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Study on integration of soil application with various organic amendments to combat sheath blight of paddy

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Abstract
Rice (*Oryza sativa* L.) is the staple food for more than half of the human population and India has the largest area of 42.75 million hectares) under rice cultivation with a production of 109.15 MT Rice cultivation is often subjected to several biotic stresses of which diseases like blast, sheath blight, stem rot and bacterial blight are the important ones Among these, sheath blight of rice caused by a soil-borne fungal pathogen, *Rhizoctonia solani* is a destructive disease in all crop-growing areas of the world. The losses due to sheath blight disease generally vary from 30 to 40 per cent and may be even 100 per cent in endemic areas 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. Under such circumstances use of Plant Growth Promoting Rhizobacteria (PGPR) offer a promising means of controlling diseases and improve the yield in the rice ecosystem. In the present study the PGPR along with some of the organic amendments are tested against *Rhizoctonia solani.* The results revealed that the combination 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 significantly reduced the sheath blight incidence to the minimum (10.01% in pot and 12.12% in field trial) at harvest with 81.42 per cent disease reduction over control. Hence the various methods of delivery system of *P. fluorescens* along with neem cake as soil application @ 250 kg/ha can be effectively used to control the rice sheath blight diseases.

Keywords: Rhizoctonia solani, *P. fluorescens,* Neem Cake, chicken manure

Introduction
Rice (*Oryza sativa* L.) is the staple food for more than half of the human population (Midha *et al*., 2017) [4], extensively grown in the tropical and sub-tropical zones across the world. Global rice production in the world is 487 MT (www.igc.int) during 2016-17 and India has the largest area of 42.75 million hectares (Sharma *et al*., 2017) under rice cultivation with a production of 109.15 MT in 2016-17(www.agricoop.gov.in). Due to high domestic consumption of rice, both production and productivity needs to be increased. Indian rice production target for the year 2025 is 140 MT which can be achieved only by increasing rice production by 2 MT per year in the coming decade (FAOSTAT, 2015). But the rice cultivation is often subjected to several biotic stresses of which diseases like blast, sheath blight, stem rot and bacterial blight are the important ones (Ou,1985). Among these, sheath blight of rice caused by a soil-borne fungal pathogen, *Rhizoctonia solani* is a destructive disease in all crop-growing areas of the world. The fungus affects the crop from tillering to heading stage (Johnson *et al.*, 2013). The losses due to sheath blight disease generally vary from 30 to 40 per cent and may be even 100 per cent in endemic areas, when the disease spreads to upper parts of the plant and panicles a total crop loss was observed (Srinivas *et al*., 2013) [10].

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. Under such circumstances use of Plant Growth Promoting Rhizobacteria (PGPR) offer a promising means of controlling diseases and improve the yield in the rice ecosystem (Mew and Rosales, 1992) [3].The promising PGPR *Pseudomonas fluorescens* is *a* gram negative, rod shaped bacterium, is a major constituent of rhizobacteria, which encourages the plant growth through its diverse mechanisms (Noori and Saud, 2012) [6]. Certain organic amendment has the capable of reducing the disease incidence and also enhances the soil fertility and crop yield to a significant level (Kumar and Kumar, 2018). Keeping in this view the present study was done to reduce the disease incidence with the use of *Pseudomonas fluorescens* and certain organic amendments.

Materials and methods

Isolation 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.

Maintenance of Pathogen

The auxenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) [8] and these were maintained on PDA slants for subsequent experiments.

Isolation of bacterial antagonist

Rhizosphere colonizing fluorescent pseudomonads were isolated from fresh roots of rice from seven different places of Tamil Nadu. The isolate Pf1 was obtained from TNAU, Madurai. The other seven isolates were isolated from Alagapuri (Pf2), Koolayanur (Pf3), Kodikulam (Pf4), Marathur (Pf5), Sathapadi (Pf6), Manaveli (Pf7) and Adoor (Pf8).

The soil suspension was prepared from each rhizosphere sample by shaking one g of soil sample in 10 ml of sterile dist. water and serial dilutions were made. One ml of soil suspension from 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 UV light. 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 (Vidhyasekaran and Muthamilan, 2010) [11].

