INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume 5, Issue 2)
Available online at: www.ijariit.com

Antifungal activity of Pseudomonas fluorescens against Rhizoctonia solani under in vitro condition

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ABSTRACT

Rice is a major food crop in India which is affected by both biotic and abiotic stresses. Rice is infected by many diseases that are caused by fungi, bacteria, and virus. Among them, Rhizoctonia solani is an important pathogen which causes a major yield loss in rice. The application is of chemicals hazardous to humans causing environmental pollution. Hence, in order to reduce these problems, there is a need for sustainable agriculture using natural resources to compact the diseases. In the present study, The PGPR Pseudomonas were collected from different places and were evaluated against the Rhizoctonia solani under in vitro conditions. Among the five isolates, Pf1 showed the highest reduction percentage against the pathogen.

Keywords— *Rhizoctonia solani, Pseudomonas fluorescens, Antifungal activity, Rice*

1. INTRODUCTION

Rice is grown in tropical and subtropical regions of the world. It serves as the staple food of the Indian people and is grown as a monsoon based cereal crop (Hossain and Pingali, 1998). Rice sheath blight, caused by Rhizoctonia solani Kuhn [Teleomorph: Thantephorus cucumeris (Frank) Donk], is a destructive disease worldwide that causes significant yield loss and quality degradation (Yellareddygari et al., 2014). A huge loss in yield of about 50% due to this pathogen was reported when susceptible cultivars were planted (Hossain et al., 2017; Suthin Raj et al., 2019). It is one of the major fungal diseases of rice in tropical Asia causing up to 50% loss in grain yield (Bhukal et al., 2015).

Many methods of plant disease control are presently being used to control rice sheath blight such as physical, chemical and cultural methods. Although, chemicals control it, their continuous , inappropriate and non-discriminative use cause undesirable effects such as residual toxicity, development of resistance, environmental pollution, health hazards to humans and animals and increase the cost of expenditure for plant protection (Suthin Raj and John Christopher, 2008; Suthin Raj and John Christopher, 2009). The organic control of soil borne plant pathogens is a potential alternative to the use of chemical pesticides.

Antagonistic bacteria especially the PGPR Fluorescent pseudomonads are the most exploited ones for the biological control of soil-borne and foliar plant pathogens. In the past three decades, numerous strains of Fluorescent pseudomonads have been isolated from the rhizosphere soil and plant roots by several workers and their biocontrol activity against soil-borne and foliar pathogens were reported (Vivekananthan, 2004; Suthin Raj et al., 2014). In this context, evaluation of Pseudomonas fluorescence against R. solani under in vitro conditions was carried out in the present study.

2. MATERIALS AND METHODS

2.1 Isolation, maintenance and identification of the pathogen

The diseased samples were washed thoroughly with tap water. A small portion of infected tissue along with adjacent small unaffected tissues was cut into 0.5 cm pieces with the help of a sterilized scalpel blade and forceps and was transferred to sterile Petri dishes. These pieces were then surface sterilized with 1% sodium hypochlorite solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to Petri dishes

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containing sterilized Potato Dextrose Agar (PDA) medium at the rate of 3-5 pieces of tissue per petri dish supplemented with streptomycin sulfate and incubated at 28±2°C in a BOD incubator. The Petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

The axenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments.

2.2 Isolation of bacterial antagonist
Rhizosphere colonizing fluorescent pseudomonads were isolated from fresh roots of rice from 5 different places. The soil suspension was prepared from each rhizosphere sample by shaking one g of soil sample in 10 ml of sterile dist. water and by making serial dilutions. One ml of soil suspension from an aliquot dilution (10-5) was aseptically added to sterile Petri dishes containing 20 ml of sterile King's B medium and incubated at 28±2°C for 48 h and after incubation, well separated individual colonies with yellow-green pigments were marked and detected by viewing under a UV light. The individual colonies were picked up with a sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in a refrigerator at 4°C for further use.

