Journal of Tropical Agriculture 61(2): 157-166, 2023

Efficacy of *Pseudomonas fluorescens* and *Rhizophora apiculata* against rice bacterial leaf blight

L. Vengadeshkumar *[1] and T. Meera [2]

[1] *Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India*
[2] *Department of Plant Pathology, School of Agriculture, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai 600 117, Tamil Nadu, India*

Received 26 July 2022; received in revision form 18 June 2023; accepted on 04 December 2023

Abstract

An investigation was carried out with the aim to study the interactive effect of *Pseudomonas fluorescens* (PF_9) and *Rhizophora apiculata* for the management of bacterial leaf blight disease of rice. In both pot and field trials, application of *P. fluorescens* as seed treatment (10 ml/kg seed) and foliar spray of *R. apiculata* @ 15% at 35 and 50 DAT significantly reduced the bacterial leaf blight incidence with maximum plant height, number of productive tillers per hill, panicle length, grains per panicle of paddy and recorded on par results with that of streptomycin (100 ppm) treatment. Untreated control treatment recorded the maximum per cent disease incidence and minimum plant height, number of productive tillers per hill, panicle length as well as grains per panicle of rice.

Keywords: BLB, *Pseudomonas, Rhizophora,* Rice.

Introduction

Rice has shaped the culture, diet and economies of millions of people and accounts for up to 60 per cent of the energy intake of 3 billion Asians (Guyer et al*.,* 1998). Rice production is constrained by diseases caused by fungal, bacterial and viral pathogens. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) Ishiyama and Dye. persists as a serious problem and threat to rice production in both tropical and temperate rice growing regions of the world (Xu et al*.,* 2010). This disease is the oldest known bacterial disease of rice in Asia (Naqvi et al*.,* 2014). In India, various workers have reported a yield loss up to 80 per cent due to this disease depending on the variety, severity and stage of infection (Shivalingaiah and Umesha, 2011; Basso et al*.,* 2011; Shivalingaiah et al*.,* 2012). Use of antibiotics and organic compounds such as cow dung supernatant was successfully evaluated for the control of bacterial leaf blight of rice (Mariappan et al*.,* 1990). Biological control of plant pathogens is a potential non-chemical, cheap, effective and eco-friendly method for the management of crop diseases (Harman, 1991). Among the biological methods, the use of Plant Growth-Promoting Rhizobacteria (PGPR) is known to be a potential alternative for synthetic pesticide in plant disease management with beneficial effects on plants due to faster multiplication and higher rhizosphere competence (Ali et al*.,* 2010). Their applicability as biocontrol agents has drawn wide attention because of the production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Khabbaz and Abbasi, 2014). The effectiveness of *P. fluorescens* in the control of bacterial diseases of various crops *viz*., carrot soft rot (Kloepper et al*.,*

* Author for correspondences: Phone: 8667806189, Email: vengadpragathi@gmail.com

1980), bacterial wilt in banana, cotton bacterial blight (Salaheddin et al., 2010) and sugar beet wilt (Jorjani et al., 2012) including rice bacterial leaf blight has also been reported (Jeyalakshmi et al., 2010; Suryadi et al., 2013; Jambhulkar and Sharma, 2014).

Many plants produce secondary metabolites which possess antimicrobial properties against pathogenic bacteria. These natural products are globally referred to as "botanical pesticides" (Gurjar et al., 2012). Among the various plant resources, mangrove plants are biochemically unique, producing a wide array of novel natural products/ bioactive compounds. A number of mangroves contain substances which show biological activities such as antiviral, antibacterial and antifungal properties (Bandaranayake, 2002). The use of plant extracts for disease management is not limited and presently gaining worldwide importance and acceptance. It has been reported that the antimicrobial activity of *Rhizophora apiculata* may be due to the presence of compounds like tannin (Lim et al., 2006) and gallic acid (Lim et al., 2011).

Pyroligneous acid (by product from *R. apiculata*) has been reported to possess antibacterial (Chalermsan and Peerapan, 2009), antioxidant (Loo et al., 2007; Loo et al., 2008) and strong antifungal activity against several plant pathogenic fungi (Oramahi and Yoshimura, 2013). Several researchers have reported the antibacterial activity of pyroligneous acid against several pathogenic bacteria (Yodthong and Niamsa, 2009; Ma et al., 2011). Also, Mahalakshmi (2019) reported that foliar application of leaf extract of *R. apiculata* @ 15% concentration significantly reduced the incidence of tomato early blight disease. Besides, *Rhizophora* spp., many plant species have been reported to have antibacterial activity and this property can be utilized for the management of bacterial diseases (Narasimhan et al., 1995) including Xoo (Khan et al., 2000; Jabeen et al., 2011; Velusamy et al., 2013; Samanta et al., 2014; Soosairaj et al., 2015). The present study was undertaken to identify an integrated approach involving *P. fluorescens* (Pf_9) and *R. apiculata* to ensure maximum suppression of BLB disease and higher yield of paddy without any deleterious effect to the ecosystem.

Materials and Methods

P. fluorescens (PF_9) and *R. apiculata* used in this study was selected to test their effect against Xoo under *in vitro* condition (Vengadeshkumar and Balabaskar 2013; Vengadeshkumar et al., 2019).

