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Antagonistic Potential of Isolates of *Trichoderma* spp. and *Pseudomonas fluorescens* Against Rice Sheath Blight Pathogen *Rhizoctonia solani In vitro*

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ABSTRACT

Seven isolates of Trichoderma spp. and eight isolates of Pseudomonas fluorescens were screened for their antagonistic potential in vitro against sheath blight pathogen Rhizoctonia solani using dual culture technique. Two of the seven Trichoderma isolates viz., ET-1 and RT-4 were found potentially antagonistic as per Bell's scale with complete overgrowth on R. solani in vitro. Two of the eight P. fluorescens isolates tested in dual culture against R. solani, viz., PF-2 and PF-5 were found potentially antagonistic in vitro sustaining inhibitory effect for even 10 days after inoculation.

Key words: *Trichoderma, Rhizoctonia, Pseudomonas, Antagonistic*

INTRODUCTION

Rice sheath blight caused by *R. solani* is second only to, and often rivals rice blast in importance. The disease is alarming due to the intensive cultivation of modern high fertilizer responsive high yielding varieties. Crop with high plant density and close canopy favors disease build up from panicle initiation onwards. Yield losses as large as 50% occur in susceptible cultivars when all the leaf sheaths and leaf blades are infected. Management of this disease at field level has become a problem due to lack of resistant varieties. Several fungicides were found effective against sheath blight[7]. However, due to unsatisfactory control and environmental pollution with chemical control, efforts are diverted towards management of this disease using biological control agents[4]. However, as isolate variation existed in the antagonistic potential of biocontrol agents with respect to the species and the isolate used[5].

Hence the present investigation was undertaken to screen six isolates of *Trichoderma* spp. and eight isolates of *P. fluorescens in vitro* using dual culture method.

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MATERIAL AND METHODS

Six isolates of *Trichoderma* spp. and eight isolates of *Pseudomonas fluorescens* obtained from the Department of Plant Pathology, S. V. Agricultural College, Tirupati (Table-1) were screened using dual culture method[6]. Potato Dextrose Agar medium was used for dual culture studies.

In case of *R. solani-Trichoderma* interactions, inoculation of 2 mm discs of both the cultures was separately inoculated in the same plate at 7.0 cm distance. In case of *R. solani-P. fluorescens* interactions, *R. solani* culture disc was inoculated at the center and *P. fluorescens* was streaked 2.5cm away from *R. solani* on both sides. Observation on radial growth of *R. solani* (radius in *R. solani-Trichoderma* interactions and diameter in *R. solani-P. fluorescens*), inhibition zone and overgrowth were recorded at periodical interval for 10 days. Bell's scale was adopted to categorize *Trichoderma* isolates for their antagonistic potential[1].

RESULTS AND DISCUSSION

Antagonistic potential of *Trichoderma* isolates against R. *solani*

In mono cultured *R. solani* check plate inoculated at the periphery, *R. solani* attained a radius of 3.2 cm, 5.2 cm and 7.3 cm on day-1, day-2 and day-3 By day-4, *R. solani* completely occupied the Petriplate in monoculture (8.0 cm) (Table 2a and 2b).

All the test isolates of *Trichoderma* showed significant reduction in the radial growth of *R. solani* starting from day-1. However, isolate variation existed in *Trichoderma* antagonistic potential from day-1 to day-4.

On day-1, *R. solani* had maximum inhibition in its growth when dual cultured with RT-6 (1.77 cm radial growth equivalent to 43.80% inhibition) followed by RT-3 (1.87 cm radial growth equivalent to 40.60% inhibition) with insignificant difference between them but differed significantly with other isolates.

On day-2, ET-1 showed significantly maximum inhibition in *R. solani* growth (2.3 cm radial growth equivalent to 55.80% inhibition) followed by RT-3 (2.50 cm), RT-2 (2.53 cm), ET-4 (2.57 cm) and RT-4 (2.63 cm) with insignificant differences among them. Among the *Trichoderma* isolates, RT-6 (3.5 cm) showed least inhibitory effect on *R. solani*. It may be remembered here that RT-6 showed highest inhibition on day-1 but could not sustain the same effect on day-2.

On day-3, *Trichoderma* isolate ET-1 (2.36 cm radial growth equivalent to 68.50% inhibition) showed significantly higher inhibition in *R. solani* growth but was on par with RT-3 and RT-2 (2.56 cm). Isolate RT-6 continued to be least inhibitory resulting in 3.50 cm of *R. solani* growth.

On day-4, isolate RT-6 continued to be the significantly least effective isolate. All the other isolates were found to be on par in affecting the growth of *R. solani*.

