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Efficacy of *Pseudomonas fluorescens* in the management of Rice Sheath blight incited by *Rhizoctonia Solani* (Kuhn)

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Abstract
Rice is a monocotyledonous annual grass which belongs to the family Gramineae and the genus *Oryza*. There is a continuous increase in the global demand for rice. Diseases are the significant limiting factors that affect rice production causing annual yield losses, conservatively estimated at the 5%. More than 70 diseases are caused by fungi, bacteria, virus and nematode on rice among which, Rice Sheath Blight disease is the most serious one. The use of chemical fertilizers and pesticides cause an incredible harm to the environment. These agents are both hazardous and may persist and accumulate in natural eco systems. These chemicals shall be replaced with biological approaches which are considered more environment friendly in the long term. *Pseudomonas fluorescens* common gram negative, rod shaped bacterium, is a major constituent of rhizobacteria, which encourages the plant growth through its diverse mechanisms. It encompasses a group of common, non pathogenic saprophytes that colonize soil, water and plant surface environments. In this present study the treatments *viz*., *Pseudomonas fluorescens* (Pf1) as seed treatment @ 10g/kg of seeds and foliar spraying of *Pseudomonas fluorescens* @ 0.2% on 30 and 45 DAT and Neem cake as soil application @ 250 kg/ha recorded the minimum disease incidence (12.12%) at harvest and maximum growth parameters of Rice.

Keywords: *Pseudomonas fluorescens*, *Rhizoctonia Solani*, Rice Sheath blight

Introduction
Rice is a monocotyledonous annual grass which belongs to the family Gramineae and the genus *Oryza*. *Oryza* includes 20 wild species and two cultivated species: *Oryza sativa* (grown throughout the world) and *Oryza glaberrima* (grown only in Africa) (Pareja *et al.*, 2011) [8]. In India it is grown over an area of 43.9 m ha with a productivity of 2.464 kg/ha. There is a continuous increase in the global demand for rice which is expected to reach 852MT by the year 2035 from its present production status of 676MT. In order to produce 176MT of more rice to fill this deficiency, there is a need to enhance the productivity of rice from 10-12 t/ha (Khush, 2005) [3].

Diseases are the significant limiting factors that affect rice production causing annual yield losses, conservatively estimated at the 5% (Song and Goodman, 2001) [11]. More than 70 diseases are caused by fungi, bacteria, virus and nematode on rice among which, Rice Sheath Blight disease is the most serious one (Manandhar *et al.*, 1998; Suthin Raj *et al.*, 2016, Anupriya *et al.*, 2019) [4, 14, 1]. Rice sheath blight caused by *R. solani* is a soil borne disease of rice, occuring throughout temperate and tropical production areas and is most prominent wherever rice is grown under intense production system (Singh *et al.*, 2016; Suthin Raj *et al.*, 2019) [10, 1].

The use of chemical fertilizers and pesticides cause an incredible harm to the environment. These agents are both hazardous and may persist and accumulate in natural eco systems. These chemicals shall be replaced with biological approaches which are considered more environment friendly in the long term. One of the emerging research area for the control of different phytopathogenic agents is the use of biocontrol plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the phytopathogen damage (Nihorembere *et al.*, 2011) [6]. *Pseudomonas fluorescens* common gram negative, rod shaped bacterium, is a major constituent of rhizobacteria, which encourages the plant growth through its diverse mechanisms (Noori and Saud, 2012) [7]. It encompasses a group of common, non-pathogenic saprophytes that colonize soil, water and plant surface environments.

Materials and Method
Survey on the occurrence of sheath blight of rice in Cuddalore district of Tamil Nadu A field

survey (fixed plot survey) was conducted to assess the extent of sheath blight occurrence of rice in Cuddalore district. The villages where rice is traditionally grown were selected for assessing the prevalence of sheath blight disease caused by *R. solani*. Ten locations were selected for the survey. During the survey, plants affected due to sheath blight disease were found and also the total number of plants observed were counted and recorded. The per cent disease incidence was worked out as per phytopathometry (Sriram *et al.*, 2000) [12]. Also the infected plants showing typical symptoms of sheath blight due to infection with *R. solani* were collected for isolation of the pathogen from the respective places. The isolates were named as Rs1, Rs2 to Rs10.