A pot culture experiment was conducted to test the efficacy of combined application of *P. fluorescens* (Pf_9) and *R. apiculata* for assessing their influence on the incidence of BLB of rice. The BLB susceptible variety BPT 5204 was used for the study. The plants were given artificial inoculation by leaf clipping method (Kauffman et al., 1973). The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. The experiment was conducted in a completely randomized design with three replications for each treatment and an inoculated control. The antibiotic streptomycin @ 100ppm was the chemical check and the standard agronomic practices as recommended by the State Agricultural Department, Government of Tamilnadu were followed. Treatments were given as per the schedule as detailed below and observations on lesion length, number of discoloured and chaffy grains, BLB disease incidence (PDI) and biometrics *viz*., plant height, number of productive tillers per hill, panicle length, grains per panicle and yield (g) per pot were recorded at harvest following standard procedures.

Treatment schedule

T_1 : Seed treatment with *P. fluorescens* (Pf_9) @10ml/ kg of seed
T_2 : Foliar spray with *P. fluorescens* (Pf_9) @ 2.0%
T_3 : T_1+T_2
T_4 : Foliar spray with leaf extract of *R.*

apiculata@15% at 35 DAT

T_5 : Foliar spray with leaf extract of *R. apiculata*@15% at 35 & 50 DAT

T_6 : T_1+T_4

T_7 : T_1+T_5

T_8 : Streptomycin @ 100 ppm as foliar spray at 35 & 50 DAT

T_9 : Un inoculated control

Separate field study was conducted to test the efficacy of seed treatment and foliar application using *P. fluorescens* (Pf_9) and foliar application *R. apiculata* (15%) for assessing their influence on the incidence of BLB of rice. The same combination of treatments tested in the pot culture experiments was evaluated in field experiment conducted during late samba season of 2013 and 2014 at Sembanarkovil, Nagapattinam district, Tamil Nadu. The BLB susceptible variety BPT 5204 was used for the study. The experiment was conducted in a randomized block design with three replications for each treatment with a suitable control. The fertilizer application was done following the blanket schedule of N:P:K (150:50:50) as recommended by the State Agricultural Department. A plot size of 5×4 m was maintained for each treatment and the crop was raised with a spacing of 20×15 cm and all the standard agronomic practices as recommended by the State Agricultural Department were followed. Treatments were given as per the schedule. The antibiotic streptomycin sulphate @ 100ppm was used for comparison. In the field trial, the observations on per cent disease index was assessed on a randomly selected set of 25 hills per plot at the time of maturity. The rice crop was harvested at maturity, threshed, winnowed and plot wise cleaned, dried and the yield was recorded and expressed as t/ha and biometrics *viz*., plant height, number of productive tillers per hill, panicle length and grains per panicle were recorded at harvest following standard procedures.

Result and Discussions

The intensive field survey has been made in delta districts of Tamilnadu for the incidence of rice BLB disease. Meanwhile, native soil sample has been collected and used to isolate the native *P. fluorescens* isolates undergoes various *in vitro* studies to assess their antagonistic potential against Xoo_2. Among the isolates, Pf_9 produced the maximum inhibition zone (12.32mm) under agar well method. Further, Pf_9 at 40 per cent concentration was found to be most effective in reducing the growth of Xoo_2 with maximum inhibition zone (9.21 mm) accounting for the highest activity index (0.51) of the pathogen over control under disc diffusion method. Likewise, isolate Pf_9 at 40 per cent concentration showed significantly the highest reduction in the growth of the pathogen with the least number of colonies (1.14×10^{-5}cfu/ml) and accounted for 90.96 per cent inhibition of Xoo_2 over control under pour plate method.

Among the different levels of *P. fluorescens* tested, the dosage level @10ml/kg of seed recorded the minimum BLB incidence (31.33%). The same treatment also recorded the maximum number of tillers per hill (12.53), plant height (85.73cm) and yield (36.41 g/pot) under pot culture experiment. Further, isolate Pf_9 was tested with different dosage level by foliar application against the disease.

Among the different dosages, *P. fluorescens* @ 3.5 lit/ha, recorded the maximum no. of tillers (10.33), plant height (84.53 cm) and yield (34.50 g/pot) of rice. However, the dosage level of *P. fluorescens* @ 2.5 lit/ha has also recorded statistically similar values on the biometrics of rice with that of the dosage level of 3.0 lit/ha. Hence, the dosage level 2.5lit/ha was used for further studies.