Thus, the present study using dual culture up to four days after inoculation indicated equal efficacy of five of the six *Trichoderma* test isolates against *R. solani*. The dual cultured plates were further incubated beyond four days up to 10 days. Incubation up to 10 days revealed over growth of all the six *Trichoderma* isolates with variation in the distance covered over the growth of *R. solani*. Bell *et al*.[1], categorized *Trichoderma* isolates based on the quantum of overgrowth on *R. solani in vitro* in dual culture. Accordingly, Bell's scale was adapted to categorize the *Trichoderma* isolates for their antagonistic potential. Isolate ET-4 could occupy less than half of the *R. solani* growth while RT-6 could occupy less than three fourth of *R. solani* growth. Isolates RT-2, RT-3, RT-4 and ET-1 could overgrow more than three fourth of *R. solani* growth. However, zone of inhibition was observed in Rs-RT-2 and Rs-RT-3 interactions while no such zone was observed in Rs-RT-4 and Rs-ET-1 interactions. Patibanda and Sen[8] while working with *Aspergillus niger-Fusarium oxysporum* f. sp. *melonis* system reported that zone of inhibition may be taken as an interaction effect rather than the individual effect. In Rs-RT-4 and Rs-ET-1 interactions, absence of zone of inhibition indicated that *R. solani* offered no resistance to the invading *Trichoderma*. Hence, *Trichoderma* isolates RT-4 and ET-1 were considered to have higher antagonistic potential.

Antagonistic potential of *P. fluorescens* isolates against *R. solani*

In monocultured plate, *R. solani* attained a radial growth of 2.90 cm, 3.90 cm and 4.50 cm dia. on day-1, day-2 and day-3 respectively. When dual cultured with *P. fluorescens* significant inhibition was observed in the radial growth of *R. solani* when compared to the monocultured check plate (Table 3)

On day-1, least growth of 1.53 cm dia. was recorded when *R. solani* was dual cultured with PF-2 isolate (47.13% inhibition) which was on par in interactions with isolates PF-5, PF-6, PF-3, PF-4, and PF-1. Both the isolates from rice eco system, *i.e.* PF-7 and PF-8 had significantly lower inhibitory effect on *R. solani* in comparison with other isolates.

On day-2, similar inhibitory effect was found when *R. solani* interacted with PF-2 (1.6 cm dia.), PF-5 (1.6 cm dia.), PF-6 (1.7 cm dia.), PF-3 (1.7 cm dia.), PF-4 (1.77 cm dia.) and PF-1 (1.77 cm dia.) isolates showing significantly lower radial growth, *i. e*., higher inhibition in *R. solani* growth compared to interaction with isolates PF-7 (2.67 cm dia.) and PF-8 (2.57 cm dia.) and also with monocultured check (3.90 cm dia.). Isolates PF-7 and PF-8 continued poor inhibitory effect on *R. solani* growth compared to other isolates.

Three days of incubation resulted in continued inhibitory effect on the radial growth of *R. solani* when dual cultured with PF-5 and PF-2 (1.60 cm dia. equivalent to 64.4% inhibition), and PF-3 (1.70 cm dia. equivalent to 62% inhibition). When interacted with other isolates *R. solani* continued to grow from day-2 to day-3 indicating low level of inhibitory effect compared to PF-2, PF-5 and PF-3. In Rs-PF-7 and Rs-PF-8 interactions zone of inhibition was nullified after 3 days, and *R. solani* continued to grow covering streaked *P. fluorescens* growth.

Continued incubation beyond three days up to 10 days resulted in static growth of *R. solani* in PF-2, PF-5 and PF-3 indicating high antagonistic effect of these three *P. fluorescens* isolates. However, sclerotial bodies were formed in Rs-PF-3, while in Rs-PF-2 and Rs-PF-5 interactions, sclerotial bodies of the fungus *R. solani* were not observed. Formation of sclerotial bodies indicated resistance from *R. solani*. Hence, among these three isolates, PF-2 and PF-5 were selected as potential antagonists which could inhibit even sclerotial production by *R. solani*.

Variability in isolates of *P. fluorescens* in antagonistic potential was earlier reported by Devi *et al.*[2], and Jayaprakashvel *et al*.[3]. Reddy *et al*.[9], reported that among the 15 isolates tested, isolate Pf003 was highly antagonistic *in vitro* with 50% reduction in the growth of *R. solani*.

