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Survey and occurrence of *Rhizoctonia solani* (Kuhn) causing sheath blight of rice and *in vitro* efficacy of bacterial antagonists against *Rhizoctonia solani* (Kuhn)

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Abstract

Sheath blight of rice caused by a soil-borne fungal pathogen, *Rhizoctonia solani* Kuhn is a destructive disease in all crop-growing areas of the world. A survey carried out to assess the occurrence of sheath blight disease incidence in major paddy growing areas of Cuddalore district of TamilNadu, India revealed the endemic nature of disease. Maximum PDI was recorded in Naduthittu (36.5%) followed by Vadakkumangudi (32.4%), Vallampadugai (26.5%), Muttlur (21.4%) villages and moderate incidence was noticed in Shivapuri, Periyakannadi, Vandurayanpattu, Keerapalayam, Vadalore, Kanthakumaran, Orathur, Permapattu, Thiththampalayam, Bhuvanagiri villages and least incidence was noticed in Ramapuram (10.5%) location. Generally, the crops grown in clay soil and at panicle initiation stage of the crop recorded fairly higher disease incidence. Among the antagonists tested under *in vitro* conditions, *P. fluorescens* was found to be more antagonistic than *R. solani*. Under pot culture conditions, the combined application of *P.fluorescens* as ST @ 10 ml kg^{-1} of seeds + seedling root dip @ 3.0 l ha^{-1} significantly reduced the incidence of sheath blight in rice to minimum, and increased the plant growth and yield of rice to maximum.

Key words

Antifungal activity, *Rhizoctonia solani,* Pathogenicity, Bacterial antagonists, Rice

Introduction

Rice (*Oryza sativa*) is an important cereal crop and staple food for large part of the world's population. Rice covers an area of 2.9 million acres worldwide, yielding an annual production of seven million tones with an average yield of 1080 kg $acre^{-1}$ (Anonymous, 2013). Rice cultivation is often subjected to several biotic stresses caused by fungal, bacterial and viral pathogens 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 *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk) anastomosis group 1 and subgroup 1A) is a destructive disease in all the crop-growing areas of the world. The disease is most prominent where ever rice is grown under intense production systems (Savary and Mew, 1996) and is second only to rice blast as the most economically important fungal disease of rice (Savary *et al*., 2006). The widespread adoption of new, susceptible high yielding cultivars with large number of tillers and the changes in cultural practices associated with these cultivars favour the development of sheath blight and contribute greatly to rapid increase in the incidence and severity of this disease in rice producing areas throughout the world (Srinivasachary *et al*., 2011). Infection usually begins near the water line of rice plants in paddy fields. Lesions develop upward to the upper leaf sheaths and leaf blades. The centre of lesion become greyish white with brown margin, later several spots coalesce and show blight symptoms (Ou,1985).

Apart from reducing plant vigour and yield, the disease also cause grain discoloration at maturity, thus reducing market value. Loss due to sheath blight disease generally vary from 30 to 40%, and may be even 100% in endemic areas. If the disease spreads to upper parts of the plant and panicles it results in total crop loss (Srinivas *et al*., 2013).

Biological control of plant pathogens and its utilization in rice ecosystem is gaining popularity and represents as an eco-friendly and cheaper strategy for managing the diseases of crop plants (Harish *et al*., 2008). However, effective management strategy of sheath blight disease is feasible only when bio-control agents in rice based cropping system survive, establish, proliferate and also have a synergistic growth promoting effect on the crop. Under such circumstances, use of plant growth promoting rhizobacteria (PGPR) offer a promising means of controlling sheath blight disease and improve the yield of rice (Pande and Chaube, 2003; Sharma *et al*., 2004; Afsharmanesh *et al*., 2010). Among the PGPR's, *P. fluorescens* and *Bacillus subtilis* have been shown to effectively control plant pathogens (Sivasakthi *et al*., 2014). These bacterial antagonists generally survive under anaerobic condition and can multiply very fast, that's why they are generally used in paddy fields (Meera and Balabaskar, 2012a).