2.3 Dual culture
P. fluorescens was grown on King's B agar medium. Than 8 mm actively growing PDA culture disc of the pathogen was placed on PDA medium in a sterilized petri dish at one side, 1.5 cm away from the edge of the plate, and incubated at room temperature (28 ± 2°C). After forty-eight hrs, actively growing 48-h-old cultures of the respective test bacteria were separately streaked on to medium at the opposite side of the plate, 1.5 cm away from the edge of the plate. The inoculated plates were then incubated at room temperature (28 ± 2°C). Three replications were maintained for identifying the antagonist. Potato dextrose agar medium inoculated with the pathogen alone served as the control. After 8 days, the radial growth of the pathogen was measured. The results were expressed as per cent growth inhibition over control. The most effective P. fluorescens isolates were used for further study.

2.4 Mycelia dry weight
PDA was prepared in 250 ml Erlenmeyer flasks and autoclaved. Culture filtrates of P. fluorescens @ 10 ml were added to 40 ml broth in a flask so as to get a final concentration of 20 per cent of the filtrate in broth. The flask was inoculated with 8mm culture disc of R. solani and incubated at 28± 1^0C for 10 days. Broth without any filtrate served as the control. Three replications were maintained and after the incubation period, the mycelial mat was harvested on a previously weighed filter paper and dried at 105^0C for 12 h in a hot air oven, cooled in desiccators and the mycelial weight was recorded and expressed an mg/50 ml/broth.

2.5 Poisoned food technique
The efficacy of P. fluorescens strains was assayed at concentrations of 10, 20, and 30 per cent. The required quantity of culture filtrates was added separately into molten and cool PDA medium so as to get the desired concentration of culture filtrates in the medium and 15 ml of poisoned medium was poured into sterile Petri plates. Each plate was inoculated at the centre with a 9mm culture disc (10 days old) of R. solani grown on PDA and incubated at room temperature for seven days. Each treatment was replicated thrice and a suitable control was maintained without adding any culture filtrate to the medium. The plates were incubated at room temperature and the mycelial growth was measured at the end of the incubation period. The efficacy of the culture filtrate was expressed as per cent inhibition of mycelial growth over the control (Vincent, 1927).

$$\text{Per cent inhibition (I)} = C - T \times 100/ C$$

Where I is the inhibition per cent, C is the radial growth in control and T is the radial growth in treatment.

3. RESULTS

3.1 Effect of Pseudomonas fluorescens against R.Solani on under in vitro
The results of the screening of five isolates of P. fluorescens against R. solani on PDA plates are presented in table 1. Among the P. fluorescens isolates, Pf1 was appeared to be the most effective against the test pathogen showing 66.67 per cent inhibition of colony growth and it was followed by the isolate Pf3 which shoved 65.33 per cent inhibition of colony growth. All the isolates significantly reduced the Mycelial growth of the pathogen over the control.

3.2 Mycelial growth
The mycelial growth of the pathogen was tested against Pseudomonas at 10, 20, 30 and 40 per cent concentrations. Among them, the Pf_1 isolates were significantly able to reduce the growth of mycelium which recorded 206, 160, 82 and 27 mg/50ml broth respectively. It was followed by Pf_3 224, 194, 99 and 34 mg/50ml broth. All the isolates significantly reduced the mycelia growth of the pathogen over the control (table 2). Hence, the superior isolate Pseudomonas was used for further studies.

3.3 Poison food technique
The mycelial growth of R. solani was found to reduce with an increase in the concentration of culture filtrates of all the isolate of the antagonists tested and the reduction was significantly maximum in the case of P. fluorescens isolate Pf1 with 20.58, 11.33and 2.81 mm at 10, 20 and 30 per cent concentration of the culture filtrate respectively as against the maximum growth of 90 mm in the control in the poison food technique. This was followed by the isolate Pf3 with 21.08, 13.61 and 3.34 mm at 10, 20 and 30 per cent concentrations of the culture filtrate respectively (table 2).

