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In vitro evaluation of bio agents against rice sheath blight pathogen *Rhizoctonia solani* Kuhn

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ABSTRACT

It is now an established fact that strains of *Trichoderma* and *Pseudomonas* are most capable among all the potent biological control agents against phytopathogenic micro-organisms recorded till now. They are effective against pathogenic microbes by the virtue of their capacity to produce some antagonistic chemicals (antibiosis), competition for food and nutrition, direct killing (parasitisation) etc. One such study was conducted to know the interaction between *Trichoderma* spp. against *Rhizoctonia solani* causing sheath blight disease in rice. Nine isolates of *Trichoderma* were tested for its parasitisation ability under dual culture shown and it was found that 77.03 – 100 per cent inhibition of Rhizoctonia after 72 hours of inoculation. Also, the effect of inhibiting volatiles was seen by inverted plate technique. It was found that none of the nine isolate of *Trichoderma* significantly reduced the growth of *R. solani* by producing volatiles and inhibition percent was found only 3.88 – 5.60 per cent. Three isolates of *Pseudomonas florescens* inhibited the growth of the test fungus by only 30.36-34.81per cent, which is very less when compared to fungal biocontrol agent *Trichoderma*.

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INTRODUCTION

Rhizoctonia solani Kühn is a an ubiquitous soil borne pathogen and thrives on large host range (Singh *et al.*, 1994 and Jia *et al.*, 2007). Various researchers have estimated losses upto 10-60 per cent due to sheath blight (Marchetti and Bollich, 1991; Bonmann *et al.*,1992; Rush and Lee, 1992; Cu *et al.*,1996; Rabindran and Vidhyasekaran, 1996 and Savary *et al.*, 2000). Its potential to cause devastation can be estimated by nature of its hosts as majority of them are prime food source to a large number of population (Qin and Zhang, 2005 and Maruthasalam *et al.*, 2007).

Soil-borne pathogens have always been a menace for agriculturists owing to various factors like

heterogeneity in the soil's physico-chemical properties rendering chemical control a futile business, hindrance in the accumulation of toxic concentration of the chemical against the pathogen, complex behavioural as well as biochemical constituents of these pathogens (Vibha, 2011 and Gaigole *et al.*,2011). Apart from all these, considering health and environmental hazard posed by use of synthetics, residual toxicity, restriction in use of certain chemicals and their doses demands a search for safer alternatives (Rathore and Nollet, 2012; Jardim and Caldas, 2012 and Akoto *et al.*, 2013).

The objective of this research was to evaluate one of such alternative for eco-friendly management of *R. solani*.

MATERIAL AND METHODS

Nine isolates of *Trichoderma harzianum* and three isolates of *Pseudomonas fluorescens* were tested under *In vitro* conditions against *R. solani*. These isolates were obtained from GBPUA and T Pantnagar and Sheath blight pathogen was isolated from infected leaf sheath collected from N.E. Borlaug Crop Research Centre, Pantnagar.

Isolation of *R. solani*:

Infected leaves of paddy exhibiting typical symptoms of sheath blight were collected from field in a paper bag. For the isolation of fungal pathogen, its sclerotia and infected leaves both were surface sterilized with mercuric chloride solution (0.1%) for 30 seconds and washed thrice with sterilized distilled water and were placed aseptically on sterilized Petri dish containing PDA. The inoculated Petri dishes were incubated in a BOD incubator at 28±1ºC for a week. The pathogenicity was proved following Koch's postulates.

For fungal antagonist:

Production of volatile metabolites:

Production of volatile metabolites by fungal bio-agent *Trichoderma* isolates were evaluated by inverted plate technique as described by Dennis and Webster (1971). Glass Petri plates of diameter 90 mm were inoculated with 5 mm disc of *R. solani* and bio agent from their actively growing culture and were sealed mouth to mouth with parafilm under aseptic conditions (Table1). Petri plates without bioagents served as control. Each treatment was replicated three times.

Inoculated Petri plates were incubated at 28± 1ºC and observations as colony diameter (mm) was recorded at every 24 hour interval till the control Petri plates were fully covered with the growth of the *R. solani*. The efficacy of volatiles was expressed as per cent inhibition of radial growth over the control which was calculated by using the following formula:

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

where,

I = Inhibition per cent

C= Colony diameter in check (mm)

T= Colony diameter in treatments (mm).