With this back ground the present study was conducted with an objective to assess the prevalence and incidence of sheath blight of rice in Cuddalore district of Tamil Nadu, and to assess the cultural and pathogenic variability among the isolates of *R . solani.* Further, effective native PGPR isolates from rice ecosystem were isolated to evaluate their antagonistic potential against *R. solani* under *in-vitro* and *in-vivo* conditions.

Materials and Methods

Survey on the occurrence of sheath blight of rice in Cuddalore District : A field survey (Fixed plot survey) was conducted at fifteen locations to assess the extent of sheath blight occurrence of rice in Cuddalore district. For assessing and scoring the disease, incidence typical assessment system for rice developed by the International Rice Research Institute (SES, 2002) was followed with the scale 0 as no infection and 1, 3, 5 ,7 , 9 as vertical spread of the lesions with 20%, 21-30%, 31-45%, 46-65%, greater than 65% of plant height, respectively. The disease severity was worked out using the following formula:

$$\text{Disease severity} = \frac{\text{Sum of disease grades x No. of infected tillers / hill}}{\text{Total No. of Tillers x Maximum disease grades x No. of tillers assessed}} \times 100$$

Isolation, maintenance of the pathogen : Diseased rice plants showing typical symptoms of sheath blight disease were collected during survey and the infected portion of sheath was cut into small bits, surface sterilized in 0.1% mercuric chloride solution for 30 sec, washed in repeated changes of sterile distilled water and plated onto PDA medium in sterile Petri dishes. The plates were incubated at room temperature (28±2p C) for five days and observed for fungal growth. The fungus was subsequently purified, maintained on PDA slants and used for further studies.

Effect of bacterial antagonists against *R. solani*

Dual culture technique : 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 obtained from department culture collections was streaked (one cm long) at equidistance just opposite to the pathogenic culture and incubated under room temperature (28±2ºC) until the control plates were covered by the pathogen. After incubation, the zone of inhibition (in mm) and mycelial growth of *R. solani* were recorded (Dennis and Webster, 1971). The per cent inhibition of mycelial growth was calculated by the following formula (Vincent, 1927) :

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

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

***In vitro* evaluation of culture filtrates of antagonist on the mycelial dry weight of *R. solani* (Liquid medium assay) :** The effective isolate identified in dual culture study was evaluated in liquid medium assay. Fifty ml of PDA broth taken in 250 ml Erlenmeyer flasks were sterilized and amended with culture filtrates of *P. fluorescens* at different concentrations and inoculated with mycelia disc of *R. solani* collected from the periphery of 10- day- old culture. The flasks were incubated for 10 days at room temperature (28±2p C) and filtered through filter paper Whatman no. 42 in vacuum. The dry weight of mycelial biomass was recorded in milligram.

Sclerotial germination assay : Different concentration of culture filtrate of *P. fluorescens* @ 0.5 ml and 0.1 % solution of carbendazim 50% WP fungicide and sclerotia of test fungus (10 No.) were mixed in cavity slide and incubated for 24, 36, 48 and 60 hr. In Petri plate glass bridge moist chamber at 28 ± 2ºC. Cavity slides with sterile distilled water having sclerotia were kept as control. The total number of sclerotia germinated under each microscopic field was recorded and

germination percentage was calculated (Macko *et al.,*1977).

Plant growth-promotion-roll towel method : Seeds treated with different levels of liquid formulations of *P. fluorescens* and Carbendazim 50% WP at 2 g kg^{-1} of seed 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 and incubated at room temperature (28 ± 2ºC) for seven days. Each treatment was replicated thrice. Normal seedlings were selected randomly from each replication, and the shoot and root length from collar to tip of the primary root was measured and their respective mean values were recorded (ISTA, 1976). Vigour index (VI) was calculated by the formulae given by Abdul Baki and Anderson (1973).

