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In-vitro Efficacy of PGPR on the Management of *Rhizoctonia solani* (KUHN) Causing Sheath Blight of Rice

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A B S T R A C T

Besides, the inherent hazards, negative public perceptions about the adverse environmental pollution of chemical pesticides, an alternative control methods are needed. Bio-stimulants are eco-friendly alternative to chemical pesticides because of their minimal impact on human health and environment, while ensuring optimal increase of crop yield, quality and tolerance to biotic stress. The soil has an enormous untapped potential of microbes, showing antagonistic effects against soil borne plant pathogenic organisms. This work validates a procedure to select one of the best Plant Growth Promoting Rhizobacteria (PGPR) as potential active ingredients for controlling Sheath blight of rice, incited by *Rhioctonia solani* (Kuhn). It is one of the most important Necrotrophic fungal diseases of rice with approximately 58% yield reduction in test plots of susceptible cultivars**.** It was arduous to manage the disease, therefore by investigating the bio-control potential of *Pseudomonas fluorescens* for the successful management of *R. solani* causing sheath blight disease. The results showed that the bacterial antagonist *P. fluorescens* has exhibited strong inhibition against *R. solani* with 76.40 Per cent over control in dual culture technique. With regard to poisoned food technique the culture filtrates of *P. fluorescens* (Pf_3) at 15 and 20 per cent conc. completely inhibited the mycelial growth of *R. solani*. In addition to the disease control, the isolate Pf_3 recorded the maximum root and shoot length; with germination percentage (96.18) and vigour index (2429.50) thereby it promotes plant growth and seed germination.

Introduction

Rice is a predominant crop, belonging to the Family Gramineae and the Genus Oryza. *Oryza sativa* and *Oryza glaberrima* are the two major cultivated species (Pareja *et al.*, 2011). Rice cultivation started at the 15th century in South East Asia and spread to India, china and Japan. Among the biotic stress factors affecting rice crop, the loss inflicted by pathogen, insect pests and nematodes are considerably more significant. The most important rice diseases like blast, sheath blight, stem rot, grain discoloration and

bacterial blight causing more damage to the crop (Sharma *et al.,* 2018). Of that, Rice sheath blight caused by *Rhizoctonia solani* Kuhn. [Sexual stage of *R. solani* was *Thanatephorus cucumeris* (A.B. Frank) Donk)], it is a universal soil saprotrophic and facultative plant parasite (Anees *et al.,* 2010) and also found to be known as soil-borne hemibiotrophic pathogen, with approximately 58% yield reduction in test plots of susceptible cultivars (Kouzai *et al.,* 2018). *R. solani* have 14 anastomosis group placed in AG-1 IA (Gonzalez-Vera et. al. 2010) The disease occurs near the water level after the infection of sheath turn in to softness leads the infection spreads to healthy plant parts resulting development of water soaked lesions and dormant sclerotia or mycelium presented in surface of the water and soil (Tsiboe *et al.,* 2017). The pathogen survive as sclerotia under unfavourable condition and it may spherical or irregular shaped and measure 4-5 mm in diameter, dark brown to black in colour, basidia and basidiospores are produced and viability for upto 3 years by its saprophytic nature (Kumar *et al.,* 2009). Sclerotia may move from one field to another through irrigation water and during movement they may produce mycelia and secondary or tertiary sclerotia (IRRI 1973). Depending upon the age of the plant, time of infection and severity, it causes yield loss to the extent of 5.9 to 69 per cent. (Roy, 1993) The maximum disease development was recorded at a temperature level of 25-30ºC and 80-100 per cent RH (Bhunkal *et al.,* 2015) also by applying high amount of nitrogenous fertilizer (Akash Datta *et al.,* 2017).

Synthetic fungicides are currently used as primary means for the control of plant disease. Besides, the inherent hazards alternative control methods are needed because of the negative public perceptions about the development of resistance by the pathogens, environmental pollution and high development cost of new chemicals (Lamichhane *et al.,* 2017). The microbial antagonist like Plant Growth Promoting Rhizobacteria (PGPR) offers a promising ability in controlling the disease (Nie *et al.,* 2015). Meanwhile, it enhance induced systemic resistant (ISR) fortifying the physical and mechanical strength of cell wall and changing physical and chemical reaction of host leading to synthesis of defense chemicals against pathogen (Jayalakshmi *et al.,* 2009). Among the PGPR's, the bio-control potential of *Pseudomonas fluorescens* was undertaken to investigate in the present study for the successful management for combating the sheath blight incidence.