Among the selected plant extracts tested against Xoo_2 at different concentrations, methanol extract of *R. apiculata* at 15% concentration recorded highest inhibition zone of 14.44mm which accounted highest activity index (0.80) over control. Further, foliar spray of *R. apiculata* extract @ 15% at 35+50 DAT recorded maximum reduction of lesion length (3.21cm) and per cent disease index (23.24%) which accounted for 63.82 per cent

Table 1: Effect of *P. fluorescens* (Pf_9) plus *R. apiculata* on BLB incidence of paddy variety BPT 5204 (Pot culture)

Tt. No	Treatments	No. of chaffy/ ill filled grains per panicle	Lesion length (cm)	Per cent disease index	Disease reduction (%)
T_1	Seed treatment with *P. fluorescens* @10ml/kg	15.44[f]	11.34[f]	41.11[f]	22.88
T_2	Foliar spray with *P. fluorescens* @ 2.0% at 35 & 50 DAT	14.27[e]	11.26[e]	34.44[e]	35.40
T_3	T_1+ T_2	9.12[b]	5.23[b]	21.38[b]	59.89
T_4	Foliar spray with *R. apiculata* @15% at 35 DAT	16.50[g]	8.34[g]	43.52[g]	18.36
T_5	Foliar spray with *R. apiculata* @15% at 35 & 50 DAT	12.53[d]	9.53[d]	24.63[d]	53.79
T_6	T_1+T_4	10.44[c]	7.62[c]	21.61[c]	59.46
T_7	T_1+T_5	7.83[a]	2.24[a]	16.72[a]	68.63
T_8	Streptomycin @ 100 ppm as foliar spray at 35&50 DAT	7.48[a]	1.31[a]	16.55[a]	68.95
T_9	Inoculated control	34.37[h]	13.24[h]	53.31[h]	0.0
	CD (0.05)	1.12	0.525	2.21	3.61

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

reduction of BLB incidence over control under pot culture experiment.

In pot culture experiments, the treatment T_7 with application of *P. fluorescens* (Pf_9) as seed treatment (@ 10 ml/kg of seed) and *R. apiculata* as foliar spray (15% at 35 and 50 DAT) significantly reduced the BLB incidence to the minimum (16.72%) with 68.63 per cent disease reduction over control, reduced the number of chaffy and ill filled grains per panicle (7.83) and lesion length (2.24cm) to the minimum and was at par with that of streptomycin (100 ppm) treatment (Table 1). This was followed by T_3 (Seed treatment with *P. fluorescens* @10ml/ kg seed and foliar spray with *P. fluorescens* @ 2.0% at 35 and 50 DAT) with 21.38 per cent disease incidence, 9.12 number of chaffy and ill filled grains per panicle and a lesion length of 5.23 cm. The test antibiotic Streptomycin (100 ppm) as foliar spray recorded 16.55 per cent BLB incidence, 7.48 numbers of chaffy and ill filled grains per panicle and a lesion length of 1.31cm while, the uninoculated control recorded the maximum disease incidence (53.31%), maximum number of chaffy and ill filled grains per panicle (34.37) and maximum lesion length (13.24 cm).

The data, on the effect of different treatments on the biometrics of rice crop are presented in table 2. Generally, all the treatments significantly increased the plant growth and yield attributes of rice when compared to control. Among the treatments, T_7 recorded maximum plant height (84.42cm), productive tillers (14.02), length of panicle (17.93cm), grains per panicle (105.20) and maximum yield of rice (36.35g/pot). This was

Table 2: Effect of *P. fluorescens* (Pf_9) plus *R. apiculata* on the biometrics of paddy variety BPT 5204 (Pot culture)

Tt. No	Treatments	Plant height at harvest (cm) DAS	No. of productive tillers/hill	Panicle length (cm)	Grains/ panicle	Yield (g/pot)
T_1	Seed treatment with *P. fluorescens* @10ml/kg	82.14[f]	9.42[f]	13.94[f]	90.41[f]	32.42[f]
T_2	Foliar spray with *P. fluorescens* @ 2.0% at 35 & 50 DAT	82.74[e]	10.33[e]	14.38[e]	94.50[e]	33.72[e]
T_3	T_1+ T_2	84.10[b]	13.36[b]	17.61[b]	102.11[b]	35.58[b]
T_4	Foliar spray with *R. apiculata* @15% at 35 DAT	80.11[g]	8.62[h]	13.50[h]	75.54[h]	34.52[h]
T_5	Foliar spray with *R. apiculata* @15% at 35 & 50 DAT	83.37[d]	12.22[d]	16.93[d]	97.50[d]	33.86[d]
T_6	T_1+T_4	83.98[c]	13.12[c]	17.50[c]	100.30[c]	34.93[c]
T_7	T_1+T_5	84.42[a]	14.02[a]	17.93[a]	105.20[a]	36.35[a]
T_8	Streptomycin @ 100 ppm as foliar spray at 35&50 DAT	81.50[f]	9.00[f]	13.70[f]	86.40[f]	31.63[f]
T_9	Inoculated control	77.13[h]	6.21[h]	11.31[h]	69.32[h]	25.83[h]
	CD (0.05)	4.62	0.46	1.16	4.97	1.93

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

followed by the treatments T_3 and T_6 in the decreasing order of merit. The antibiotic treatment recorded a plant height of 81.50cm, productive tillers (9.00), panicle length (13.70 cm), grains per panicle (86.40) and yield (31.63g/pot). The untreated control recorded the minimum plant biometric values of rice. Also, the treatments considerably increased the activity of defense enzymes *viz*., peroxidase (2.98), polyphenol oxidase (2.06), phenyl alanine ammonia lyase (19.01), catalase (2.01), chitinase activities (5.12), â-1,3 glucanase activity (54.56) phenolics activity (21.84) on 9th day and thereafter a gradual decrease was observed.