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

Efficacy of seed treatment plus seedling root dip with *P. fluorescens* for the management of rice sheath blight : The antagonist *P. fluorescens* as liquid formulation having not less than 1x 10^{8} cfu ml^{-1} (Manikandan *et al*., 2010) was treated as per schedule. Rice seeds sown in pot soil mixed with the inoculum of *R. solani* alone served as control. Seed treatment @ 2 g kg^{-1} plus soil drench @ 0.1% with Carbendazim 50% WP served as comparison. The experiment was conducted with three replications in a randomized block design. All the observations *viz*., sheath blight disease incidence (PDI- at different intervals), plant height (cm), number of tillers, and yield (Grain and straw yield gm/pot) were assessed and recorded at harvest.

Statistical analysis : The data collected were subjected to statistical analysis using computer aided IRRISTAT Version 92 software developed by the International Rice Research Institute, Philippines.

Results and Discussion

Survey on the incidence of sheath blight of rice in Cuddalore district of Tamil Nadu : The data presented on the fixed plot survey conducted in some major rice growing areas of Cuddalore district, indicated that the Per cent Disease Index (PDI) varied with low to high level. The maximum PDI was recorded in Naduthittu of (36.5%) followed by Vadakkumangudi (32.4%), Vallampadugai (26.5%).The least level of incidence was noticed in Ramapuram (10.5%). Generally, the crop grown in clayey soil and at panicle initiation stage of the crop recorded fairly higher incidence of disease. Similar to the present observations, Jia *et al*. (2012) reported that all the cultivars of rice are susceptible to sheath blight, but the degree of susceptibility varied and sheath blight is usually severe on cultivars that are short, high tillering and responsive to high fertilizer in comparison to tall cultivars with fewer tillers.

Cultural characters of *R. solani* isolates : All the fifteen isolates of sheath blight pathogen *R. solani* produced light brown to brown colour sclerotia. Maximum sclerotial population was observed in Naduthittu isolate of 1 to 3 mm. The diameter of sclerotia ranged from 1.4-2.5 mm. Similar to the present results, Sunder *et al*. (2003) also reported that colony colour ranged from brown, light brown, dark brown and yellowish brown. Discoloration of growth media is mainly attributed to the production of pigments by the pathogen. Difference in the intensity of the colour might also correspond to the amount of pigments released by their respective isolate in the media.

Efficacy of bacterial antagonists against *R. solani* : The results of dual culture technique indicated that all the antagonists inhibited the growth of test fungus significantly when compared to control. Among the antagonists *P. fluorescens* was found to be more antagonistic to *R. solani* as it recorded maximum per cent inhibition (75.86%), which was followed by *Bacillus subtilis* (73.88%) and *Serratia marcescens* (62.71%) in the decreasing order of merit. Minimum growth inhibition was observed in *B. cereus* (59.7%).

Effect of culture filtrate of *P. fluorescens* on the mycelial growth and mycelial dry weight of *R. solani* : The results revealed an increasing trend in the per cent inhibition with an increase in the concentration of culture filtrates of *P. fluorescens*. In solid media, the culture filtrate of *P. fluorescens* at 20% and above completely inhibited the mycelial growth of *R. solani* which was statistically on par with carbendazim 50 WP. 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 *R. solani* alone (control) recorded maximum mycelial dry weight (301.28 mg). Minimum mycelial dry weight (0.81 mg) of *R. solani* was recorded in 40% concentration of the culture filtrate of *P. fluorescens.* Among all the concentrations used, *P. fluorescens* @ 5% concentration was found to be least effective (61.54 %) (Table 1).Similar to the present study, Intesar Ali Mezeal (2014) reported that the culture filtrate of *P. fluorescens* effectively inhibited the mycelial growth of *R. solani*.