Materials and Methods

Isolation, maintenance and identification of the test pathogen

Diseased rice plants with characteristic symptoms of sheath blight disease were collected from ten traditional rice growing areas of Mayiladuthurai district, Tamil Nadu. The infected portion of the sheath was cut into small bit, surface sterilized with 0.1% sodium hypochlorite solution for 1min and washed thrice with sterile distilled water. Further, a piece of specimen was transferred to sterile Petri dishes containing PDA medium. The plates were incubated at room temperature (28±2°C) for 5 days and the isolates were purified by single hyphal tip method. Characterization and identification of viruent isolate of *R. solani* was confirmed and maintained on PDA slant for further studies.

Isolation and characterization of bacterial antagonist (*P. fluorescencs*)

Rhizoplane-colonizing *P. fluorescens* was isolated from fresh roots of paddy grown in different locality of Mayiladuthurai district and were designated as Pf_1 – Pf_8. The soil

particles loosely adhering to the roots were teased out and used for the isolation of *P. fluorescens*. A soil suspension was prepared from each rhizosphere sample by shaking 1g of soil sample in 10 ml of sterile dist. water and serial dilutions were made. 1ml of soil suspension from aliquot dilutions (10^{-5} to 10^{-8}) was aseptically added to sterile Petri dishes containing 20ml of sterile King's B medium and incubated at 28 ± 2°C for 48 h after incubation, well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light. Also, the colony and type of colony, shape of cell were observed. The individual colonies were picked up with sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in refrigerator at 4°C for further use.

Efficacy of bacterial antagonists against *R. solani* (*in vitro*)

Dual culture technique

20 ml of PDA medium was poured into sterile petridishes. After solidification, 10 days old, 9mm disc of *R. solani* culture was inoculated 1cm away from the edge of the petridish. At opposite end of the sterile plate dish 2 days old culture of *P. fluorescens* one cm long streak was gently made onto the medium and incubated at room temperature (28±2°C) until the control plates covered by the pathogen. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelia growth of the pathogen and percent reduction over control was calculated by using the formula (Vincett, 1927)

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

∴ C- Mycelial growth of pathogen in control; T- Mycelial growth of pathogen in dual plate.

Bioassay of culture filtrates on the Mycelial dry weight of *R. solani* (Liquid medium assay)

The culture filtrates of the *P. fluorescens* were separately incorporated into sterilized 50 ml PDA broth at 10, 20, 30 and 40 per cent by adding the calculated quantity of the culture filtrates to the broth. The PDA broth without the culture filtrate served as control. The amended media were dispensed in 250 ml Erlenmeyer flasks, autoclaved at 1.4 kg / cm^2 for 20 min and cooled. The each flask was inoculated separately with a 10 day old 9 mm culture disc of *R. solani* and incubated at room temperature (28±2°C) for fifteen days. Three replications were maintained for each medium. After incubation the mycelial mat was filtered through a pre weighed Whatman No. 1 filter paper and then dried in hot air oven at 60°C till a constant weight was obtained. The mycelial dry weight was calculated by subtracting from the weight of the filter paper and recorded. The results were expressed as per cent growth inhibition over control.

Preparation of the cultural filtrate of *P. fluorescens*

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

Efficacy of culture filtrates on the mycelial growth of *R. solani*

The culture filtrate of the antagonists was separately incorporated into sterilized PDA media at 10, 20 and 30 per cent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The

PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @ 15ml and allowed to solidify. Each plate was inoculated at the centre with five days old (9mm) PDA culture disc of *R. solani*. Three replications were maintained for each treatment. The diameter of the mycelia growth (in mm) of *R. solani* was measured when the mycelia growth fully covered the control plates.

