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ANTAGONISTIC ACTIVITY OF *SERRATIA MARCESCENS* AGAINST *PYRICULARIA ORYZAE*

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Abstract. Rice is an important crop, widely affected by quite a number of diseases that results in higher yield losses. Among the fungal diseases, blast incited by *Pyricularia oryzae* is a major disease. The biological method of plant disease management seems to be an alternative to chemical fungicides in managing the blast disease. A new bio control agent *viz., Serratia marcescens* appears to be an ideal agent for the control of *P. oryzae*, because it produces chitinolytic enzymes which causes degradation of the fungal cell walls, induction of plant defence reaction and certain antifungal low molecular weight molecules. A study was undertaken to investigate the effect of a new bio control agent like *S. marcescens* against *P. oryzae.* The talc based formulation of *S. marcescens* (@ 1.0, 1.5, 2.0 and 2.5 kg/ha) was sprayed on old IR 50 rice plants in fields. Out of the six-bio protectants tested, *S. marcescens* was found very effective against *P. oryzae* under *in vitro* conditions. *S. marcescens* could be isolated from shoots as well as roots emerging from the treated seeds and the plant parts from treated seeds inhibited *P. oryzae.* The antagonist *S. marcescens* survived in the phyllosphere even 80 days after spray. The results revealed that rice blast control was achieved by spraying *S. marcescens* @ 1.0 kg/ha. The increasing dose of talc-based inoculum when applied on foliage increased the phyllosphere population of *S. marcescens* and controlled rice blast. The maximum disease control was achieved when inoculum was applied at 2.5 kg/ha

Key words: Systemic movement, Rice blast, *Serratia marcescens*

INTRODUCTION

Rice, which is being cultivated for several years in the Indian country, it is not just a grain, it is the lifeline and the second most important crop next to wheat.

India is one leading producer of rice in Asia (Tony Cisse, 2005). Rice crop has been under cultivation from time immemorial, being grown under varying climatic conditions in different parts of the country. It is widely affected by quite a number of diseases caused by fungi, bacteria, viruses and mycoplasma that results in higher yield losses (Ou, 1985).

Among the fungal diseases, blast incited by *Pyricularia oryzae* Cavara is a major disease occurring in almost all the rice-growing areas of the world and is the most destructive fungal disease of rice causing yield loss up to 90 per cent (Mehrotra, 1998) despite decades of research towards its management.

The management of blast disease is done by using fungicides, growing resistant varieties, application of organic amendments, balanced nutrition, biological agents and resistance inducing chemicals. The extraordinary use of chemical fungicides resulted in environmental pollution and ill health to biotic community as a whole. Therefore, the

biological method of plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. In addition, the biological control of plant pathogens is an attractive preposition as it mimics the nature's own way of balancing the population of living organisms (Mukerji et al., 1992). However, rice being a crop under inundated conditions and the pathogen *P. oryzae* infecting the foliage, the bio-inoculant should survive in the anaerobic soil conditions. Furthermore, the organism should survive on the leaf surface because *P. oryzae* affects the foliar parts of rice. Hence, a new bio control agent *viz., Serratia marcescens* appears to be an ideal agent for the control of *P. oryzae* as it is known to survive in the rhizosphere of plants grown under inundated conditions, as well as in the phyllosphere of many plants (Akutsu et al., 1993). *Serratia marcescens* produces chitinolytic enzymes, which causes degradation of the fungal cell walls, induction of plant defence reaction and certain antifungal low molecular weight molecules (Someya et al., 2000).

Therefore, the present study was undertaken to investigate the effect of *Serratia marcescens*, the management of Paddy blast.

MATERIALS AND METHODS

In vitro screening

In vitro studies were carried out to screen the various *viz., Aspergillus clavatus, A. nidulans, A. terreus, Pencillium chrysogenum, P. citrinum, P. minioluteum* and *Serratia marcescens* against *P. oryzae.*

In vitro efficacy of certain bio control agents against *P. oryzae* [Dual culture (Dennis and Webster, 1971)].

Twenty ml of sterilized and cooled Potato Dextrose Agar (PDA) medium was poured in to each sterilized Petri plates. The mycelium (9 mm) obtained from seven days old culture of the test pathogen *P. oryzae* was placed 25 mm away from the periphery of the Petri plates and on the opposite direction, the antagonist was placed at a distance of 25 mm from the periphery. Petri dishes inoculated with the pathogen alone served as control. Three replications were maintained. The plates were incubated at 28 ± 2° C. After incubation, the mycelial growth of *P.oryzae* was recorded. The per cent inhibition of mycelial growth was calculated according to Vincent (1927).

