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Management of Brown Spot of Rice (*Oryza sativa* L.) Caused by *Bipolaris oryzae* by Bio-Control Agents

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A B S T R A C T

Under laboratory conditions using dual culture techniques, bio-control agents (*Trichoderma viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis*) were tested against *Bipolaris oryzae* the causal organism of brown spot of rice. Maximum inhibition (65.33 %) of mycelial growth of *B. oryzae* was caused by *T. harzianum*, followed by *T. viride* (57.76%) and *P. flourescens* (32.68%). Under field conditions three rice varieties, Basmati-370, Jaya and PC-19, were used to test the bio-control agents, *T. viride*, *T. harzianum* and *P. fluorescens* each at 1×10^9 cfu/ml concentration. The foliar application of *T. harzianum* exhibited maximum disease reduction (64.00%) and also resulted in increased yield in PC-19 (8.00%) as compare to their respective control.

Introduction

Brown spot of rice caused by *Bipolaris oryzae* Subr. and Jain (teleomorph=*Cochliobolus miyabeanus*) is an important disease of rice throughout the world (Ou, 1985; Savary *et al.*, 2000a), and is responsible for the reduction of 26 to 52 per cent gain in yield (Chakrabarti, 2001). The disease is known to occur in resource poor farmers' fields where there is deficiency of water supply and nitrogenous fertilizers (Zadoks, 1974). In India, the disease was reported to cause Bengal Famine in 1942 with 50-90 per cent in yield loss (Padmanabhan, 1973). The disease has been reported to occur in all the rice growing countries including Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, India, North America, Philippines, Saudi Arabia, Australia, Malaya and Thailand (Gangopadhyay, 1983; Ou, 1985; Khalili *et al.*, 2012). The disease is responsible for reduction in leaf area index (LAI), early senescence of the diseased plants, reduction in number of tillers, reduction in shoot elongation and lowered quality and weight of individual grains (Vidhyasekharan *et al.*, 1973; Klomp, 1977). Initiation of the

symptoms appears as minute spots on leaves which may reach upto 1 cm or more in length. Similar symptoms also appear on leaf sheaths and coleoptiles. The affected nursery can often be recognized from a distance by scorched appearance due to the death of the seedlings. Fungicides, such as iprodione, propiconazole, azoxystrobin, trifloxystrobin, and carbendazim (Moletti *et al.*, 2000; Cortesi and Giuditta, 2003; Mandal and Jha, 2008) have been reported as an effective means to manage the disease. Inspite of the availability of chemicals for the effective management of brown spot disease, continuous, inappropriate and indiscriminate use of chemicals is known to cause undesirable effects such as residual toxicity, development of chemical resistance, environmental pollution, health hazards to humans and animals and increased expenditure for plant protection. Therefore, application of beneficial microbes for the management of plant diseases has emerged as a viable alternative in the recent past. Keeping in view the economic value of the crop and extensive damage caused by brown spot of rice, the present study was undertaken to evaluate different biocontrol agents for the management of brown spot of rice.

Materials and Methods

In vitro evaluation of biocontrol agents against *Bipolaris oryzae*

The bio-control agents *viz*., *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested on Potato Dextrose Agar against the *B. oryzae* by dual culture technique (Dennis and Webster, 1971). The experiment was laid in Completely Randomized Design (CRD) with three replications.

The per cent inhibition of the mycelial growth of the pathogen was calculated using the following formula (Vincent, 1947).

$$\text{Inhibition (\%)} = \frac{\text{Radial growth in control (mm)} - \text{Radial growth in treatment (mm)}}{\text{Radial growth in control (mm)}} \times 100$$

Effect of foliar application of bio-control agents on the severity of brown spot of rice

Rice varieties, Basmati-370, Jaya and PC-19 were selected for evaluation of biocontrol agents. The seeds of the selected rice varieties were obtained for the Division of Plant Breeding and Genetics SKUAST-J, Chatha, Jammu. The field experiment was laid out in factorial randomized block design with three replications during kharif cropping season for two consecutive years having plot size of 3× 2.5m^2 consisting of 15 rows, each 2.0m long and 22.5cm apart. Spraying of bio control agents *T. viride* and *T. harzianum* each at @1×10^8 cfu/ml and *P. flourescens* @ 1×10^8 cfu/ml were done in the month of August after the appearance of symptoms. Observations regarding disease severity were recorded periodically and yield/plot was calculated at the time of harvesting of crop. Data was statistically analysed by using OP software.

