Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci.[Spl. Issue 2] 2016: 88-91

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
http://doi-ds.org/doilink/05.2016-13212626/

OPEN ACCESS

Mycoparasitic effect of Pseudomonas fluorescens on *Bipolaris oryzae* the incitant of brown spot of rice (*Oryza sativa*)

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ABSTRACT
Rice (*Oryza sativa* L.) is one of the major cereal crops of the world used as the staple food by 60% of the world's population. Among the diseases affecting rice, brown spot of rice caused by *B. oryzae* is most disastrous and reported in all rice growing area of the world. Among the four different bacterial antagonists tested, *P. fluorescens* exhibited strong inhibition on the mycelia growth and conidial germination of *B. oryzae.* Thus, the mycoparasitic effect of *P. fluorescens* can be well exploited for the control of *B. oryzae* in rice.
Key words: Rice, brown spot, *B.oryzae*, mycelial growth, spore germination, antagonist

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major cereal crops of the world used as the staple food by 60% of the world's population [5]. Diseases are the significant limiting factors that affect rice production causing annual yield losses, conservatively estimated at 5% [6]. Brown spot of rice caused by *Bipolaris oryzae* (Breda de Haan) Subram. and Jain (*Helminthosporium oryzae)* telemorph=*Cochliobolus miyabeanus*) occurs in almost all the rice growing areas. Several chemicals are suggested to control rice diseases. However, it is imperative that, with the growing concerns on environmental pollutions due to chemical pesticides, it was thought to use *P. fluorescens* which performs extremely well under paddy environment. Biological control of plant pathogens by antagonists is an important part of plant pathological research all over the world these days. A great success has been achieved in this direction as evidenced by several workers as the studies are usually cheap, self maintaining, eco-friendly non hazardous and fits well in the frame work of integrated disease management.

MATERIALS AND METHODS

Isolation of *B. oryzae* culture

A virulent culture of *B. oryzae (*Breda de Haan) was isolated from brown spot infected leaves of rice variety BPT 5204 of Puducherry and Karaikal districts of Puducherry (U.T.) by the tissue culture technique. The affected leaf portions were cut in to small bits, surface sterilized with 0.1 per cent mercuric chloride solution for 1 min, washed thoroughly with sterile distilled water (3-4 times), placed on special medium, viz., Rice polish agar medium (Previously added to the sterile Petri plates along with streptomycin sulphate at 50 ppm concentration) and incubated at room temperature (28±2°C) for 48-72 h. The fungus was subsequently purified by single spore isolation.

Antagonist used

Virulent bacterial antagonists *viz., Pseudomonas fluorescens, Serratia marcescens, Bacillus cereus, B. subtilis* obtained from the culture collections of Department of Plant Pathology were used in the present study.

Preparation of the culture filtrate *P. fluorescens*

The effective *P. fluorescens* isolate was inoculated into Erlenmeyer flasks containing 50 ml of sterile King's B broth and kept on a rotary shaker at 100 rpm for 48 h. The cultures were then filtered through bacteriological filter under vacuum and the filtrate thus obtained was used for the studies.

Effect of antagonists on mycelial growth (Dual culture)

The antagonistic activity of bacterial bio control agents against *B .oryzae* was tested by dual culture technique [2]. A 9 mm actively growing PDA culture disc of *B. oryzae* was placed at one end, 1.5 cm away from the edge. Just opposite to the pathogen one cm long streak of bacterial bio control agents was gently made in the medium using two days old culture at equidistance. A control was maintained by inoculating *B. oryzae* alone at one end of the Petri dish. The plates were incubated at room temperature (28±2°C) for seven days. Three replications were maintained for each antagonist. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula proposed by Vincent, [7].

Per cent inhibition (I) = C-T/C x 100

Where, C- mycelial growth of pathogen in control,

T- Mycelial growth of pathogen in dual plate.

Among the antagonists *P. fluorescens* recorded the maximum inhibition of the mycelial growth of *B.oryzae* and hence the same isolate alone was tested further using poisoned food technique (liquid medium assay).