Assessment of systemic movement of *S. marcescens* in rice plants

One kg of surface sterilized IR 50 seeds were treated with two ml culture filtrate (containing 10^9 cfu / ml) of *S. marcescens* and air dried for one h. in a laminar cabinet. After one h., the seeds were taken with a sterile forceps and plated on moistened blotter paper in Petri dishes @ 10 seeds per plate. The seeds were incubated for 10 days by moistening the plates daily with sterile water. After 10 days, the germinated seeds were taken, the roots and shoots were separated and cut in to bits of one cm using sterile blade and the population of *S. marcescens* in plant bits (2, 4, 6 and 8 cm away from seed coat) was estimated.

Ten g of shoot and root bits from the experiment above were ground individually in a sterile pestle and mortar with 10 ml of sterile dist. water. The extract was taken and serially diluted. One ml aliquots were transferred to sterile Petri dishes to which molten Nutrient agar medium was added, gently rotated and incubated at 28 ± 2° C for 48 h. The colonies were counted against a UV lamp.

One cm bits of root and shoot (2, 4, 6 and 8 cm away from seed coat) from the experiment above were taken, surface sterilized and placed on Nutrient agar medium at one end. After incubation over night, 8 mm discs of *P. oryzae* were placed just opposite the plant parts, incubated for five days and the inhibition zone was measured.

To study the survival of *S. marcescens* in the phyllosphere of rice plants, 25 ml of spore suspension (containing 10^9 cfu/ml) of the antagonist was diluted in one liter of water and sprayed onto 15 days old IR 50 rice plants grown in cement pots. Samples were taken at 0, 20, 40, 60 and 80 days after spraying and the phyllosphere survival of *S. marcescens* was assessed (Kishore et al., 1998).

From the experiment above, one g of surface sterilized leaf bits were taken in 10 ml of sterile dist. water, shaken vigorously and serial dilutions were prepared. From the dilution of 10^4, one ml aliquot was transferred into sterile Petri dishes, over which 20 ml of molten Nutrient agar medium was poured, rotated gently and incubated for 48 h. The colonies were counted under a UV lamp (Kishore et al., 1998).

From the experiments above, 10 g of surface sterilized leaf samples were taken at 0, 20, 40, 60 and 80 days after spraying. The leaf samples were ground in 10 ml sterile dist. water in a sterile pestle and mortar. From this, one ml of extract was transferred to 9 ml sterile dist. water and serial dilutions were prepared. From the final dilution of 10^4, one ml aliquot was transferred to sterile Petri dishes. Nutrient agar medium was poured over the aliquot, gently rotated and incubated at $28 \pm 2^{\circ}$ C for 48 h. The colony count was taken against the UV lamp.

Effect of different doses of talc-based *S. marcescens* treatment on the management of rice blast (Pot culture)

One kg of IR 50 seeds was taken in a cloth bag soaked in two litres of water for 24 h. and the excess water was drained. *S. marcescens*, maintained in talc based formulation was added to the seed @ 2.5, 5.0, 7.5 and 10.0 g per Kg of seeds with two per cent CMC and sown in cement plots. The pathogen was inoculated on 35^{th} day after sowing and the incidence of blast was recorded.

Effect of foliar application of talc-based inoculum of *S. marcescens* on the management of rice blast (Field experiment)

S. marcescens in talc based formulation (@ 1.0, 1.5, 2.0 and 2.5 kg/ha) was dissolved in water, allowed to settle for 2 h., filtered through muslin cloth and the filtrate was sprayed at 500 litres / ha on to 34 days old IR 50 rice plants in fields. On 35^{th} day, the pathogen was inoculated and the second spray with antagonist was given on 37^{th} day after sowing. The blast incidence and phyllosphere antagonist population were estimated on 95 days after sowing as described earlier.

RESULTS AND DISCUSSION

Efficacy of certain bio-control agents against *P. oryzae* (Dual culture)

Results of the experiments (Table 1) clearly showed that *S. marcescens* was found to be more antagonistic to *P. oryzae*. Statistically all the bio-control agents were found superior over control. *S. marcescens* recorded 68.88 per cent growth inhibition. The other bio-control agents *viz., Pencillium chrysogenum* and *Aspergillus terreus* recorded 53.33 per cent and 50.0 per cent growth inhibition with a colony diameter of 42 mm and 45 mm, respectively. The lowest growth inhibition recorded in *Aspergillus nidulans* (40.0 %).