In this study two field trials were conducted, the treatment T_7 with *P. fluorescens* @ 10ml/kg as seed treatment and foliar spray with leaf extract of *R. apiculata* @ 15% at 35 and 50 DAT produced results which were on par with that of the test antibiotic S treptomycin (100ppm) in respect of reducing disease intensity and increasing the biometrics of paddy in both the trials (Tables3&4).The results revealed that the treatment T_7 resulted in minimum

Table 3: Effect of *P. fluorescens* plus *R. apiculata* on BLB incidence of paddy variety (BPT 5204) (Field Trial 1 and 2)

Tt. No.	Treatments	Lesion length (cm)		Per cent disease index		Per cent disease reduction	
		1	2	1	2	1	2
T_1	Seed treatment with *P. fluorescens* @10ml/kg	10.43^{f}	10.54^{f}	13.33^{f}	14.24^{f}	62.16	60.59
T_2	Foliar spray with *P. fluorescens* @ 2.0% at 35 & 50 DAT	10.35^{e}	10.46^{e}	13.14^{e}	14.15^{e}	62.70	60.84
T_3	T_1+ T_2	8.62^{b}	8.73^{b}	11.36^{b}	12.27^{b}	67.75	66.04
T_4	Foliar spray with *R. apiculata* @15% at 35 DAT	7.43^{g}	7.54^{g}	13.54^{g}	14.45^{g}	61.56	60.01
T_5	Foliar spray with *R. apiculata* @15% at 35 & 50 DAT	6.71^{d}	6.82^{d}	12.12^{d}	13.13^{d}	65.59	63.66
T_6	T_1+T_4	4.32^{c}	4.43^{c}	11.43^{c}	12.34^{c}	67.55	65.85
T_7	T_1+T_5	2.33^{a}	2.44^{a}	11.23^{a}	12.14^{a}	68.12	66.40
T_8	Streptomycin @ 100 ppm as foliar spray at 35&50 DAT	1.52^{a}	1.63^{a}	11.14^{a}	12.15^{a}	68.37	66.38
T_9	Uninoculated control	11.54^{h}	11.65^{h}	35.23^{h}	36.14^{h}	0.0	0.0
	CD (0.05)	0.52	0.54	0.57	0.59	3.46	3.14

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 4: Effect of *P. fluorescens* plus *R. apiculata* on the plant biometrics of paddy variety-BPT 5204 (Field Trial 1&2)

Tt. No	Treatment	Plant height at harvest (cm)		No. of productive tillers/hill		No. of grains/		Panicle length panicle		Yield t/ha (cm)	
		1	2	1	2	1	2	1	2	1	2
T_1	Seed treatment with *P. fluorescens* @10ml/kg	81.64^{f}	80.73^{f}	12.43^{f}	11.52^{f}	184.57^{f}	180.66^{f}	16.52^{f}	15.63^{f}	6.32^{f}	6.13^{f}
T_2	Foliar spray with *P. fluorescens* @ 2.0% at 35 & 50 DAT	82.24^{e}	81.33^{e}	12.72^{e}	11.81^{e}	191.34^{e}	187.43^{e}	16.83^{e}	15.84^{e}	6.77^{e}	6.58^{e}
T_3	T_1+ T_2	82.83^{b}	81.82^{b}	14.17^{b}	13.26^{b}	198.28^{b}	194.37^{b}	18.75^{b}	17.86^{b}	7.68^{b}	7.48^{b}
T_4	Foliar spray with *R. apiculata* @15% at 35 DAT	73.43^{h}	72.52^{h}	11.64^{h}	10.73^{h}	161.31^{h}	157.41^{h}	15.31^{g}	14.42^{g}	5.42^{h}	5.23^{h}
T_5	Foliar spray with *R. apiculata* @15% at 35 & 50 DAT	82.21^{d}	81.33^{d}	13.47^{d}	12.56^{d}	193.76^{d}	189.85^{d}	17.54^{d}	16.65^{d}	7.29^{d}	7.11^{d}
T_6	T_1+T_4	82.61^{c}	81.72^{c}	14.11^{c}	13.21^{c}	196.12^{c}	192.21^{c}	18.43^{c}	17.54^{c}	7.52^{c}	7.33^{c}
T_7	T_1+T_5	83.12^{a}	82.23^{a}	14.76^{a}	13.85^{a}	204.36^{a}	200.45^{a}	20.12^{a}	19.23^{a}	7.74^{a}	7.55^{a}
T_8	Streptomycin @ 100 ppm as foliar spray at 35&50 DAT	79.51^{g}	78.64^{g}	12.21^{g}	11.32^{g}	181.22^{g}	177.31^{g}	16.41^{f}	15.52^{f}	6.22^{g}	6.03^{g}
T_9	Uninoculated control	67.21^{i}	66.35^{i}	11.22^{i}	10.31^{i}	129.12^{i}	125.22^{i}	12.38^{h}	11.47^{h}	4.34^{i}	4.15^{i}
	CD (0.05)	4.56	4.14	0.63	0.58	9.26	8.82	0.61	0.56	0.42	0.41

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

BLB disease incidence (11.23 and 12.14%), maximum per cent disease reduction (68.12 and 66.40%), the minimum lesion length (2.33 and 2.44 cm), maximum plant height (83.12 and 82.23cm), number of productive tillers per hill (14.76 and 13.85), maximum length of panicle (20.12 and 19.232cm) and maximum number of grains per panicle (204.36 and 200.45) and was on par with streptomycin (100ppm) which recorded 68.37 and 66.38per cent reduction of BLB incidence over control. The untreated control treatment recorded the maximum disease incidence (35.23 and 36.14%), lesion length (11.54 and 11.65cm), minimum plant height (67.21 and 66.35cm), minimum tillers (11.22 and 10.31), minimum length of panicle (12.38 and 11.47), minimum number of grains per panicle (129.12 and 125.22) and least yield (4.34 and 4.15 t/ha) in both the trials.