Disease Scale

0 = No infection
1 = Less than 5% of the area of leaf sheath affected
2 = 6-10% of the area of leaf sheath affected
3 = 11-25% of the area of leaf sheath affected
4 = 26-50% of the area of leaf sheath affected
5 = More than 50% of the area of leaf sheath affected

The Percent Disease Index (PDI) was calculated as given by McKinney (1923).

$$\text{Per cent Disease Index} = \frac{\text{Sum of the grade}}{\text{Number of observations}} \times \frac{100}{\text{Maximum grade}}$$

Isolation, maintenance and identification of pathogen

The diseased samples were washed thoroughly with tap water. Small portion of infected tissues along with adjacent small unaffected tissues were cut in to 0.5 cm pieces with the help of sterilized scalpel blade and by using flame -sterilized forceps, they were 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 containing sterilized Potato Dextrose Agar (PDA) medium at the rate of 3-5 pieces of tissues per Petri dish supplemented with streptomycin sulfate, and incubated at 28±2 °C in BOD incubator. The Petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

The auxenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) [9] and these were maintained on PDA slants for subsequent experiments.

Evaluation of virulence of *R. solani* isolates

30 kilograms of top soil collected from rice growing fields was steam pasteurized and filled in cement pots (30 x 15 cm). Thirty days old rice seedling var. BPT 5204 was transplanted at the rate of 6 per pot. Rice sheath were collected, cut into small pieces (4cm), transferred to open mouthed bottles and closed with a cotton wool plug. The desired quantity of water was added and the bottles were sterilized at 15 psi for 2 h for three successive days. The medium was used to grow *R. solani* pathogen. From 20 days old culture of the pathogen grown in PDA, six discs of 9mm were taken and inoculated into each bottle. The bottles were then incubated at room temperature (28±2°C) for 14 days and the inoculum thus prepared. The prepared inoculum is then inoculated to seedlings at 30 DAT.

They were observed periodically for the incidence of disease. The intensity of sheath blight was calculated as per cent disease index (PDI) as per the grade chart proposed by (Sriram *et al.*, 2000) [12].

Combination effect of *P. fluorescens* as seed treatment, foliar spraying and neem cake as soil application on the management of sheath blight of rice

A pot culture experiment was conducted with the rice variety BPT 5204. The experiment has been designed in RBD with nine treatments each replicated thrice and a suitable control is maintained. The seeds and seedling source were collected from the Experimental farm of Annamalai University. Rectangular cement pots of size 18"×12"×12" filled with 45 kg of paddy field soil under puddled condition were used for the study. The treatments were given as per schedule and the pots were maintained in green house. The standard agronomic practices as recommended by the State Agricultural Department were followed.

Treatment details

T1- *Pseudomonas fluorescens* (Pf1) as seed treatment @ 10g/kg of seeds
T2- *Pseudomonas fluorescens* (Pf1)as foliar spraying @ 0.2% on 30 and 45 DAT
T3- Neem cake as soil application @ 250 kg/ha
T4- T1 + T2
T5- T1 + T3
T6- T2+ T3
T7- T1 + T2 + T3
T8- Hexaconazole 5 SC as seed treatment @ 2g/kg of seeds and foliar spraying @ 0.2% on 30 and 45 DAT
T9- Control

All observations *viz.*, The sheath blight disease incidence (PDI), plant height (cm), number of tillers, and yield (Grain gm/pot) were assessed and recorded at harvest.

Biometrics and Yield parameters

Plant height

The height of the plant was measured from the surface of the soil to the neck of the panicle plant height was measured at maturity stage on the sample plants and the mean height was calculated in cm.

Number of productive tillers per clump

The productive tillers per clump were counted from total number of tillers at maturity stage on the sample plants and the mean value was calculated and recorded.

Straw yield

After threshing and separation of grains, the straw was dried pot wise or plot wise in sun light for two days. Later the straw weight was recorded.

Grain yield

After the harvest, the grains were separated, winnowed and dried in the sun light and dry weight was recorded.

Results and Discussion

The fixed plot survey conducted during the year 2016 – 2017 in the different locations of Cuddalore district, Tamil Nadu indicated the endemic occurrence of rice sheath blight disease and the results are presented in table 1. Among different locations surveyed, the maximum per cent disease index was recorded in Rayanallur village (23%) followed by Thiruvadigai (22%), Sakkangudi (19%), Orathur (16%), Bhuvangiri (14%) Alapkkam (13%), Thiruvendhipuram (11%),

Kurinjipadi (9%) and Keerapalaym (8%) in the decreasing order of merit. The minimum sheath blight per cent disease index was recorded in Pannapattu (7%). In general sheath blight disease incidence was more in cultivar BPT 5204 and ADT 36 compared to other cultivars. Similar to the present observation, a survey was carried out in selected areas of Allahabad (India) to evaluate the incidence of sheath blight disease of rice. The incidence ranged between 15-42% (Yaduman *et al*., 2018). Jia *et al*., 2012 opined that the amount of crop and yield loss (5-60 per cent) by the disease varied from place to place because of the existence of different races, biotypes of strains of the pathogen. All these earlier reports are in line with the present observations.