Effect of culture filtrate of *P. fluorescens* on the sclerotial germination of *R. solani* (cavity slide method) : Among various concentrations of *P. fluorescens* culture filtrate tested, the sclerotial germination of *R. solani* was completely inhibited by 40% concentration of culture filtrate and it was found to be on par with carbendazim @ 0.1% concentration

P. fluorescens @ 20% concentration of the culture filtrate ranked next by significantly reducing the sclerotial germination by 9.42, 7.35, 6.74% and 592 % after 24, 36, 48 and 60 hrs of observation respectively. Five per cent concentration of the culture filtrate was found to be least effective. Carbendazim at 0.1% concentration recorded complete inhibition of sclerotial germination of test fungus at all the intervals tested (Table 2).

Inhibitory effect *P. fluorescens* on the conidial/ sclerotial germination of pathogen has been reported (Umamaheswari *et al.,* 2002). Kumar and Sood (2005) reported that *P. fluorescens* produced siderophore, pseudobactin, which can efficiently control conidial germination of *F. oxysporum* f. sp. *lycopersici* of tomato**.** The above results lend support to the present findings.

Among the antagonists tested in the study, *P. fluorescens* was found to be more antagonistic to *R. solani* as it recorded maximum per cent inhibition of the mycelial growth, mycelia dry weight and conidial germination when tested with dual culture, poisoned food technique and cavity slide techniques. The mycoparasitic potential of *Pseudomonas* spp. is well documented (Keel and Defago, 1997; Elad *et al*., 1980). These earlier reports lens support to the present findings. Several strains of *Pseudomonas* spp. have been reported to produce wide array of antibiotics *viz*., 2, 4, diacetylphloroglucinol, oligomycin, oomycinA, phenazine, pyoluterin, pyrolnitrin, pyocyanin, iturin, surfactin and several other uncharacterized molecules (Ramamoorthy and Samiyappan, 2001) which could be attributed as the reason for growth inhibition.

Efficacy of *P. fluorescens* on plant growth promotion (Roll towel method) : Maximum germination percentage, shoot length, root length and vigour index was observed in *P. fluorescens* seed treatment @ 10.0 ml (95.79%, 7.48 cm, 10.12 cm and 1685.90) treated seeds. This was followed by *P. fluorescens* seed treatment @ 7.5 ml (94.76%, 7.12 cm, 9.81cm and 1604.28 respectively). Minimum germination percentage, shoot and root length was noticed in control (58.65%, 4.50cm, 3.20 cm and 451.60) (Table 3).

Kloepper and Schroth (1981), reported that specific rhizosphere bacteria applied to seeds could colonize roots and promote plant growth which was later called as Plant Growth Promoting Rhizobacteria (PGPR). Several workers have reported about the growth promoting effect of

Table 1 : Effect of culture filtrate of *P. fluorescens* on the mycelial growth and mycelial dry weight of *R. solani*

Tr. No.	Conc. of the culture filtrate (%)	Mycelial growth (mm)	Per cent inhibition	Mycelial dry weight (mg)	Per centinhibition
1	5	34.61	61.54	158.25	47.47
2	10	28.29	68.56	98.00	67.47
3	15	09.31	89.65	43.71	85.49
4	20	NG	—	1.18	99.60
5	40	NG	—	0.81	99.73
6	Carbendazim 50%WP @ (0.1%)	NG	—	1.00	99.66
7	Control	90.0	_	301.28	-
	SEdCD (P=0.05)	2.5995.726	0.5611.241	1.1852.774	0.4711.038

NG - Nil growth

Table 2 : Effect of culture filtrate of *P. fluorescens* on the Sclerotial germination of *R. solani* (cavity slide method)