Results clearly showed that *S. marcescens* was found to be more antagonistic to *P. oryzae*. Hence *S. marcescens* was selected for the subsequent studies as it proved to be the best bio-protectant against *P. oryzae*. Several reports are available about the antagonistic effect of *S. marcescens* against various fungal pathogens (Someya et al., 2000; Akutsu et al., 1993; Iyozumi et al., 1996; El-Tarabily et al., 2000).

Table 1

Efficacy of certain bio-control agents against *P. oryzae* (Dual culture)

Tt. No	Bio-control agents	Colony diameter (mm)	Growth inhibition (%)
1	*Aspergillus terreus*	45	50.00
2	*A. clavatus*	51	43.33
3	*A. nidulans*	54	40.00
4	*Penicillium citrinum*	50	44.44
5	*P. chrysogenum*	42	53.33
6	*Serratia mercescens*	28	68.88
7	Control	90	-
	SE	3.21	-
	CD (P=0.5)	5.84	-

Systemic movement of *S. marcescens* in rice plants

After seed treatment

When rice seeds were treated with *S. marcescens* and allowed to germinate the bacteria could be isolated from emerging shoots, as well as roots, after surface sterilization. The plant parts obtained from treated seeds showed inhibition against *P. oryzae.* The plant parts (shoot and root) obtained from a distance of 2 cm away from the seed coat recorded higher *S. marcescens* population (4×10^4, 11×10^4 g^{-1}) compared to other treatments (Table 2).

Table 2

Systemic movement of *S. marcescens* in rice plants

Tt. No	Plant part distance in cm away from seed coat	Seeds treated with *S. marcescens*		Seeds untreated	
		S. marcescens population (10^{-4})	Inhibition zone (mm)	*S.marcescens* population (10^{-4})	Inhibition zone (mm)
Shoot					
1	2	4	76.0	0	0
2	4	3	60.0	0	0
3	6	2	56.0	0	0
4	8	1	52.0	0	0
Root					
1	2	11	71.0	0	0
2	4	8	54.0	0	0
3	6	7	50.0	0	0
4	8	4	41.0	0	0
	SE	0.57	6.12	-	-
	CD (P=0.5)	0.93	10.82	-	-

After foliar spray

When *S. marcescens* was sprayed to 25 days old plants, the antagonist was found to survive in phyllosphere even up to 80 days after sowing. The bacterium could be isolated even from surface sterilized leaf tissues after 80 days of spraying with a population of 1.1×10^4 g^{-1} (Table 3).

In the present study, when *S. marcescens* was treated to rice seeds, the bacteria moved through the emerging roots and shoots to a height of 8 cm. Since blast pathogen cause aerial symptoms, the movement of bacteria is important to enact biological control. Geels and Schippers (1983) have observed similar movement of antagonistic bacteria in potato. Weller (1984) isolated *Pseudomonas fluorescens* from the entire length of wheat root, maximum near seed and root tip. In case of groundnut *S. marcescens* move in roots, coleoptile and the first and second leaves (Kishore et al., 1998). All these reports lend support to the present findings.

Effect of different doses of talc based *S. marcescens* treated seeds of rice on the incidence of blast (Pot culture).

Table 3

Survival of *S. marcescens* in the phylloplane of paddy

Tt. No	Days after spraying *S. marcescens*	Phylloplane population of *S. marcescens* (10^4 / g leaf)	Population of *S. marcescens* in leaf tissues (10^4 / g leaf)
1	0	19.2	0.0
2	20	4.5	3.0
3	40	3.4	4.3
4	60	1.8	3.5
5	80	1.1	3.0
	SE	0.68	0.42
	CD (P=0.5)	1.03	0.74

Data on effect of different doses of talc based *S. marcescens* treated seeds of rice on the incidence of blast are given in Table 4.

Table 4

Effect of different doses of talc-based *S. marcescens* inoculum treated seed on the management of Paddy blast (Pot culture experiment)

Tt. No	Dosage of talc based inoculum (g/kg seed)	Disease incidence (%)
1	2.5	58.0
2	5.0	45.0
3	7.5	33.0
4	10.0	18.0
5	Control	74.0
	SE	1.56
	CD (P=0.5)	2.69

Among the different dosages, seeds treated with 10 g/kg recorded the least disease incidence (18.0 per cent). It was followed by seeds treated with 7.5 g and 5.0 g, which recorded 33.0 per cent and respectively 45.0 per cent of the disease incidence. The maximum disease incidence was recorded in the control (74.0 per cent).

Effect of foliar application of talc based inoculum of *S. marcescens* on the management of rice blast (Filed experiment)

The data on the effect of foliar application of *S. marcescens* on the management of rice blast was found significant.

Data regarding the rice blast incidence are presented in Table 5.