In both pot and field trials, the application of *P. fluorescens* as seed treatment @ 10ml/kg of seed plus foliar application of *R. apiculata* @15% at 35 and 50 DAT recorded minimum BLB incidence of rice during both the years. The results indicated that different plant colonization pattern and different mechanism of disease suppression elicited by the combination of *P. fluorescens* and the leaf extract of *R. apiculata* might have offered greater protection to the rice crop against the attack of Xoo causing BLB disease. Similar to the present observations a positive correlation was observed by Akila et al. (2011) on the suppression of *Fusarium* wilt of banana in the treatment with combination of botanicals and bacterial antagonist such as *P. fluorescens* and *B. subtilis*. Uppal et al. (2013) also opined that application of bacterial antagonist *P. fluorescens* (DF37) and Canada milk vetch extract effectively reduced the potato *Verticillium* wilt.

Besides bacterial diseases, Vimala and Suriachandraselvan (2008) recorded minimum powdery mildew disease and increased yield of bhendi in the treatment combination with NSKE 5% + *P. fluorescens* I_{18} 0.2%. Latha et al. (2009) reported that the treatment combination consisting of PGPR mixture + zimmu as seed treatment and foliar spray was superior in reducing the early blight of tomato. Muthukumar et al. (2010) reported that the combination of *T. viride* + *P. fluorescens* + zimmu leaf extract showed the highest inhibition of *P. aphanidermatum*, causing chilli damping-off. All these earlier reports corroborate and add value to the present findings. Strains of *Pseudomonas* spp. have been shown to produce wide array of antibiotics which includes DAPG, HCN, kanosamine, phenazine, pyoluteorin and pyrrolnitrin as well as several other uncharacterized moieties (Keel and Defago, 1997; Whipps, 1997; Thrane et al*.,* 1999). Besides, *P. fluorescens* could have also contributed to the disease suppression through the induction of ISR. The PGPR mediated ISR against pests and diseases in several crops have been demonstrated under field conditions (Nandakumar et al*.,* 2001a, b; Ramamoorthy and Samiyappan, 2001).

Combination of different methods of application was found to be more effective in disease management than a single method of application (Nandakumar et al*.,* 2001b). Thus, it is quite reasonable to assume that *P. fluorescens* applied to the seed could have moved on to foliage and offered protection to the crop from the primary infections of the pathogen. Likewise, *R. apiculata* sprayed on to the foliage could have created a toxic barrier on the plant surface and protected the crop from secondary infections. Also, the antibacterial compounds produced by both *P. fluorescens* and *R. apiculata* could have exerted a synergism and suppressed Xoo.

In the present study, besides disease suppression, the treatment T_7 consisting of *P. fluorescens* as seed treatment @ 10ml/kg of seed plus foliar application of *R. apiculata* @15% conc. at 35 and 50 DAT recorded maximum plant growth and yield of rice during both years. As observed in the present study, increased plant growth and yield was observed due to treatment with NSKE 5% + *P. fluorescens* I_{18} 0.2% in bhendi (Vimala and Suriachandraselvan, 2008),

PGPR mixture + zimmu in tomato (Latha et al., 2009) and *T. viride* + *P. fluorescens* + zimmu leaf extract in chilli (Muthukumar et al., 2010) were also reported. These earlier reports are in line with the present findings. Mahalakshmi et al. (2020) reported that foliar application of *R. apiculata* significantly reduced the incidence of early blight of tomato. Mahalakshnli et al. (2020b) proved that extract of *R. apiculata* as a potential bio-inducer of early blight disease resistance in tomato. Vengadeshkumar et al. (2023) reported that foliar application of *R. apiculalu* and *Ampellomyces quisyualis* significantly reduced the, incidence of powdery mildew of blackgram.Mary Sharmila et al. (2021) observed that the antifungal activity of *R. apiculalu* against rigsheath blight disease. Vengadeshkumar et al. (202 1) found that the combination of *R. apiculata* and P. flourescens are as a best bio inducer in rice BLB management.

The ability of *P. fluorescens* strains to increase plant growth and yield in various crops has been well established (Vivekananthan et al., 2004; Saravanakumar and Samiyappan, 2007). In general, PGPR can promote plant growth mainly by following means; (1) producing ACC deaminase to reduce the level of ethylene in the roots of developing plants (Dey et al., 2004) (2) producing plant growth regulators like gibberellic acid (Narula et al., 2006), cytokinins (Castro et al., 2009), ethylene (Saleem et al., 2007) and indole acetic acid (IAA) (Mishra et al., 2011). In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, phosphate solubilization and production of siderophores that chelate iron and make it available to the plant roots (Lalande et al., 1989; Bowen and Rovira, 1999). Such mechanisms could be attributed as the reason for the enhanced biometric of rice observed in the present study.