Dual culture technique:

Dual culture technique as described by Morton and Stroube (1955) was followed to evaluate efficacy of direct mechanism (mycoparasitism) of bio-control as indirect mechanism has been stroked off for the tested isolates of fungal bio-agent.

Fifteen ml of sterilized melted potato dextrose agar medium was poured aseptically near burner flame in sterilized laminar flow into 85 mm sterilized Petri plates. Five mm discs of the fungal antagonist and the test fungus were cut with the help of a sharp sterilized cork borer from the edge of 3 days old culture and placed on the solidified medium the by placing them in a straight line equidistant from each other maintaining a distance of 3 mm from the edge of PDA plates. Each treatment was replicated three times. Inoculated Petri plates were incubated at 28± 1ºC and observations as colony diameter (mm) was recorded at every 24 hour interval.

Assessment of per cent parasitisation:

Growth diameter of the *R. solani* (mycelial growth) at the time of meeting with mycelial growth of fungal antagonist served as check for every treatment and was compared every 24 hour interval until complete parasitisation (Table 2). This was done to assess mycoparasitisation capacity of antagonist.

Per cent parasitisation was calculated using the following formula:

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

where,

I = Inhibition per cent

C = Colony diameter in control (mm)

T = Colony diameter in treatments (mm).

For bacterial antagonist:

Dual culture technique:

One 5-mm disc of a pure culture of *R. solani* was placed at one corner of a Petri plate containing PDA and at the opposite end sterilized paper disc dipped in bacterial suspension of antagonistic bacteria (5 x 10^9-cfu ml^1), was placed, a check was maintained by replacing bacterial suspension by sterile distilled water. Each isolate of *Pseudomonas fluorescens* was considered as a treatment and was replicated three times. Inoculated Petri plates were incubated at 28± 1ºC and observations as colony diameter (mm) was recorded at every 24 hour interval and growth diameter of the pathogen (fungal growth) was measured and compared to control growth (Table 3). Per cent inhibition was calculated using the following formula:

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

where,

I = Inhibition per cent

C = Colony diameter in check (mm)

T = Colony diameter in treatments (mm).

RESULTS AND DISCUSSION

Among nine isolates of *Trichoderma* screened only three *viz*., Th-69, 89-N and 82-N had shown volatile metabolite activity against *R. solani* by reducing mycelial growth by 5.18, 3.88, 5.60 per cent, respectively (Table 1). However, a very high per cent parasitisation was recorded in all the nine isolates (100 -77%) where maximum parasitisation was recorded in 89 N, 82 N and L Th- 14 (100.00%) whereas minimum in T-93 (77.03%) (Table 2). Overall reduction in growth of *R. solani* was also found maximum in 89 N, 82 N and L Th-14 (100.00%) and minimum was recorded in T-93 (92.70%).

Among bacterial bio-agent isolates PsF_2 and PsB_{31} were found inhibiting mycelial growth of *R. solani* by 34.81 and 33.85 per cent, respectively followed by PsF_{11} (30.36%) (Table 3).

Isolates of *Pseudomonas fluorescens* used in the present study showed very low per cent inhibition as compared to the fungal antagonist. The fact cannot be denied that *Pseudomonas fluorescens* appear to be promising in induction of ISR and referred as PGPR for promoting growth and development of plant (Nandakumar *et al.*, 2001; Rao, 2007 and Khan and Khan, 2001) and this could also be a reason for their low potential *in vitro* condition.

Trichoderma strains exert biocontrol against fungal phytopathogens either indirectly, directly or by coordination of both (Benitez *et al.*, 2004), Here in case of isolate 89-N and 82-N cent per cent reduction in the growth of *R. solani* may be by virtue of combined mechanism of production of volatile metabolite (indirect) and mycoparasitisation (direct) while for rest of the isolate may have mycoparasitisation as their prime mechanism of biocontrol. Isolate Th-69 has shown 91.04 per cent parasitisation while its volatile activity was recorded 5.18 per cent and therefore, the overall reduction in the mycelial growth of *R. solani* was recorded 97.64 per cent which may be considered as synergistic effect of two mechanism (volatile activity and mycoparasitisation) on overall reduction.