Dual culture technique (Dennis and Webster, 1971).

Twenty ml of sterile PDA medium was poured into Petri dishes under aseptic conditions and allowed to solidify. After solidification, 9 mm culture disc obtained from the periphery of 10-day-old culture of *R. solani* was inoculated 1.5 cm away from the edge of the Petri dish. Similarly, 2-day-old bacterial antagonist which was isolated before was streaked (one cm long) at equidistance just opposite to the pathogenic culture and incubated under room temperature (28±2°C) until the control plates were covered by the pathogen.

After incubation, the zone of inhibition (in mm) and mycelial growth of *R. solani* was recorded by measuring the distance between the edges of the fungal mycelium and the antagonistic bacterium. The per cent inhibition of mycelial growth was calculated by the following formula (Vincent, 1927) [12]

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

Where, C- mycelial growth of pathogen in control,
T- Mycelial growth of pathogen in dual plate.

Efficacy of culture filtrate *P. fluorescens* against the mycelial growth of *R. solani* (Poisoned food technique)

The efficacy of *P. fluorescens* strains was assayed at the conc., of 10, 20, and 30 per cent. 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 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 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 control (Vincent, 1927) [12].

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

Where, I is the inhibition per cent
C- Mycelial growth of pathogen in control,
T- Mycelial growth of pathogen in dual plate.

Effect of different organic amendments (soil application) on incidence of sheath blight of rice and plant growth promotion (Pot culture)

Seven organic amendments viz., chicken litter (12.5 t/ha), fishmeal (12.5 t/ha), neem cake (250 kg/ha), groundnut cake (250 kg/ha), FYM (12.5 t/ha), mahua cake (250 kg/ha) and sheep manure (12.5 t/ha) were used for this study. The organic amendments were incorporated with the soil. Surface sterilized rice seeds were treated with the antagonists as per the schedule mentioned earlier. Rice seeds sown in pot soil mixed with the inoculum of

R. solani alone served as control. The experiment was conducted with three replications in a randomized block design. All observations *viz.*, the sheath blight disease incidence (PDI), plant height (cm), number of tillers, and yield (Grain g/pot) were assessed and recorded at harvest.

Combined 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.

Combined effect of *P. fluorescens* as seed treatment, foliar spraying and neem cake as soil application on the management of sheath blight of rice (Field trial)

Field trials were conducted during October to December 2017 in sheath blight prone area in the farmer's fields. A plot size of 5×4 m was used for each treatment and the experiment has been designed in RBD with nine treatments each replicated thrice. The rice susceptible variety BPT 5204 was used for the study. The blanket fertilizer schedule of 150:50:50 NPK/ha and all the other agronomic practices as recommended by the state agricultural department was followed. A suitable control plot without any treatment was also maintained. Hexaconazole 5 SC as seed treatment @ 2 g/kg of seeds and as foliar spray @ 0.2% conc. on 30 and 45 DAT was used for comparison. The treatments with *P. fluorescens* and neem cake were given as per the schedule followed in the pot culture experiment.

Results and Discussion

Screening of *P. fluorescens* isolates against *R. solani*

Dual culture and poison food technique

The isolates Pf2, Pf3, Pf4, Pf5, Pf6, Pf7, Pf8 along with the isolate Pf1 obtained from TNAU, Madurai were tested against *R. solani*. Among the *P. fluorescens* isolates, Pf1 produced significantly the maximum inhibition zone (17.24mm) and minimum mycelial growth (21.45mm) accounting for 76.16 per cent reduction on the mycelial growth of

R. solani over control followed by the isolate Pf4 and Pf7 was the least effective among those isolates (Table 1). Likewisethe mycelial growth of *R. solani* was found reduced with an increase in the concentration of culture filtrates of all the isolates of the antagonists tested and the reduction was significantly the maximum in the case of *P. fluorescens* isolate Pf1 with 19.58, 10.33and 1.81 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 poison food technique. (Table 1).