The isolate Rs 9 was significantly the most virulent one which recorded the highest per cent disease index of 55% followed by Rs1 (53%), Rs3 (51%), Rs5 (47%), Rs7 (44%), Rs2 (41%), Rs4 (38%), Rs6 (36%) and Rs8 (33%) in the decreasing order of merit. The isolate Rs10 recorded the least virulence with 31 per cent disease index (Table 2).

Results depicted in table 3 showed that the treatment T7 with application of *P. fluorescens* (Pf1) as seed treatment @ 10 g/kg of seeds, *P. fluorescens* (Pf1) as foliar spray (@ 0.2% on 30 and 45 DAT) and neem cake as soil application @ 250 kg/ha reduced the sheath blight incidence (12.12%) at harvest, with maximum per cent disease reduction (78.26%) and was on par with which Hexaconazole 5 SC as seed treatment @ 2 g/kg and foliar spraying @ 0.2% on 30 and 45 DAT recorded 76.07 per cent reduction of sheath blight incidence over control. It was followed by the treatment T4 (*P. fluorescens* as seed treatment @ 10g/kg of seeds (Pf1) and *P. fluorescens* as foliar spraying @ 0.2% (Pf1) on 30 and 45 DAT) with 14.64 per cent disease index at harvest with maximum per cent disease reduction (73.74%) and T6 (*P. fluorecsens* as foliar spraying @ 0.2 % (Pf1) on 30 and 45 DAT and neem cake as soil application @ 250 kg /ha)with 15.52 per cent disease index at harvest with maximum per cent disease reduction (72.16). The control treatment recorded the maximum disease incidence of 55.76 per cent disease index at harvest (Table 3).

Combined modes of delivery system i.e., the application of *P. fluorescens* as seed treatment, foliar spraying and neem cake as soil application might have enhanced the resistance of rice plants against *R. solani*.

Table 1: Survey on the incidence of sheath blight of rice incited by *Rhizoctonia solani* in Cuddalore district of Tamil Nadu

SI. No.	Locality	Isolates	Variety	Crop stage	Soil type	Sheath blight incidence (%)
1.	Thiruvadigai	Rs1	ADT 36	Tillering stage	Clay loam	22^{b}
2.	Alapakkam	Rs2	BPT 5204	Panicle initiation	Clay	13^{f}
3.	Sakkangudi	Rs3	BPT 5204	Panicle initiation	Clay	19^{c}
4.	Thiruvendhipuram	Rs4	ADT 43	Tillering stage	Clay loam	11^{g}
5.	Orathur	Rs5	ADT 38	Panicle initiation	Clay loam	16^{d}
6.	Kurinjipadi	Rs6	ADT 38	Panicle initiation	Clay loam	9^{h}
7.	Bhuvanagiri	Rs7	ADT 36	Grain filling	Clay	14^{e}
8.	Keerapalayam	Rs8	BPT 5204	Grain filling	Clay	8^{i}
9.	Rayanallur	Rs9	BPT 5204	Panicle initiation	Clay	23^{a}
10.	Pannapattu	Rs10	ADT 36	Tillering stage	Clay	7^{j}

Rs* – *Rhizoctonia solani* isolate

* Values are expressed as means for three replications in each group

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

Table 2: Evaluation of virulence of *R. solani* isolates (Pot culture)

Sl. No	Isolates	Per cent disease index
1	Rs1	53^{b}
2	Rs2	41^{f}
3	Rs3	51^{c}
4	Rs4	38^{g}
5	Rs5	47^{d}
6	Rs6	36^{h}
7	Rs7	44^{e}
8	Rs8	33^{i}
9	Rs9	55^{a}
10	Rs10	31^{j}

* Values are expressed as means for three replications in each group

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

Table 3: Effect of *Pseudomonas fluorescens* and neem cake on Sheath blight incidence of rice variety BPT 5204 (Field condition)

Tr. No.	Treatments	Sheath blight incidence (%)			Per cent disease over control		
		60 DAT	90DAT	At harvest	60DAT	90DAT	At harvest
1.	*Pseudomonas fluorescens* as seed treatment @ 10g/kg of seeds (Pf1)	14.31^{g} (22.23)	16.90^{g} (24.27)	19.64^{g} (26.31)	56.46	61.49	64.77
2.	*Pseudomonas fluorescens* as foliar spraying @ 0.2% (Pf1) on 30 and 45 DAT	13.62^{f} (21.66)	15.71^{f} (23.35)	18.31^{f} (25.33)	58.56	64.20	67.16
3.	Neem cake as soil application @ 250kg/ha	15.18^{h} (22.93)	17.39^{h} (24.65)	20.88^{h} (27.19)	53.81	60.37	62.55
4	T1 + T2	10.60^{c} (19.00)	12.42^{c} (20.64)	14.64^{c} (22.50)	67.75	71.70	73.74
5	T1 + T3	12.67^{e} (20.85)	14.32^{e} (22.24)	16.82^{e} (24.21)	61.45	67.37	69.83
6	T2 + T3	11.35^{d} (19.69)	13.84^{d} (21.84)	15.52^{d} (23.20)	65.47	68.46	72.16

