 2, 4, 6, 8 days. The lid of each Petri plates was replaced with the bottom of PDA plates inoculated with *Sclerotium oryzae*. (test fungus) at the centre. The bottom of the Petri plates containing centrally inoculated mycelial disc of test fungus served as check. The pair of each plate was taped together with cellophane adhesive tape. Three replications of each treatment were maintained. The plates were incubated at 28 ± 1^0C. The colony diameter of the test fungus in the treatment in comparison with that of check gave growth inhibition per cent.

Glass house experiment :

Plant growth promotion activity of the *Trichoderma* isolates on rice was studied under glass house conditions during 2007-08. Seeds were treated with powdered formulation of *Trichoderma* isolates (@ 10g/kg seed: cfu=10^9/g powder) except for control. Ten seeds were sown per pot having autoclaved soil. Three replications were maintained for each treatment including control. Pots were irrigated daily. Ten days after germination only 5 plants were maintained in each pot. Observations were recorded for root and shoot length and compared with control.

RESULTS AND DISCUSSION

All the isolates had variations in their radial growth. Among 15 isolates of *Trichoderma* spp., 8 isolates attained a radial growth of 85.00 mm after 72 hours whereas isolates 4, 10, 11, 37, 44, *T. virens, T. hamatum* exhibited mycelial growth of 51.0, 80.0, 51.7, 76.0, 84.7, 79.3 and 80.7 mm, respectively (Table 1).

Results obtained for the dual culture technique indicated that of the 15 isolates of *Trichoderma* spp., isolates (1, 12, 22, 24, 32 and 43) attained a radial growth of 75 mm after 72 hour. Whereas, *T. virens* and *T. hamatum* and *T. harzianum* exhibited mycelial growth of 39, 30 and 35.0 mm, respectively. After 96 hour of incubation *Trichoderma* spp. isolates 1, 12, 31, and 44 exhibited more than 70 mm mycelial growth. Mycelial growth of pathogen was checked to a little extent with the hyphae of these biocontrol agents which continued its fast growth. Yellow band was noticed at the point of contact between mycelium of the pathogen and biocontrol agent which was clearly visible from underside of the Petri plates. In case of *Trichoderma* sp., at the beginning, it grew together on the same medium. After colonies met, the mycelium of *Trichoderma* sp. grew into areas that have already been occupied by *Sclerotium oryzae.* The pathogen was checked and its colony was overrun by *Trichoderma* sp. which covers entire plate very quickly. Re-isolation of the same area where pathogen has been growing resulted in the recovery of *Trichoderma* sp. alone. It shows that *Trichoderma* sp. is capable attacking and killing of *S. oryzae* in dual culture (Table 2). Antagonism of *Trichoderma* spp. on *Sclerotium rolfsii* had been reported by Yaqub and Shahzad (2005) and Puri *et al.* (1998).

Result of experiment on the effect of volatile compounds of fungal biocontrol agents on the radial growth of *Sclerotium oryzae* indicated that actively growing fungal biocontrol

Table 1: Radial growth of fungal isolates (*Trichoderma* spp.) in monoculture

Trichoderma isolate	Radial growth* (mm)		
	24 hrs	48 hrs	72hrs
T_1	26.30	64.00	85.00
T_3	28.70	58.70	85.00
T_4	26.70	48.00	51.00
T_{10}	31.30	63.70	80.00
T_{11}	19.30	28.70	51.70
T_{12}	34.00	72.30	85.00
T_{22}	34.70	78.00	85.00
T_{24}	28.00	54.00	85.00
T_{31}	28.30	68.30	85.00
T_{32}	32.00	62.00	85.00
T_{37}	25.30	54.30	76.00
T_{44}	25.30	65.70	84.70
T. harzianum	30.00	72.70	85.50
T. virens	20.70	51.70	79.30
T. hamatum	21.30	48.70	80.70
C.D. at 5%	7.26	7.30	5.71

* Mean of three replications

agents on PDA produced volatile compounds which significantly inhibited the radial growth of the test pathogen (Table 3). Maximum inhibition of the mycelial growth of *Sclerotium oryzae* (28.88%) was recorded by *Trichoderma* sp. (isolate no. 4) which was followed by isolate no. 10 (24.66%), 43 (26.22%), 11 (24.11%), 12 (22.11%) and 3 (21.11%), when 2 days old cultures were used. As the culture of biocontrol agents got older their effect on the growth of pathogen was significantly reduced. The vapour action was minimum on the pathogen from 8 days old culture of biocontrol

Table 2: Linear growth of *Trichoderma* spp. in dual culture with *Sclerotium oryzae.*

Trichoderma isolate	Linear growth*(mm)		
	48hour	72 hour	96 hour
T_1	38.00	56.00	77.30
T_3	23.60	46.00	66.30
T_4	30.00	51.30	69.00
T_{10}	30.00	51.60	70.60
T_{11}	21.30	35.30	53.60
T_{12}	30.00	50.00	71.30
T_{22}	34.60	54.00	66.60
T_{24}	31.00	50.60	65.60
T_{31}	31.60	57.30	73.30
T_{32}	29.30	50.00	67.30
T_{37}	22.60	33.30	56.60
T_{44}	26.30	47.00	73.30
T. harzianum	23.60	41.30	69.00
T. virens	22.00	39.30	68.30
T. hamatum	18.30	35.00	61.60
C.D. 5%	3.74	3.42	5.87