Results and Discussion

In-vitro evaluation of bio-control agents against *Bipolaris oryzae*

The data presented in the Table 1 reveal that among the different bio-control agents tested against *Bipolaris oryzae*, *T.harzianum* was most effective with the inhibition of mycelial radial growth of 65.33 per cent, followed by *T. viride* and *P. flourescens* (57.76 and 32.68 %, respectively). The least effective was *B. subtilus* with the per cent inhibition of 26.84. Amin *et al.*, (2010) had also observed that *T. harzianum* (Th-1), *T. viride* (Tv-1) and *T. viride* (Tv-2) were significantly effective in reducing the mycelial growth of *B. oryzae* by 37.16, 36.75 and 36.21 per cent, respectively.

Table.1 Evaluation of bio-control agents against *Bipolaris oryzae* causing brown spot of rice

Bio-control agent	*Bipolaris oryzae*	
	Radial growth of mycelium (mm)	Per cent inhibition
Trichodermaharzianum	29.72	65.33
Trichodermaviride	36.21	57.76
Pseudomonas flourescens	57.72	32.68
Bacillus subtilus	62.50	26.84
Control	85.75	0.00
S.E(±)	1.27	
C.D. (p=0.05)	3.75	

Table.2 Effect of foliar application of bio-control agents on the severity of brown spot of rice and yield

Variety	Mean Disease Severity (%)				Mean Yield (q/ha)			
	Control	Th	Tv	Pf	Control	Th	Tv	Pf
Basmati-370	42.46	16.54	18.05	21.69	22.87	25.81	24.36	23.67
Jaya	20.30	9.47	13.40	13.78	36.93	39.72	38.29	37.41
PC-19	21.35	7.78	9.91	10.08	34.84	42.00	39.82	37.96
CD (P=0.05)	Bio control agents 4.6 Varieties 2.7 Interaction (B*V) 8.07				Bio control agents 1.08 Varieties 1.17 Interaction (B*V) 2. 41			

Th: *Trichoderma harzianum*
Tv: *Trichoderma viride*
Pf: *Pseudomonas fluorescens*

Fig.1 Effect of bio-control agents on per cent reduction of severity of brown spot of rice

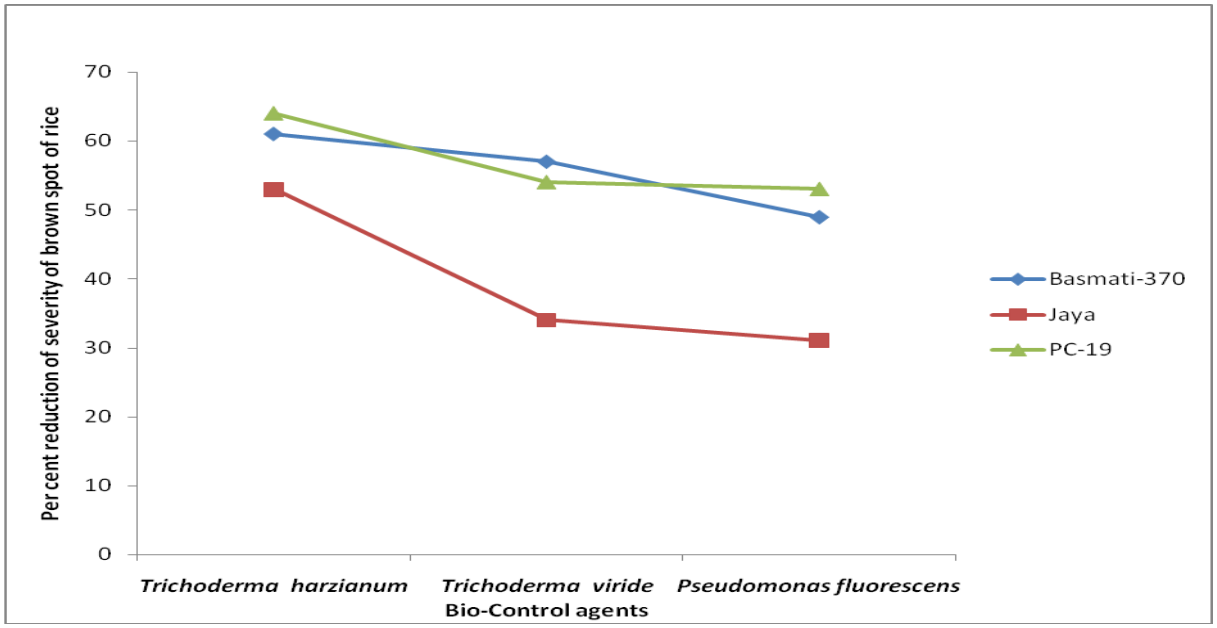
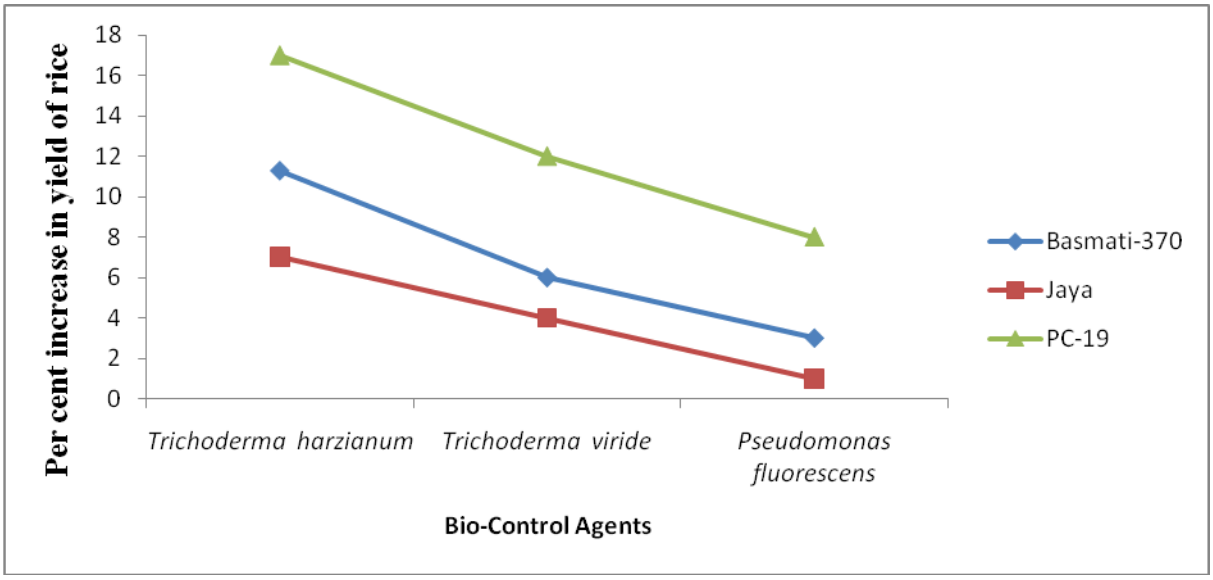