Table 1: Effect of volatiles of fungal antagonist (*Trichoderma harzianum*) on mycelial growth of *Rhizoctonia solani* at 28± 1°C

Sr. No.	Fungal bioagents (Isolates of *Trichoderma harzianum*)	Colony diameter of *Rhizoctonia solani* (mm)	Per cent growth inhibition of *Rhizoctonia solani*
1.	T-93	90.00	0.00
2.	T-60	90.00	0.00
3.	LTh-14	90.00	0.00
4.	Th-69	85.33	5.18
5.	Th-75	90.00	0.00
6.	89-N	86.50	3.88
7.	82-N	84.96	5.60
8.	TCMS-4	90.00	0.00
9.	TCMS-65	90.00	0.00
10.	Check	90.00	-
C.D. (P=0.05)		1.97	

Table 2: Effect of fungal antagonist (*Trichoderma harzianum*) on mycelial growth of *Rhizoctonia solani* at 28± 1°C

Sr. No.	Treatments	Incubation period (24 hours)		Incubation period (48 hours)			Incubation period (72 hours)		
	Fungal bioagents (Isolates of *Trichoderma* spp.)	Colony diameter of *Trichoderma* sp.(mm)	Colony diameter of *Rhizoctonia solani* (mm)	Colony diameter of *Trichoderma* sp.(mm)	Colony diameter of *Rhizoctonia solani* (mm)	Per cent parasitization of *Rhizoctonia solani*	Colony diameter of *Trichoderma* sp. (mm)	Colony diameter of *Rhizoctonia solani* (mm)	Per cent parasitization of *Rhizoctonia solani*
1.	T-93	58.00	27.00	69.33	15.33	43.22	78.80	06.20	77.03
2.	T-60	57.35	27.65	65.00	20.00	27.67	80.00	05.00	81.91
3.	LTh-14	72.67	12.33	80.00	05.00	59.44	85.00	00.00	100.00
4.	Th-69	62.67	22.33	78.00	07.00	68.65	83.00	02.00	91.04
5.	Th-75	63.67	21.33	76.00	09.00	57.80	84.00	01.00	95.31
6.	89-N	64.33	20.67	79.00	06.00	70.97	85.00	00.00	100.00
7.	82-N	67.33	17.67	78.67	06.33	64.2	85.00	00.00	100.00
8.	TCMS-4	55.00	30.00	67.33	17.33	42.23	79.65	05.35	82.16
9.	TCMS-65	68.33	16.67	74.33	10.67	35.99	82.60	02.40	85.60
C.D. (P=0.05)					A : 05.28				
					B : 11.21				
					C : 15.85				

Table 3: Effect of bacterial antagonist (*Pseudomonas florescens*) on mycelial growth of *Rhizoctonia solani* at 28± 1°C

Sr. No.	Treatments	Incubation period (24 hours)		Incubation period (48 hours)		Incubation period (72 hours)	
	Bacterial bioagents (Isolates of *Pseudomonas florescens*)	Colony diameter of *R. solani* (mm)	Per cent growth inhibition of *R. solani*	Colony diameter of *R. solani* (mm)	Per cent growth inhibition of *R. solani*	Colony diameter of *R. solani* (mm)	Per cent growth inhibition of *R. solani*
1.	PsF_{11}	44.67	15.18	52.75	22.04	59.19	30.36
2.	PsF_{2}	37.33	29.12	49.33	27.10	55.41	34.81
3.	PsB_{31}	39.80	24.43	48.98	27.61	56.22	33.85
	Check	52.67	-	67.67	-	85.00	-
C.D. (P=0.05)			1.29				

Conclusion:

On the basis of *In-vitro* assay of selected isolates of *Pseudomonas fluorescens* and *Trichoderma* against *Rhizoctonia solani* causing Sheath blight of rice it can be concluded that both the bioagents are potent to check the growth of fungal plant pathogen. Hence, the study can be further taken to field experimentation for validation and evaluation under natural condition.

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