Thus, the results of the present study have clearly revealed that integration of *P. fluorescens* along with plant extract like *R. apiculata* would have exerted a synergism and also different mechanisms of disease control which certainly enhanced greater disease suppression, enhanced plant growth and yield of rice and improved the consistency of biological control under varied climatic conditions.

Conclusion

The susceptibility of varieties, resistance to antibiotics and possible pollution to the environment have created concerns worldwide and also necessitated scientists to look for alternative eco friendly ways of managing BLB of rice. Use of antibacterial antibiotics and pseudomonads with limited success has been the practice followed for the management of BLB of rice. But the results of the present study have proved that application of *P. fluorescens* as seed treatment along with leaf extract of *R. apiculata* as foliar spray exhibited a general trend towards maximum suppression of BLB of rice caused by Xoo. The combined action of different mechanisms exerted by *P. fluorescens* and *R. apiculata* might be the reason for the enhanced suppression of BLB disease and improved consistency of biological control under varied climatic conditions in rice. The treatment with *R. apiculata* and *P. fluorescens* showed no phytotoxic symptoms and was safe to the crop and environment. In addition to disease control, the plant growth promotion observed in the study adds another advantage over the use of fungicides in disease management strategies. Besides, the treatment combination involving *P. fluorescens* and plant extract (*R. apiculata*) 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.

Acknowledgement

Sincere thanks to Directorate of Research, Annamalai University, Tamilnadu, India.

Reference

Akila, R., Rajendran, L., Harish, S., Saveetha, K., Raguchander, T. and Samiyappan, R. 2011.

Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f.sp. *cubense* causing *Fusarium* wilt in banana. *Biol.Cont.,* 57: 175-183.

Ali, A., Khan, M.H., Bano, R., Rashid, H., Raja, N. I. and Chaudhry, Z. 2010. Screening of Pakistani rice (*Oryzae sativa*) cultivars against *Xanthomonas oryza* pv. *oryzae. Pak. J. Bot*., 41(5): 2595-2604.

Bandaranayake, W.M. 2002. *Wetlands Ecology and Management*. 10:421 – 52.

Basso, A.A, Onasanya, S., Issaka, A.Y. and Sido, A. 2011. Bacterial leaf blight of rice in Nigeria Pathological diversity of isolates collected on irrigated lands. *J. Applied. Biosci.,*38: 2551-2563.

Bowen G.D. and Rovira A.D., 1999. The rhizosphere and its management to improve plant growth. Adv. in Agron. 66.

Castro, R.O., Cornejo, H.A.C., Rodriguez, L.M. and Bucio, J.L. 2009. The role of microbial signals in plant growth and development. *Pl. Signal Behav.,*4(8): 701 – 712.

Chalermsan, Y. and Peerapan, S. 2009. Wood vinegar Byproduct from rural charcoal kiln and its role in plant protection. *Asian J. Food Agri. Ind.,* Special Issue, 189-195.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan, S.M. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L) by application of plant growth-promoting rhizobacteria. *Microbiol. Res.* 159: 371-394.

Gurjar M.S., Shahidali, Masood Akhtar. And Kangabam Suraj Singh. 2012. Efficacy of plant extracts in plant disease management. *Agricult. Sci.,* 3: 425-433.

Guyer, D., Tuttle, A. S., Johnson, M., potter, S., Gorlach, J., Forff, S. and Land, C. L. 1998. Activation of latent transgenes in *Arabidopsis* using a hybrid transcription factor. *Genetics*, 149: 633-639

Harman, G.E. 1991. Seed treatments for biological control of plant diseases. *Crop Prot.*, 10: 166-171.

Jabeen, R., Ashraf, M., Ahmad, I. and Iftikhar, T. 2011. Purification and bioassays of bioactive fraction from *Curcuma longa* against *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. *Pak. J. Bot*., 43(2): 1335 – 1342.

Jambhulkar, P. P. andSharma, P. 2014. Development of bioformulation and delivery system of *Pseudomonas fluorescens* against bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*).*J. Environ. Biol.,* 35(5): 843 – 849.

Jeyalakshmi, C., Madhiazhagan, K. and Rettinassababady, C. 2010. Effect of different methods of application of *Pseudomonas fluorescens* against bacterial leaf blight under direct sown rice. *J. of Biopesti.*,3(2): 487 – 488.

Jorjani, M., Heydari, A., Zamanizadeh, H., Rezaee, S., Naraghi L. and Zamzami, P. 2012. Controlling sugar beet mortality disease by application of new bioformulations. *J. Pl. Prot. Res.,*52: 303-307.

Kauffman, H.E., Reddy, A.P.K., Hsieh, S.P.Y. and Merca, S.D. 1973. An improved technique for evaluating resistance in rice varieties to *Xanthomonas oryzae*. *Pl. Dis. Report*., 57: 537-541.

Keel, C. and Defago, G. 1997. Interactions between beneficial soil bacteria and root pathogens: mechanisms and ecological impact. In: Gange A. C, Brown V. K, (Eds.) *Multitrophic interactions in terrestrial system*. Oxford: Blackwell science, 27-47.