Table 1: Evaluation of various isolates of P. fluorescens against R. solani by dual culture technique and mycelial dry weight

S.no	Isolates	Linear growth (mm)		% Growth inhibition	Mycelial dry weight (mg/50m/broth)				
		Antagonist	*R. solani*		10%	20%	30%	40%	Mean
1	Pf_1(TNAU- Coimbatore)	60.00	30.00	66.67^a	206.00	160.00	82.00	27.00	118.75^a
2	Pf_5 (Cuddalore)	49.70	40.30	55.22^e	322.00	244.00	154.00	54.00	193.50^e
3	Pf_2 (Aduthurai)	58.80	31.20	65.33^b	224.00	194.00	99.00	34.00	137.75^b
4	Pf_3(Annamalainagar)	56.60	33.40	62.89^c	265.00	217.00	115.00	47.00	161.00^c
5	Pf_4 (Virudhasalam)	53.50	37.50	58.33^d	292.00	221.00	134.00	50.00	174.25^d
6	Control	90.00		0.00	478.00	478.00	478.00	478.00	478.00

Table 2: Evaluation of various isolates of *P. fluorescens* against *R. solani* by Poison food technique

S no.	Isolate number	Poison food Technique					
		Mycelial growth (mm)					
		10%	Per cent inhibition over control	20%	Per cent inhibition over control	30%	Per cent inhibition over control
1	P. fluorescens (Pf_1)	20.58^a(26.97)	77.13	11.33^a (19.67)	87.41	2.81^a (8.73)	96.88
2	P. fluorescens(Pf_2)	21.86^e (27.87)	75.71	16.02^e (23.59)	82.20	6.72^e (14.84)	92.53
3	P. fluorescens (Pf_3)	21.08^b (28.02)	75.47	13.61^b (21.65)	84.88	3.34^b (9.80)	96.28
4	P. fluorescens (Pf_4)	23.60^c (29.06)	73.78	14.17^c (22.11)	84.26	4.64^c (12.00)	94.84
5	P. fluorescens (Pf_5)	24.33^d (28.86)	72.97	16.86^d (24.24)	81.27	5.48^d (13.22)	93.91
6	Control	90.00^i (71.57)	-	90.00^i (71.57)	-	90.00^i(71.57)	-

4. DISCUSSION

In the present study, all the ten isolates of P. fluorescens showed a varying degree of antagonism against R. solani. Among the isolates, Pf1 was the most antagonistic and formed the maximum inhibition zone against the growth of R. solani. Similarly several isolates of P. fluorescens were reported to effectively inhibit the growth of R. solani with varying degree of antagonism and formed higher inhibition zones (Jayalakshmi et al., 2003; Kazempour, 2004; Krishna Kumari, 2016; Singh et al., 2016; Suthin Raj et al., 2016; Suman et al., 2017).

In poison food technique, the growth of R. solani was found to be reduced with an increase in the concentration of culture filtrates of all the isolates tested and the reduction was significantly the maximum in the case of P. fluorescens isolate Pf1 at a 30 per cent conc. The volatile compounds of the bacterial antagonists have a remarkable inhibitory effect on mycelial growth of the pathogen. Extra cellular enzymes and antibiotics produced by P. fluorescens inhibited the mycelial growth of R. solani (Kazempour, 2004. Suthin Raj et al., 2013). There are various modes of actions such as antibiosis, competition for iron through the production of siderophores, parasitism that may involve production of extracellular enzymes and induction of plant resistance mechanisms (Naureen et al., 2015; Suthin Raj et al., 2012). Such versatile mechanisms exerted by P. fluorescens might be attributed as the reason for the in vitro suppression of R. solani. Reports by these earlier workers lend support to the present investigations.

5. CONCLUSION

In the present study, all the ten isolates of P. fluorescens showed varying degree of antagonism against R. solani. Among the isolates, Pf_1 was the most antagonistic and formed the maximum inhibition zone against the growth of R. solani.

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