Table 5

Effect of foliar application of talc-based *S. marcescens* on the management of paddy blast (Field experiment)

Tt.No	Dosage of talc based inoculum (kg/ha)	Disease incidence (%)	Phylloplane Population of *S.marcescens* (10^4)
1	*S.marcescens* @ 1.0	52.4	1.1
2	*S.marcescens* @ 1.5	40.4	1.3
3	*S.marcescens* @ 2.0	29.8	2.1
4	*S.marcescens* @ 2.5	18.4	3.0
5	Control (Un inoculated)	56.2	0.0
6	Control (Inoculated)	82.1	0.0
	SE	1.44	0.07
	CD (P=0.5)	2.47	0.13

The results of the present investigation revealed that the control of rice blast was achieved by spraying *S. marcescens* @ 1.0 kg/ha. However, the maximum efficacy was obtained at 2.5 kg/ha. The increasing dose of foliar spray increased phyllosphere population of *S. marcescens*. The maximum bacterial population (3×10^4 g^{-1}), was found in 2.5 kg/ha. It was followed by 2.0 kg/ha with a bacterial population of 2.1×10^4 g^{-1}. The lowest bacterial population was found in 1.0 kg/ha with 1.1×10^4 g^{-1}.

Efficacy of seed treatment in the control of blast increased with increasing the inoculum dosage and the maximum efficacy was observed when the talc-based inoculum was applied at 10 g/kg of seed. Akutsu et al., (1993) in Cyclamen obtained similar results for the control of *Rhizoctonia solani.*

Efficacy of foliar application in the control of blast increased with the increase in inoculum dosage and the maximum efficacy was observed when the talc-based inoculum was sprayed at 2.5 Kg/ha. Numerous reports on the control of foliar diseases with *S. marcescens* applied to foliage are available in literature. Some of the pathogens controlled are *Cercosporidium personatum* (Kishore et al., 1998), *R. solani* (Someya et al., 2000). *Botrytis* sp. (Akutsu et al., 1993), *Colletotrichum orbiculare* (Wei et al., 1991). The above reports are in accordance with the present findings.

CONCLUSIONS

Out of the six-bio protectants tested, *S. marcescens* was found very effective against *P. oryzae* under *in vitro* conditions. *S. marcescens* could be isolated from shoots, as well as roots emerging from the treated seeds and the plant parts from treated seeds inhibited *P. oryzae.* The antagonist *S. marcescens* survived in the phyllosphere even 80 days after spray. The increasing dose of talc based inoculum when applied on foliage increased the phyllosphere population of *S. marcescens* and controlled rice blast. The maximum disease control was achieved when inoculum was applied at 2.5 kg/ha.

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REZUMAT

ACTIVITĂȚI ANTAGONICE ALE *SERRATIA MARCESCENS* ÎMPOTRIVA *PYRICULARIA ORYZAE*

Orezul este o importantă plantă de cultură, dar şi o specie afectată de un număr mare de boli, ceea ce duce la mari pierderi de producție. Există mai multe putregaiuri care atacă orezul, dintre care *Pyricularia oryzae* este principala boală a orezului**.** În combaterea acesteia, metodele biologice par să fie o alternativă viabilă la tratamentele chimice cu fungicide. Un nou agent biologic *viz., Serratia marcescens* pare să fie agentul ideal de control pentru *P. oryzae*, pentru că produce enzime chitinolitice, care cauzează degradarea pereților celulelor fungilor inducând o reacție sigură şi defensivă a plantelor împotriva moleculelor fungilor. Un studiu a fost efectuat pentru a investiga efectele noului bioagent *S. marcescens* împotriva *P. oryzae*. Formula s-a bazat pe faptul că *S. marcescens* (@ 1,0, 1,5, 2,0 şi 2,5 kg/ha) a fost utilizat prin stropirea plantelor de orez, în câmp. În condiții *in vitro* pe şase testeri protejați, *S. marcescens* a fost găsit eficace împotriva *P. oryzae. S. marcescens* a putut fi izolat din muguri, dar şi din vârfurile rădăcinilor plantelor provenite din semințele tratate, şi părțile plantelor tratate au inhibat *P. oryzae. S. marcescens* a supraviețuit în atmosferă 80 de zile după pulverizare. Rezultatele au relevat faptul că boala a putut fi controlată prin pulverizarea plantelor cu*. S. marcescens* @ 1.0 kg/ha. Doza crescută, bazată pe inocularea foliajului, a asigurat creşterea populației de *S. marcescens* în filosferă şi controlul bolii. Controlul maxim al bolii s-a obținut când inoculii au fost aplicați în cantitate de 2,5 kg/ha.