Tr. No.	Culture filtrate	Sclerotial germination (%)			
	conc. (%)	24 hr	36 hr	48 hr	60 hr
1	5	60.25(50.91)	70.43(57.05)	86.43(68.38)	88.21(69.91)
2	10	40.56(39.55)	46.75(43.13)	51.66(45.95)	54.87(47.79)
3	15	32.74(34.90)	36.35(37.07)	37.14(37.54)	38.42(38.30)
4	20	9.42(17.87)	7.35(15.73)	6.74(15.04)	5.92(14.08)
5	40	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
6	Carbendazim (0.1 %)	0.00	0.00	0.00	0.00
7	Control	73.25(58.85)	81.33(64.39)	90.65(72.19)	97.53(80.95)
	SEdCD (P=0.05)	0.5751.267	0.6191.364	0.5571.226	0.5611.241

Data in parentheses indicate angular transformed values

Pseudomonas in various crops (Ramamoorthy and Samiyappan, 2001; Singh *et al*., 2005). The results of the present study are in line with these earlier reports.

Effect of combination of seed treatment plus seedling root dip with *P.fluorescens* for the management of *R. solani* causing sheath blight of Paddy : The data depicted in Table 4 revealed that the treatment with *P. fluorescens* showed significant influence on the incidence of sheath blight of rice. The treatment (T_5) *P. fluorescens* as ST @ 10.0 ml kg^{-1} of seeds +SRD @ 3.0 l ha^{-1} of seeds recorded minimum sheath blight incidence (13.33 %) at harvest, which was better than carbendazim treatment. This was followed by T_4 and T_3. Maximum sheath blight disease incidence was observed with untreated control. The results of the present study is in agreement with Patil *et al*. (2003) who opined that the biological control agents were more effective and economical when applied using combination of delivery systems. Further, combination of seed treatment and seedling root dip of *P. fluorescens* would have ensured adequate population of the antagonists in the rhizosphere. Commercially available antagonistic microbes, mostly belonging to the genera *Pseudomonas*, can reduce the damage by direct effects on the pathogens (mycoparasitism, antibiosis, competition for iron) or by improving plant immunity (induced resistance, IR) (Singh *et al*., 2005). Such multiplicity of mechanisms would have contributed to the suppression of pathogen which resulted in reduced incidence of sheath blight disease.

Effect of combination of seed treatment plus seedling root dip with *P. fluorescens* on the biometrics of rice var. BPT 5204 (pot culture) : Treatment with *P. fluorescens* as ST @ 10.0 ml Kg^{-1} of seeds +SRD @ 3.0 l ha^{-1} recorded maximum growth parameters of plant height (25.52), germination (94.66%), No. of productive tillers of (16.99) , grain yield and straw yield were 48.23 and 90.12 g per^{-1} pot. This was followed by the treatment with *P. fluorescens* as ST @ 10.0 ml kg^{-1} of seeds +SRD @ 2.5 l ha^{-1}. The dosage of biocontrol agents at 2.5 l ha^{-1} of soil was proved to be insufficient as it

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

Tr. No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	Seed treatment @ 2.5 ml Kg^{-1} of seeds	90.79(72.33)	5.43	7.94	1213.86
2	Seed treatment @ 5.0 ml Kg^{-1} of seeds	91.55(73.10)	6.78	8.23	1374.16
3	Seed treatment @ 7.5 ml Kg^{-1} of seeds	94.76(76.76)	7.12	9.81	1604.28
4	Seed treatment @ 10.0 ml Kg^{-1} of seeds	95.79(78.15)	7.48	10.12	1685.90
5.	Carbendazim 50 % WP as ST @ 2.0 g Kg^{-1} of seeds	94.12 (75.96)	7.00	9.00	1505.92
6.	Control	58.65(49.98)	4.50	3.20	451.60
	SEdCD (p=0.05)	1.2352.721	1.209 2.684	0.682 1.583	1.019 2.246

Table 4 : Effect of combination of seed treatment plus seedling root dip with *P.fluorescens* for the management of *R. solani* causing sheath blight of paddy