Plant growth promotion- Roll towel method

Plant growth promoting activity of the antagonists was based on the seedling vigour index by the standard roll towel method. The germination paper was soaked in water for 2-4 hrs. The 25 seeds treated with the selected antagonists were kept over the pre-soaked germination paper. The seeds were held in position by placing another pre-soaked germination paper strip over it and gently pressed. The sheets along with seeds were then rolled carefully ensuring no pressure on the seed, wrapped with polythene sheet to reduce surface evaporation and kept in germination chamber in an upright position and incubated at room temperature at 28±2ºC for ten days. Three replications were maintained for each treatment. The root length and shoot length of individual seedlings were measured and the per cent germination of seeds was also calculated. The seedling vigour index was calculated by (Abdul Baki and Anderson 1973).

The germination percentage was calculated by using the formula,

$$\text{Germination}(\%) = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The vigour index was calculated by using the formula

Vigour index = (Shoot length + Root length) × Germination (%)

Results and Discussion

Efficacy of bacterial antagonists against *R. solani* (*in vitro*)

Dual culture technique and Liquid medium assay

Eight isolates of *P. fluorescence* were isolated from different parts of Tamil Nadu and tested for their efficacy against *R. solani*. All the isolates significantly reduced the mycelial growth of the pathogen over control under dual culture technique. The results presented in Table 1. revealed varying degree of antagonism by the isolates of *P. fluoresecens* against *R. solani* (RS_7) among the isolates, Pf_3 produced significantly the maximum inhibition zone (13.43 mm) and minimum mycelial growth (21.24 mm) accounting for 76.40 per cent reduction on the mycelial growth of *R. solani* (RS_7) over control.

This was followed by isolate Pf_1 which recorded 73.64 per cent reduction on the mycelial growth over control. The isolate Pf_7 was the least effective as it produced only the inhibition zone of 5.18 mm and 62.22 per cent reduction on the mycelial growth. Suman *et al.*, (2017) reported that *P. fluorescence* in dual culture technique effectively inhibited the mycelial growth of *R. solani*.

The mycelial dry weight of the pathogen was also recorded on 10, 20, 30 and 40 per cent concentration of culture filtrate of the antagonists.

Table.1 Antagonistic activity of native isolates of *P. fluorescens* against *R. solani* (RS_7) (Dual culture technique)

Isolates	*R. solani* (RS_7)			Mycelial dry weight (mg/50 ml broth)				
	Mycelial growth (mm)	Per cent inhibition over control	Inhibition zone	10%	20%	30%	40%	Mean
Pf_1	23.72[b]	73.64	11.76	233	193	105	35	141.5[b]
Pf_2	29.17[d]	67.58	7.93	296	253	135	46	182.5[d]
Pf_3	21.24[a]	76.40	13.43	217	165	85	27	123.5[a]
Pf_4	26.43[c]	70.63	9.82	249	225	115	38	156.75[c]
Pf_5	28.78[d]	68.02	7.35	269	239	125	40	168.25[d]
Pf_6	33.77[e]	62.47	5.73	325	258	150	49	195.5[e]
Pf_7	33.64[e]	62.62	5.18	315	266	139	53	193.25[e]
Pf_8	35.19[f]	60.90	3.71	346	263	130	54	198.25[f]
Control	90.00[f]	-	-	390	390	390	390	390

*Values in the column followed by common letters do not differ significantly by DMRT (P= 0.05)

Table.2 Effect of culture filtrate of native isolates of *P. fluorescens* on the mycelial growth of *R. solani* (RS_7) (Poisoned food technique)

Isolates No.	Mycelial growth (mm)							
	5% Concentration	Per cent inhibition over control	10% concentration	Per cent inhibition over control	15% Concentration	Percent inhibition over control	20% Concentration	Per cent inhibition over control
Pf_1	25.33[b]	71.85	17.66[b]	80.37	7.65[b]	91.50	1.60[b]	98.22
Pf_2	37.65[d]	58.26	22.05[d]	75.50	11.65[d]	87.05	11.65[d]	87.05
Pf_3	20.00[a]	77.77	11.32[a]	87.42	0.00[a]	100.00	0.00[a]	100.00
Pf_4	30.00[c]	66.66	20.66[c]	77.04	8.60[c]	90.44	1.82[c]	97.97
Pf_5	37.56[d]	58.16	24.60[d]	72.66	19.20[d]	78.66	19.20[d]	78.66
Pf_6	39.00[e]	56.66	28.00[e]	68.88	19.33[e]	78.52	19.33[e]	78.52
Pf_7	40.66[f]	54.82	30.00[f]	66.66	20.10[f]	77.66	20.10[f]	77.66
Pf_8	43.20[f]	52.00	31.32[f]	65.20	23.32[f]	74.08	23.32[f]	74.08
Control	90.00[g]	-	90.00[f]	-	90.00[g]	-	90.00[g]	-