Khabbaz, S.E. and Abbasi, P.A. 2014. Isolation, characterization and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. *Can. J. Microbiol.,*60 (1): 25-33.

Khan, M.A, Rashid, A. and Chohan, R.A. 2000. Development of bio formulations of antagonistic bacteria for the management of damping-off in chilli. *Pak. J. Agrl Sci*., 37: 3 – 4.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. 1980. Enhanced plant growth by siderophore produced by plant growth promoting rhizobacteria. *Nature,* 286: 885-886.

Lalande, R., Bissonnette, N., Coutlee, D. and Antoun, H. 1989. Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. *Plant Soil,* 115: 7-11.

Latha, G.S., Reddy, C.S., Krishnaveni, D., Sundaram, R.M., Srinivasa Prasad, M., Ram, T., Muralidharan, K. and Viraktamath, B.C. 2009. Bacterial blight of rice and its management. Technical Bulletin No. 41. Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad-500 030, A.P., India. 37.

Lim, S.H., Darah, I. and Jain, K. 2006. Antimicrobial activity of tannin extracted from *Rhizophoraapiculata*Blume barks. *J. Tropi. Forest Sci*., 18(1): 59-65.

Lim, S.H., Darah, I., Jain, K. and Suraya, S. 2011. Gallic acid: An anticandidal compound in hydrolysable tannin extracted from the barks of *Rhizophora apiculata* Blume *J. Appl. Pharma. Sci.,*1(6): 75-79.

Loo A., Jain, K. and Darah I. 2007. Antioxidant and

radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. *Food Chem.,* 104(1): 300 – 307.

Loo A.Y., Jain K. and Darah, I. 2008. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. *Food Chem.,*107:1151 – 1160.

Ma, X.H., Wei, Q., Zhang, S.S., Shi, L. and Zhao, Z. 2011. Isolation and bioactivities of organic acids and phenols from walnut shell pyrolgneous acid from *Rosmarinus offininalis* leaves. *J. Anal. Appl. Pyrol.,*91(2): 33 – 343.

Mahalakshmi, G. 2019.Use of mangrove leaf extracts on the management of tomato early blight disease. M.Sc., Thesis, Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Mahalakshmi, G., Vengadeshkumar, L., Sanjaygandhi, S., Rajamohan, K., Udhayakumar, R. and Mary Sharmila, A. M. 2020a. Management of tomato early blight disease using mangrove leaf extract. Plant Aichives., 20(1):3 130-3 134.

Mahalakshmi, G., Vengadeshkumar, L., Rajamohan, K., Sanjaygandhi, S. and Sharmila, A.M. 2020b. Leaf extract of *Rhizophoru apiculalcr* as a potential bio-inducer of early blight disease resistance in tomato plant Novel Res. in Microbiol. J. 4(2): 714-724.

Mariappan, V., Durairaj, F., Natarajan, S. and Jeyarajan, R. 1990. Antibiotic fungicide combination in the control of bacterial leaf blight of rice. Proc. *Nat. Symp. Phytobact*. Univ., Madras, India. 91-93.

Mary Sharmila, A., Vengadeshkumar, L., Sanjaygandhi, S. and Meera, T. 2021. Antifungal activity of *Rhizophora apiculata* leaf extract for the management of rice sheath blight. Res. J. of Agril. Sci.12: 1153-1 157.

Mishra, D.S., A.K. Gupta, C.R. Prajapati and U.S. Singh. 2011. Combination of fungal and bacterial antagonists for management of root and stem rot disease of soybean. *Pak. J. Bot.,* 43: 2569-2574.

Muthukumar, A., Eswaran, A., Nakeeran, S. and Sangeetha, G. 2010. Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. *Crop prot.,*29:1483-1488.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchandera, T. and Samiyappan, R. 2001a. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil. Biol. Biochem*., 33: 603 – 612.

Nandakumar, R., Viswanathan, R., Babu, S., Sheela, J., Raguchander, T., Samiyappan, R., 2001b. A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. *Biocont.,*46: 493–510.

Naqvi, S.A.H., Perveen, R., Din Umer,U., Malik, O., Rehman, A., Wazeer, S. and Majid, T. 2014. Determination of antibacterial activity of various broad spectrum antibiotics against *Xanthomonas oryzae* pv. *oryzae*, a cause of bacterial leaf blight of rice. *Intl.J.Microbio.Mycol*.,2(3):12 – 19.

Narasimman, V., Selvam, R. and Marippan, V. 1995. Neem for the management of crop diseases. New Delhi: associated Publishing Co.115-21.

Narula, N., Deubel, A., Gans, W., Behl, R.K. and Merbach, W. 2006. Paranodules and colonization of wheat roots by phytohormone producing bacteria in soil. *Plant Soil Environ.,*52(3): 119-129.

Oramahi, H.A. and Yoshimura, T. 2013. Antifungal and antitermitic activities of wood vinegar from *Vitex pubescens*Vahl. *J Wood Sci*,, *Ind. Phy*. 59(1): 124 – 125.

Ramamoorthy, V. and Samiyappan, R. 2001. Induction of defense-related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici*. *J. Mycol. Pl. Pathol.*, 31:146-155.