Effect of different organic amendments on incidence of sheath blight of rice (Pot culture)

Application of organic amendments significantly reduced the sheath blight incidence and enhanced the plant height, number of productive tillers/hill and yield. Among them, neem cake @ 250 kg/ha recorded minimum disease incidence (37.82) and significantly increased the plant height (84.91 cm), number of productive tillers/hill (12.87) and yield (39.86 g/pot). It was followed by FYM @ 12.5 t/ha which recorded 38.84 per cent disease incidence with increased plant height (83.62), number of productive tillers/hill (12.02) and yield (38.31 g/pot). From this experiment neem cake was found to best for reducing the disease incidence and hence it was used in further studies (Table 2).

Effect of *P. fluorescens* (Pf1) and neem cake on sheath blight incidence of rice variety BPT 5204 (Pot culture)

In pot culture studies, 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 significantly reduced the sheath blight incidence to the minimum (10.01%) at harvest with 81.42 per cent disease reduction over control and was at par with that of Hexaconazole 5 SC as seed treatment @ 2 g/kg and foliar spraying @ 0.2% treatment on 30 and 45 DAT which recorded 10.56 per cent disease incidence at harvest with 80.40 per cent disease reduction (Table 3). It was followed by T4 (Seed treatment with *P. fluorescens* (Pf1) @ 10g/kg and foliar spray with *P. fluorescens* (Pf1) @ 0.2% at 30 and 45 DAT) which recorded11.30 per cent disease index at harvest with 79.03 per cent disease reduction over control and T6 (Foliar spray with *P. fluorescens* (Pf1)

@ 0.2% on 30 and 45 DAT and neem cake as soil application 250 kg/ha which recorded 12.01 per cent disease index with 77.71 per cent disease reduction over control. The control treatment recorded the maximum disease incidence of 53.89 per cent disease index at harvest.

Effect of *P. fluorescens* and neem cake on sheath blight incidence of rice variety BPT 5204(Field trial)

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 recorded the maximum disease incidence of 55.76 per cent disease index at harvest (Table 4).

Table 1: Screening of *P. fluorescens* isolates against *R. solani*

Sl. No.	Isolate number	Dual Culture			Poison food Technique					
					Mycelial growth (mm)					
		Mycelial growth (mm)	Per cent inhibition over control	Zone of inhibition	10%	Per cent inhibition over control	20%	Per cent inhibition over control	30%	Per cent inhibition over control
1	*P. fluorescens* (Pf1)	21.45[a] (27.59)	76.16	17.24	19.58[a] (26.26)	78.24	10.33[a] (18.75)	88.52	1.81[a] (7.73)	97.99
2	*P. fluorescens* (Pf2)	25.15[e] (30.09)	72.05	13.28	24.86[e] (29.91)	72.38	15.02[e] (22.80)	83.31	5.72[e] (13.84)	93.64
3	*P. fluorescens* (Pf3)	26.17[f] (30.77)	70.92	12.67	25.12[f] (30.08)	72.09	16.34[f] (23.84)	81.84	6.16[f] (14.37)	93.16
4	*P. fluorescens* (Pf4)	22.19[b] (28.10)	75.34	16.94	21.08[b] (27.33)	76.58	12.61[b] (20.80)	85.99	2.34[b] (8.80)	97.40
5	*P. fluorescens* (Pf5)	27.47[g] (31.61)	69.48	11.12	26.34[g] (30.88)	70.73	17.28[g] (24.56)	80.80	7.34[g] (15.72)	91.84
6	*P. fluorescens* (Pf6)	23.85[c] (29.23)	73.50	15.43	22.60[c] (28.39)	74.89	13.17[c] (21.28)	85.37	3.64[c] (11.00)	95.56
7	*P. fluorescens* (Pf7)	28.39[h] (32.19)	68.46	10.84	27.92[h] (31.90)	68.98	18.65[h] (25.59)	79.28	8.70[h] (17.15)	90.33
8	*P. fluorescens* (Pf8)	24.56[d] (29.71)	72.71	14.00	23.33[d] (28.88)	74.08	15.86[d] (23.47)	82.38	4.48[d] (12.22)	95.02
9	Control	90.00[i] (71.56)	–	–	90.00[i] (71.57)	–	90.00i (71.57)	–	90.00[i] (71.57)	–