Table 1 Efficacy of PGPR's against *Bipolaris oryzae*

Tr. No.	Antagonists	Growth pattern		
		Colony diameter of pathogen (mm)	Per cent inhibition (%)	Inhibition zone (mm)
1.	*Pseudomonas fluorescens*	22.3	75.22	10.22
2.	*Serratia marcescens*	24.5	72.78	9.37
3.	*Bacillus cereus*	30.7	65.89	7.44
4.	*Bacillus subtilis*	26.5	70.56	8.61
5.	Control	90.0	--	--
	SE CD (p=0.05)	0.12 0.39	--	--

***In vitro* evaluation of culture filtrates of *P. fluorescens* on the mycelia of *B. oryzae* (Liquid medium assay)**

50 ml of PDA broth taken in 250 ml Erlenmeyer flasks were sterilized and amended with culture filtrates of *(P. fluorescens*) at different concentrations like 5, 10, 15, 20 and 40 per cent and inoculated with mycelial disc (9mm) of *B. oryzae* collected from the periphery of seven days old culture. The flask amended with Mancozeb (0.1%) was used for comparison and a suitable control was also maintained. The flasks were incubated for 10 days at room at 28 ± 2^0C and thereafter, filtered through filter paper Whatman no. 42 in vacuum. The dry weight of mycelial biomass was recorded in mg.

Poisoned food technique (Liquid medium assay)

Different conc. of the antagonist *P. fluorescens viz.,* 5, 10, 15, 20 and 40 per cent was added to the sterile Petri plate by using a sterile pipette followed by adding PDA. Each treatment was replicated thrice and a suitable control was maintained. The Petri plates containing antagonist impregnated medium were inoculated with 9 mm mycelial disc of *B. oryzae* collected from the periphery of seven days old culture. The plates were incubated for ten days at room temperature 28 ± 2°C .The mycelial growth and the per cent inhibition was recorded.

Table 2 Effect of culture filtrate of *P. fluorescens* on the mycelial growth and mycelial dry weight of *B.oryzae*

Tr. No.	Conc. of the culture filtrate (%)	Solid medium assay		Liquid medium assay	
		Mycelial growth (mm)	Per cent Inhibition (%)	Mycelial dry weight (mg)	Per cent Inhibition (%)
1	5	36.63	59.3	159.27	46.95
2	10	29.31	67.43	99.00	67.02
3	15	19.43	78.41	45.74	84.75
4	20	9.8	89.11	20.19	93.28
6	40	NG	-	0.84	99.72

7	Mancozeb 75 % WP (0.1%)	NG	-	1.00	99.67
8	Untreated Control	90.0	-	300.25	-
	SE CD (p=0.05)	0.92 2.12	--	0.25 0.68	--

Plant growth promotion-Roll Towel Method [3]

The germination paper was soaked in water for 2 to 4 h to moist it evenly and to remove water soluble toxic substances present in it. The seeds treated with different levels of liquid formulation of *P. fluorescens* and were placed equidistantly between the two sheets of paper towel, rolled carefully ensuring no pressure on seeds, wrapped with a polythene sheet to reduce surface evaporation and kept in germination chambers in an upright position. Each treatment was replicated thrice. They were incubated at room temperature (28 ± 2^{o}C) for seven days. Ten normal seedlings were selected at random from each replication and the shoot and root length from the collar at the tip of the primary root was measured and the respective mean values were recorded. The vigour index (VI) was calculated by using the formula suggested by Abdul Baki and Anderson [1].