Salaheddin, K., Valluvaparidasan, V., Ladhalakshmi, D. and Velazhahan, R. 2010. Management of bacterial blight of cotton using a mixture of *Pseudomonas fluorescens* and *Bacillus Subtilis*. *Pl. Protec. Sci*., 46 (2): 41- 50.

Saleem, M., Arshad, M., Hussain, S., and Bhatti, A.S. 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J. Ind. Microbiol. Biotechnol.,*34 (10): 635-648.

Samanta, T.T, Samanta, P. and Das, A. 2014: Isolation and characterization of *Xanthomanas oryzae* isolation from different regions of Midnapore district of West Bangal and their ecofriendly management by some medicinal plant. *Int. J.Phytomedicine*, 6: 29-42.

Saravanakumar, D. and Samiyappan, R. 2007. ACC deamynase for *Pseudomonas fluorescens* mediated saline resistance in ground nut (*Arachis hypogea*) plants. *J. Appl. Microbiol.,*102 (5): 1283-1292.

Shivalingaiah, P. and Umesha, S. 2011. Characterization of *Xanthomonas oryzae* pv. *oryzae* from major rice growing regions of Karnataka. *The Bioscan,*6(1): 5-10.

Shivalingaiah, P.,Umesha, S. and Sateesh, M.K. 2012. Molecular Detection of *Xanthomonas oryzae* pv. *oryzae* in rice seeds. *Asia and Austral Journal of Pl. Sci. and Biotech.,*6(1) : 44-47.

Soosairaj, S., Kala A., Mathiyazhagan, S. and Raja, P. 2015. Isolation and identification of *Xanthomonas oryzae* pv. *oryzae* the causal agent of rice bacterial leaf blight and its activities against of six medicinal plants. *Asian J. of Pl. Sci. and Res.,*5(6): 80-83.

Suryadi, C., Susilowati, D.N., Suryani K., Zaffan, Z.R., Nurul, H. and Rachmania Mubarik, N.R. 2013. Bioformulation of antagonistic bacterial consortium for controlling blast, sheath blight and bacterial blight diseases on rice. *Asian J. of Pl. Pathol*.,7: 92-108.

Thrane, C., Olsson, S., Nielsen, T.H. and Sorensen, J. 1999. Vital fluorescent strains for detection of stress in *Pythium ultimum* and *Rhizoctonia solani* challenged with viscosinamide from *Pseudomonas fluorescens* DR54. *Microbiol. Ecology.*,30:11-23.

Uppal, M.A., Kafizas, A. and Ewing, M.B. 2013. The room temperature formation of gold nanoparticles from the reaction of cyclohexanone and auric acid; a G. (1971). *A Dictionary of Plants Used by Man*; (1 Indian Eds. 1984), CBS Pub. and *Distr. Print Orient. Delhi,*1: 7351-7359.

Velusamy, P., Rajalakshmi, S., Pitchaimuthu, S. and Kannan, N. 2013. Photo decoloration of Brilliant Green dye using b-CD modified TiO2. *Ind. J Environ. Prot.,* 33(7): 583 – 589

Vengadeshkumar, L. and Balabaskar, P. 2013. Survey, isolation, characterization and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight disease in rice. *Ann. of pl. Prot. Sci.* 48 (2):456-59

Vengadeshkumar L, Meera T, Balabaskar P, Sanjaygandhi S, Rajamohan K, Udhayakumar R, Sudhasha, S. 2019. Antibacterial activity of *Rhizophora apiculata* leaf extract for the management of rice bacterial blight disease. Plant Pathol. J., 18: 39-46

Vengadeshkumar, L., Meera, T., Sanjaygandhi, S., Udhayakumar, R. and Rajamohan, K. 2021. *Rhizophora apiculata* and *Pseudonzonas fluorescens:* Novel Bio Inducers in rice bacterial leaf blight management. Biopestic. Int.17(2): 15 1-158.

Vengadeshkumar, L., Sanjaygandhi, S., Meera, T. and Vignesh, K. 2023. Evaluation of different treatments on the management of blackgram Powdery mildew disease caused by *Erysiphepolygoni* (De Candolle). Curr. Agril. Res. J. Vol. 11(3):890-894.

Vimala, R. and Suriachandraselvan, M. 2008. Influence of antagonistic agent, plant products and chemical agents on the powdery mildew disease of bhendi and its Product. *J. Biopest*., 1(2):130 – 133.

Vivekanandhan, R., Ravi, M., Sible, G.V., Prakasam.V. and Samiyappan, R. 2004. *Pseudomonas fluorescens* (FP7) amended with chitin bioformulation for the management of anthracnose pathogen in mango cultivar Alphonso. *Madras Agric. J.,*91(7-12): 475-482.

Whipps, J. M. 1997. Developments in the biological control of soil-borne plant pathogens. *Adv.Bot. Res.*, 26:1-134.

Xu, Y., Zhu, M.G., Zhou, J., Kuang, Y., Zhang, Y., Shang, J.X. and Wang, J. 2010. *Phytopathol.,* 158: 601-608.

Yodthong, B. and Niamsa, N. 2009. Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. *Biomass and Bioenergy*, 33(6): 994-998.