Tr. No.	Treatments	Sheath blight incidence (%)			Per cent decrease over control		
		60 DAT	90 DAT	At harvest	60 DAT	90 DAT	At harvest
1.	*P.fluorescens* as ST@ 2.5 ml Kg^{-1} of seeds+SRD @1.0 lit ha^{-1}	18.12 (25.19)	20.69 (27.05)	21.33 (27.50)	40.70	50.91	60.25
2.	*P.fluorescens* as ST@ 5 ml Kg^{-1} of seeds +SRD @1.5 l ha^{-1}	16.66 (24.08)	19.78 (26.40)	20.82 (27.14)	45.48	53.07	61.20
3.	*P.fluorescens* as ST @ 7.5 ml Kg^{-1} of seeds +SRD @2.0 l ha^{-1}	15.28 (23.01)	17.10 (24.42)	18.23 (25.27)	50.00	59.43	66.03
4.	*P.fluorescens* as ST @ 10.0 ml Kg^{-1} of seeds +SRD @ 2.5 l ha^{-1}	11.90 (20.17)	14.18 (22.12)	15.76 (23.39)	61.06	66.35	70.63
5.	*P.fluorescens* as ST @ 10.0 ml Kg^{-1} of seeds +SRD @ 3.0 l ha^{-1}	10.12 (18.54)	11.23 (19.57)	13.33 (21.41)	66.88	73.35	75.16
6.	Carbendazim 50 % WP @ 2.0 g Kg^{-1} of seeds	13.58 (21.62)	14.00 (21.97)	16.52 (23.98)	55.56	66.78	69.21
7.	Control	30.56 (33.56)	42.15 (40.48)	53.67 (47.10)	_	_	_
	SEdCD (p=0.05)	0.633 1.394	0.805 1.773	0.593 1.306	0.244 0.538	0.382 0.842	0.462 1.018

Table 5 : Effect of combination of seed treatment plus seedling root dip with *P.fluorescens* on the biometrics of rice var. BPT 5204 (pot culture)

Tr. No.	Treatments	Germination (%)	Plant height	No. of productive tillers	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})
1	*P.fluorescens* as ST@ 2.5 ml kg^{-1} of seeds +SRD @1.0 l ha^{-1}	88.41(70.09)	16.39	10.26	39.12	83.69
2	*P.fluorescens* as ST@ 5 ml kg^{-1} of seeds +SRD @1.5 l ha^{-1}	90.55(72.09)	19.66	13.23	41.66	84.23
3	*P.fluorescens* as ST @ 7.5 ml kg^{-1} of seeds +SRD @2.0 l ha^{-1}	93.74(75.51)	24.33	15.90	44.33	87.87
4	*P.fluorescens* as ST @ 10.0 ml kg^{-1} of seeds +SRD @ 2.5 l ha^{-1}	94.00(75.82)	24.52	16.43	46.75	89.23
5	*P.fluorescens* as ST @ 10.0 ml kg^{-1} of seeds +SRD @ 3.0 l ha^{-1}	94.66(75.82)	25.52	16.99	48.23	90.12
6	Carbendazim 50 % WP @ 2.0 g kg^{-1} of seeds	93.12(74.79)	20.79	14.20	41.55	84.98
7	Control	60.65(51.14)	11.75	8.22	18.32	38.54
	SEdCD (p=0.05)	0.1960.433	0.4350.958	0.2650.585	0.3730.822	0.6781.476

recorded higher level of disease incidence with all the antagonistic treatment (Table 5). It can be assumed that various growth promoting substances produced by *P. fluorescens* would have contributed significantly to the growth promotion and yield enhancement observed in the present study.

The use of *P. fluorescens* for the management of soil borne pathogen like *R. solani* without chemical pesticides, as demonstrated in this study will be of interest to the growing organic crop industry, where the product is to be certified as organic. In addition to disease control, the plant growth promotion provided by *P. fluorescens* adds another advantage over the use of fungicides in disease management strategies.

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