Table 1. Isolates of *Trichoderma* and *Pseudomonas fluorescens* used the present study

Isolate	Isolated from	Designated as
T. flavofuscum	Groundnut root endophyte	ET-1
T. virens	Groundnut root endophyte	ET-4
T. fertile	Groundnut rhizosphere	RT-2
T.hamatum	Groundnut rhizosphere	RT-3
T. polysporum	Groundnut rhizosphere	RT- 4
T. konigii	Groundnut rhizosphere	RT-6
P. fluorescens	Groundnut rhizosphere	PF-1
P. fluorescens	Groundnut rhizosphere	PF-2
P. fluorescens	Groundnut rhizosphere	PF-3
P. fluorescens	Groundnut rhizosphere	PF-4
P. fluorescens	Groundnut rhizosphere	PF-5
P. fluorescens	Groundnut rhizosphere	PF-6
P. fluorescens	Rice phyllosphere	PF-7
P. fluorescens	Rice rhizosphere	PF-8

Table 2a. Effect of *Trichoderma* isolates on the radial growth (radius) of *Rhizoctonia solani* in dual culture *In vitro*

S. No	Dual cultured with *Trichoderma* isolate	Growth of *R. solani* in cm							
		Day-1		Day-2		Day-3		Day-4	
		Growth (cm)	Inhibition (%)	Growth (cm)	Inhibition (%)	Growth (cm)	Inhibition (%)	Growth (cm)	Inhibition (%)
1	RT_2	2.43^{bc}	28.10	2.53^{c}	51.90	2.56^{cd}	65.80	2.63^{c}	67.10
2	RT_3	1.87^{e}	40.60	2.50^{c}	51.90	2.56^{cd}	65.80	2.56^{c}	67.90
3	RT_4	2.33^{cd}	28.10	2.63^{c}	50.00	2.66^{c}	64.40	2.66^{c}	66.70
4	RT_6	1.77^{e}	43.80	3.50^{b}	32.70	3.50^{b}	52.10	3.50^{b}	56.70
5	ET_1	2.03^{d}	37.50	2.30^{d}	55.80	2.36^{d}	68.50	2.50^{c}	68.80
6	ET_4	2.47^{b}	21.90	2.57^{c}	51.90	2.70^{c}	63.00	2.633^{c}	67.10
7	*R. solani* monoculture	3.20^{a}	-	5.20^{a}	-	7.30^{a}	-	8.00^{a}	-
	C.D (P=0.01)	0.10		0.16		0.21		0.19	
	SEm (±)	0.03		0.05		0.07		0.06	
	C.V (%)	2.69		3.42		4.32		4.02	

Note: The figures with similar alphabets do not differ significantly

Table 2b. Categorization of *Trichoderma* isolates based on over growth on *R. solani* in dual cultured plate *in vitro* following Bell's scale

Group	Over growth on *R. solani* in dual cultured plate	Isolate(s)
1	< 1/4 over growth of *Trichoderma*	Nil
2	1/4 to 1/2 over growth of *Trichoderma*	ET-4
3	1/2 to 3/4 over growth of *Trichoderma*	RT-6
4	>3/4 over growth of *Trichoderma*	RT-2, RT-3, ET-1, RT-4
5	over growth of *R. solani* on *Trichoderma*	Nil

Table 3. Effect of *Pseudomonas fluorescens* on the radial growth (dia.) of *Rhizoctonia solani in vitro* in dual culture

S. No.	Treatments	Period of incubation in days					
		Day 1		Day 2		Day 3	
		R solani growth (cm)	Inhibition (%)	*R solani* growth (cm)	Inhibition (%)	*R solani* growth (cm)	Inhibition (%)
1	PF-1	1.78^{c}	39.08	1.77^{c}	54.70	1.92^{c}	57.41
2	PF-2	1.53^{c}	47.13	1.60^{c}	58.97	1.60^{d}	64.44
3	PF-3	1.70^{c}	41.56	1.70^{c}	56.41	1.70^{d}	62.22
4	PF-4	1.77^{c}	39.08	1.77^{c}	54.70	1.93^{c}	57.04
5	PF-5	1.60^{c}	44.86	1.60^{c}	58.97	1.60^{d}	64.44
6	PF-6	1.60^{c}	44.86	1.70^{c}	56.41	2.67^{b}	40.74
7	PF-7	2.57^{b}	11.48	2.67^{b}	31.62	3.07^{b}	31.85
8	PF-8	2.37^{b}	18.39	2.57^{b}	34.19	2.73^{b}	39.26
9	Control	2.90^{a}	-	3.90^{a}	-	4.50^{a}	-
	C.D (P=0.01)	0.32		0.33		0.31	
	SEm (±)	0.11		0.11		0.10	
	C.V (%)	9.33		9.79		8.20	

Note: The figures with similar alphabets do not differ significantly

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