VI= (Root length + Shoot length) × (Germination percentage)

Table 3 Efficacy of liquid formulation of *P. fluorescens* on plant growth promotion under *in vitro* (Roll towel method)

S. No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	ST @ 2.5 ml / Kg of seeds	89.77(71.34)	5.25	7.99	1188.55
2	ST @ 5.0 ml / Kg of seeds	90.55(72.09)	6.79	8.28	1364.58
3	ST @ 7.5 ml / Kg of seeds	93.15(74.92)	7.12	8.78	1481.09
4	ST @ 10.0 ml / Kg of seeds	94.76(76.77)	7. 63	11.15	1779.59
5	ST with Mancozeb 75 % WP @ 2.0 g / Kg of seeds	93.72(75.48)	7.35	9.81	1608.23
6	Control	57.63(49.39)	4.75	5.25	576.30
	SE CD (p=0.05)	0.12 0.58	0.01 0.05	0.02 0.06	--

Spore germination assay (Macko *et al*.., 1977)

Different concentrations of the antagonist *P. fluorescens* @ 0.5 ml. and the spore suspension of test fungus (0.1 ml of 1x10^{6}/ml) were mixed in cavity slide. Cavity slides with sterile distilled water having only the spore suspension were kept as control. The slides were incubated for 24, 36 and 48 h. in Petri plate glass bridge moist chamber at (28 ± 2^{0}C). Spore germination was examined at 24, 36 and 48 h. of incubation. Observations of the conidial germination from each slide were observed at ten different microscopic fields and the germination percentage was calculated and recorded.

Table 4 Effect of culture filtrate of *P. fluorescens* on the conidial germination of *B. oryzae* (Cavity slide method)

Tr. No.	Culture filtrate conc. (%)	Conidial germination (%)		
		24 h.	36 h.	48 h.
1	5	60.18 (50.87)	69.47 (56.45)	87.63 (69.40)
2	10	40.23 (39.36)	46.55 (43.02)	51.12 (45.64)
3	15	32.45 (34.72)	36.78 (37.33)	37.88 (38.98)
4	20	6.41 (7.66)	7.69 (9.09)	8.25 (9.69)
5	40	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
6	Mancozeb 75 % WP (0.1 %)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
7	Control	70.27 (81.23)	76.36 (83.58)	89.93 (95.61)
	SE CD (p=0.05)	1.03 2.09	0.73 1.51	0.62 1.32

Data in parenthesis indicate angular transformed values.

RESULTS AND DISCUSSION

Among the antagonists *P. fluorescens* was found to be more antagonistic to *B. oryzae* as it recorded the maximum percent inhibition (75.22%) which was followed by *S. marcescens* (72.78%) and *B. subtilis* (70.56%) in the decreasing order of merit. The minimum growth inhibition was recorded by *B. cereus*

(65.89%). From the results recorded on the effect of culture filtrate of *P. fluorescens* on the conidial germination of *B. oryzae,* it is observed that, among the various conc. of culture filtrate of *P. fluorescens* tested, the conidial germination of *B. oryzae* was completely inhibited by 40 per cent conc. of the culture filtrate of *P.f*. The *P. fluorescens* @ 20% conc. of the culture filtrate ranked next, and as a result, a significant reduction in the conidial germination percentage was observed (6.41 %, 7.69% and 8.25 % at 24 h, 36 h and 48 h. respectively). The 5% conc. of the culture filtrates was found to be the least effective. The results of the *in vitro* studies conducted to find out the effect of culture filtrate of *P. fluorescens* on the mycelial growth and mycelial dry weight of *B. oryzae* revealed, an increasing trend in the percent inhibition with an increase in the conc. of culture filtrates of *P. fluorescens*. In solid media, the culture filtrate of *P. fluorescens* at 40 per cent completely inhibited the mycelial growth of *B. oryzae.* In liquid medium assay, the flasks inoculated with pathogen and amended with culture filtrate of *P. fluorescens* recorded significant reduction in the mycelial dry weight whereas, the flasks inoculated with *B. oryzae* alone (control) recorded the maximum mycelial dry weight (300.25 mg). The minimum mycelial dry weight (0.84 mg) of *B. oryzae* was recorded in 40 per cent conc. of the culture filtrate of *P. fluorescens.* Among all concentrations used, *P. fluorescens* @ 5% conc. was found to be the least effective (59.3 % inhibition) Seed treatment with *P. fluorescens* @ 10.0 ml/Kg of seeds recorded the maximum seed germination, shoot length, root length and vigour index of rice. In general, the treatment with *P. fluorescens* showed significant increase in seed germination and plant growth parameters of rice when compared to control.

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