* 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)
Within parenthesis are Arc Sin transformed values

Table 2: Effect of different organic amendments on the incidence of sheath blight of rice (Pot culture)

Sl. No	Organic amendments	Sheath blight incidence %	Per cent decrease over control	No. of tillers/hill	Plant height (cm)	Yield (g/pot)
1	Chicken litter (12.5 t/ha)	45.98[d] (42.69)	43.00	10.85[d]	82.60[d]	37.51[d]
2	Fishmeal (12.5 t/ha)	50.54[f] (45.30)	37.34	9.12[f]	79.67[f]	35.26[f]
3	Neem cake (250 kg/ha)	37.82[a] (37.95)	53.11	12.87[a]	84.91[a]	39.86[a]
4	Groundnut cake (250 kg/ha)	52.36[g] (46.35)	35.09	8.97[g]	77.65[g]	34.25[g]
5	FYM (12.5 t/ha)	38.84[b] (38.55)	51.85	12.02[b]	83.62[b]	38.31[b]
6	Mahua cake (250 kg/ha)	42.65[c] (40.77)	47.13	11.23[c]	82.62[c]	38.06[c]
7	Sheep manure (12.5 t/ha)	48.67[e] (44.23)	39.67	10.12[e]	81.15[e]	35.65[e]
8	Control	80.67[h] (63.91)	_	7.07[h]	72.53[h]	31.5[h]

*Values are expressed as means for three replications n 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

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

Tr. No.	Treatments	Sheath blight incidence (%)			Per cent disease over control		
		60 DAT	90 DAT	At harvest	60 DAT	90 DAT	At harvest
1.	*Pseudomonas fluorescens* as seed treatment @ 10g/kg of seeds (Pf1)	13.73[g] (21.75)	14.82[g] (22.64)	16.12[g] (23.67)	55.07	65.08	70.08
2.	*Pseudomonas fluorescens* as foliar spraying @ 0.2% (Pf1) on 30 and 45 DAT	12.48[f] (20.69)	13.56 (21.61)	14.82[f] (22.64)	59.16	68.05	72.49
3.	Neem cake as soil application @ 250kg/ha	14.56[h] (22.43)	16.71[h] (24.13)	18.32[h] (25.34)	52.35	60.63	66.00
4	T1 + T2	9.36[c] (17.81)	10.52[c] (18.93)	11.30[c] (19.64)	69.37	75.21	79.03
5	T1 + T3	11.28[e] (19.62)	12.34[e] (20.57)	13.64[e] (21.67)	63.08	70.93	74.68
6	T2 + T3	10.74[d] (19.13)	1136[d] (19.70)	12.01[d] (20.28)	64.85	73.23	77.71
7	T1 + T2 + T3	7.86[a] (16.28)	8.16[a] (16.60)	10.01[a] (18.44)	74.28	80.77	81.42
8	Hexaconazole 5 SC as seed treatment @ 2 g/kg of seeds and foliar spraying @ 0.2% on 30 and 45 DAT	8.36[b] (16.81)	9.42[b] (17.87)	10.56[b] (18.96)	72.64	77.80	80.40
9	Control	30.56[i] (33.56)	42.45[i] (40.66)	53.89[i] (47.23)	-	-	-

*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)
*Within parenthesis is Arc Sin transformed value

Table 4: 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	90 DAT	At harvest	60 DAT	90 DAT	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)	-	-	-

*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)

* Within parenthesis are Arc Sin transformed values

Discussion

In the present study, all the ten isolates of *P. fluorescens* showed 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*. In poison food technique, the growth of *R. solani* was found 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 30 per cent conc. Neem cake @ 250 kg/ha recorded minimum disease incidence (37.82) and significantly increased the plant height, number of productive tillers/hill and yield. Similar to the present observations in pot and field trial Mishra *et al.*, 2009 recorded minimum sheath blight incidence in rice and significant increase in grain yield and 1000g weight in the treatment combination with neem cake @ 250 kg/ha, *Trichoderma harzianum* and *P. fluorescens* @ 5kg/ha.

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