*Mean of three replication

Table 3: Effect of volatile compounds produced by *Trichoderma* spp. on radial growth of *Sclerotium oryzae*

Trichoderma isolates	Radial growth (mm) of *S. oryzae*			
	2 days	4 days	6 days	8 days
T_1	73.83	66.66	70.83	74.16
T_3	71.00	80.66	74.00	61.00
T_4	64.00	85.93	69.33	70.33
T_{10}	67.83	72.16	74.66	77.50
T_{11}	76.16	76.33	74.33	84.66
T_{12}	70.00	89.00	73.33	87.33
T_{22}	79.33	87.00	70.33	85.00
T_{24}	79.66	87.16	85.33	88.00
T_{31}	79.50	85.33	81.66	85.66
T_{32}	83.83	86.00	85.00	88.00
T_{37}	75.83	90.00	90.00	90.00
T_{43}	90.00	90.00	86.33	89.33
T_{44}	83.66	90.00	90.00	89.33
T. harzianum	84.33	89.33	90.00	88.00
T. virens	85.83	80.33	73.66	75.00
T. hamatum	78.66	77.33	76.00	76.00
Check	90.00	90.00	90.00	90.00
C.D. at 5 %		6.20		

agents. There was a linear relationship: fresher the biocontrol agent cultures, greater the amount of volatile compounds and consequently less radial growth, more inhibition percentage. *Trichoderma* isolates no. 3 (32.77%), 4 (21.88%), 1 (17.56%), *T. hamatum (*16.66%), and 10 (13.89%) resulted in a maximum inhibition of mycelial growth of *S. oryzae*, when eight days old culture of biocontrol agents were used. Eight days old culture of isolates nos. 37, 43, 44, and 101 did not inhibit mycelial growth of *S. oryzae* The production of volatile compounds by different isolates and specific group of *Trichoderma* / against several test fungi (*R. solani*, *Sclerotium* sp., *Fusarium* sp. etc.) was demonstrated by Dennis and Webster (1971). Hutchinson and Cowan (1972) who observed the release of antibiotic from *Trichoderma* sp. which were inhibitory to *Sclerotium rolfsii*. The inhibitory action of volatile compounds produced by *Trichoderma* sp. on rice isolates of *Rhizoctonia solani* was reported by Bhuyan *et al.* (1994) and Dubey (1995). Similar observations indicating inhibitory effect of volatile antibiotics produced by *Trichoderma* sp. against sugarbeet isolate of *Sclerotium solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* (Saxena and Mukhopadhyay, 1987) were also reported. The effect of cultural filtrate of selected biocontrol agents on radial growth of *Sclerotium oryzae* indicated that the biocontrol agents produced non-volatile antifungal antibiotics (Table 4). With the increase in the concentration of culture filtrates of biocontrol agents in the medium the radial growth of test pathogen was proportionally decreased, in general. Maximum inhibition (94.44%) of mycelial growth of *Sclerotium oryzae* was recorded with the culture filtrate of all four *Trichoderma* spp. isolates, (1, 4, 12 and 43) used at 50 per cent concentration. At 25 per cent concentration of cultural filtrate maximum inhibition of mycelial growth (94.44%) of *Sclerotium oryzae* was observed in case of *Trichoderma* spp. isolate 1 and 12 followed by 4 (82.59%) and 43 (82.22%), respectively. At 10 per cent concentration of cultural filtrate maximum inhibition of mycelial growth of *Sclerotium oryzae*

Table 4: Effect of non volatile compounds (cultural filtrate) *Trichoderma* isolates on radial growth (mm) of *Sclerotium oryzae*

Trichoderma isolates	Concentrations (%) of cultural filtrate of *Trichoderma*			
	Radial growth* (mm)			
	50	25	10	Mean
T_1	5.00	5.00	13.00	7.66
T_4	5.00	15.67	60.66	27.11
T_{12}	5.00	5.00	13.66	7.88
T_{43}	5.00	16.00	80.00	33.66
Check	90.00	90.00	90.00	90.00
C.D. at 5%		2.27		

Table 5 : Effect of seed treatment of *Trichoderma* isolates formulations on plant growth

Trichoderma isolate	Root Length (cm) 21 DAT	Shoot Length (cm) 21 DAT
T_1	31.50	51.10
T_3	14.10	51.30
T_4	13.30	50.80
T_{10}	17.20	51.30
T_{11}	10.00	38.20
T_{12}	7.10	40.20
T_{22}	6.70	42.20
T_{24}	7.40	44.90
T_{31}	10.60	56.10
T_{32}	7.80	48.40
T_{37}	11.30	37.60
T_{44}	9.40	39.00
T. harzianum	14.00	46.20
T. virens	7.10	45.50
T. hamatum	12.10	53.70
Control	6.20	35.10
C.D. (5%)	12.04	0.20

was observed in case *Trichoderma* spp. isolate 1 (85.55%) followed by 12 (84.81%), 4 (32.59%) and 43 (11.11%).

All the isolates were found effective regarding to root growth promotion as compare to check (Table 5). Maximum root growth increment was recorded with isolate T_{10} whereas minimum root increment was given by isolate T_{22}. All isolates gave promising results regarding shoot growth increment as compared to check. Isolates T_{31} was the best isolate in terms of shoot growth (Table 5).

The findings of the present investigation clearly indicate the potential of *Trichoderma* spp. against *Sclerotium oryzae.* However field trials are required to evaluate the efficacy of potential isolates of *Trichoderma* sp against stem rot.

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