7	T1 + T2 + T3	9.19[a] (17.65)	11.01[a] (19.38)	12.12[a] (20.37)	72.04	74.91	78.26
8	Hexaconazole 5 SC as seed treatment @ 2 g/kg of seeds and foliar spraying @ 0.2% on 30 and 45 DAT	9.84[b] (18.28)	11.96[b] (20.23)	13.34[b] (21.42)	70.06	72.75	76.07
9	Control	32.87[i] (34.98)	43.89[i] (41.49)	55.76[i] (48.31)	-	-	-

*V alues are expressed as means for three replications in each group
*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)
* Within parenthesis are Arc Sin transformed values

Effect of *P. fluorescens* (Pf1) and neem cake on biometrics of rice variety BPT 5204

Plant height

The data depicted in table 4 shows that all the treatments significantly increased the plant height when compared to untreated control. Among the treatments, the maximum plant height of 81.01 cm was observed in treatment T7 which was on par with T8 with height of 80.09 cm. This was followed by T4 (79.86 cm) which was on par with T6 (79.05 cm). The untreated control recorded the minimum plant height of 63.60 cm. Increased number of productive tillers per hill was recorded in the treatment T_7 (15.98) which was on par with T8 (Hexaconazole 5 SC as seed treatment @ 2 g/kg and foliar spraying @ 0.2% on 30 and 45 DAT) which recorded 15.03 productive tillers/hill. This was followed by T4 (14.79), T6 (13.98), T5 (13.08), T2 (12.86), T1 (12.04) and T3 (11.15) in the decreasing order of merit. The untreated control recorded the minimum number of tillers (10.43) (Table 4).

Generally, all the treatments significantly increased the grain yield (g/plant) compared to control. The effect of different treatments on the grain yield (g/ plant) was recorded in Table 4. Among the treatments, the maximum grain yield (49.50 g/ plant) was observed in treatment T7 which was on par with T8 which recorded grain yield of 48.95 g/ plant. The untreated control recorded the minimum grain yield (19.45 g/ plant). On the straw yield all the treatments significantly increased the straw yield (g/ plant) when compared to control. Among the treatments, the maximum straw yield (90.26 g/ plant) was observed in treatment T7 which was on par with T8 which recorded 89.37 g/pot. This was followed by T4 (88.63 g/ plant), T6 (87.41 g/ plant, T5 (86.95 g/ plant), T2 (86.05 g/ plant, T1 (85.64 g/ plant) and T3 (84.69 g/ plant) in the decreasing order of merit. The untreated control recorded the minimum straw yield (39.45 g/ plant) (Table 4).

Table 4: Effect of *Pseudomonas fluorescens* and neem cake on growth and yield attributes of rice variety BPT 5204 (Field condition)

Tr. No.	Treatments	Plant height (cm)	No. of productive tillers / hill	Grain yield (g. per plant)	Straw yield (g. per plant)
1	*Pseudomonas fluorescens* as seed treatment @ 10g/kg of seeds (Pf1)	76.81[g]	12.04[g]	44.67[g]	85.64[g]
2	*Pseudomonas fluorescens* as foliar spraying @ 0.2% (Pf1) on 30 and 45 DAT	77.62[f]	12.86[f]	45.74[f]	86.05[f]
3	Neem cake as soil application @ 250kg/ha	75.07[h]	11.15[h]	43.29[h]	84.69[h]
4	T1 + T2	79.86[c]	14.79[c]	48.02[c]	88.63[c]
5	T1 + T3	78.88[e]	13.98[e]	46.52[e]	86.95[e]
6	T2 + T3	79.05[d]	13.08[d]	47.56[d]	87.41[d]
7	T1 + T2 + T3	81.01[a]	15.98[a]	49.50[a]	90.26[a]
8	Hexaconazole 5 SC as seed treatment @ 2 g/kg of seeds and foliar spraying @ 0.2% on 30 and 45 DAT	80.09[b]	15.03[b]	48.95[b]	89.37b
9	Control	63.60[i]	10.43[i]	19.45[i]	39.45[i]

*Values are expressed as means for three replications in each group
*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

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