* Mean of three replication

* Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table.3 Efficacy of *P. fluorescens* on the plant growth promotion under in vitro conditions

Isolates	Mean root length (cm)	Mean shoot length (cm)	Germination percentage (%)	Vigour index
Pf_1	15.97	8.10	95.75	2304.70[b]
Pf_2	13.98	7.42	93.40	1998.76[d]
Pf_3	17.10	8.16	96.18	2429.50[a]
Pf_4	15.72	7.90	95.15	2247.44[b]
Pf_5	14.20	7.78	94.53	2077.76[c]
Pf_6	12.36	5.86	90.28	1644.90[f]
Pf_7	13.58	6.35	92.35	1840.53[e]
Pf_8	11.31	5.28	90.05	1493.92[g]
Carbendazim 50% WP	16.56	8.65	96.11	2422.93[a]
Control	10.55	4.33	78.15	1162.87[h]

* Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

All the treatments were found to be effective in reducing the growth of pathogen. However, among the different treatments *P. fluorescens* isolates (Pf_3) showed minimum mycelial growth recording 217, 165, 85and 27 mg/50 ml broth at 10, 20, 30 and 40 per cent conc. respectively, followed by Pf_1 and Pf_4 recording a vegetative growth of 233,193,105,35 and 249,225,115 and 38 mg/50ml/broth respectively (Table 1). The results of the experiment showed the superiority of Pf_3 isolate. The secondary metabolites like 2,4, diacetylphloroglucinol, oligomycin, oomycinA, phenazzine, pyoluterin, pyrolnitrin, pyocyanin, iturin, hydrogen cyanide, antibiotics and lytic enzymes, β 1,3-glucanase and chitinase were reduced by *P. fluorescence* (Kumar *et al.,* 2013; Mishra and Arora 2017; Purkayastha *et al.,* 2018).

Efficacy of culture filtrates on the mycelial growth of *R. solani* (RS_7)

Studies on the effect of different conc. of culture filtrate of *P. fluorescens* on the mycelial growth of *R. solani* (RS_7) under *in vitro* condition. The results revealed that the culture filtrate of isolate Pf_3 totally inhibited the mycelial growth of *R. solani* (RS_7) at 15 % and 20% conc. under *in vitro* condition followed by the isolate Pf_1 which recorded 98.22 per cent inhibition of mycelial growth over control at 20 %. However, incorporation of 5% conc. of culture filtrate was not effective against *R. solani* (RS_7) due to irrespective of various isolates tested in the present investigation (Table 2). Similar findings was reported by (Tewari and arora 2016; Neha 2016; Meena *et al.,* 2018).

Bioassay of *P. fluorescens* on the seed germination and plant growth promotion under *in vitro* conditions

The seed treatment with *P. fluorescence* was induced plant growth promotion in paddy under *in vitro* condition. However, the data depicted in Table 3 revealed that all the eight isolates of *P. fluorescens* increase the vigour index of rice seedlings. Among them, the isolate Pf_3 recorded the maximum root (17.10) and shoot (8.16) length, germination percentage (96.18) and vigour index (2429.50). This was followed by the isolate Pf_1 recorded 15.97cm, 8.10cm, 95.75% root and shoot length, and germination percentage respectively. Pf_8 isolate recorded the least vigour.

Kloepper and scroth 1981], reported that specific rhizosphere bacteria applied to seeds could colonize roots and promote rhizobacteria PGPR. Several workers have reported about the growth promoting effect of *Pseudomonas* in various crops (Chatterjee *et al.,* 2012; Singh *et al.,* 2015; Prasad *et al.,* 2016). The result of present study is in line with these earlier reports

The use of *P. fluorescens* for the management of *R. solani* without chemical pesticides will be of interest to the organic fertilizer industry. In addition to disease control, *P. fluorescens* provided plant growth promotion along with notable benefits. Hence *P. fluorescens* were found to superior in inhibiting the growth of *R. solani*

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