Fig.2 Effect of bio-control agents on per cent increase in yield of rice



Trichoderma species are known to produce a large number of allelopathic fungal exudates such as antibiotics, peptides, cyclic polypeptides, volatile and non-volatile compounds that suppress the mycelial growth of the plant pathogens (Angelica *et al.*, 2001; Vinale *et al.*, 2005; Harish *et al.*, 2008).

Effect of foliar application ofbio-control agents on the severity of brown spot of rice

The data depicts that among the three rice test varieties the mean brown spot severity ranged from 21.35 to 42.46 per cent in control plots (Table 2). In Basmati-370, the bio-control agent, *T. harzianum* and *T. viride* exhibited maximum control of brown spot, showing 16.64 and 18.00 per cent mean disease severity, respectively, which was followed by *P. flourescens* with mean disease severity of 21.69 per cent. In the variety Jaya also, *T. harzianum* and *T. viride* exhibited maximum control (9.47 and 13.40% disease severity, respectively), followed by *P. flourescens* having diseases severity of 13.78 per cent. Application of *T. harzianum* and *T. viride* were again most effective in variety PC-19 exhibiting maximum disease control with 7.78 and 9.20 per cent mean disease severity, respectively, followed by *P. flourescens* (10.08%). The maximum per cent reduction of 61.00, 53.00 and 64.00 in the brown spot severity was observed with the application of *T. harzianum* in Basmati-370, Jaya and PC-19, respectively (Fig. 1). Kumawat *et al.*, (2008) also found that pre-application of spore suspension of *T. harzianum* and *T. viride* reduced the infection of *B. oryzae*, which was attributed to increased level of total soluble protein and total phenol contents. Two strains of *T. harzianum* were also reported to control the disease in Iran (Khalili *et al.*, 2012). Isolates of fluorescent *Pseudomonas* from soil have been reported to reduce the fungal growth and brown spot incidence (Ray *et al.*, 1990).

Effect of foliar application of bio-control agents on the yield of rice

The data presented in the Table 2 further reveal that in rice variety Basmati-370, the application of *T. harzianum* resulted in maximum yield of 25.81q/ha which was followed by the application of *T. viride* 24.36q/ha while, *P. flourescens* showed the

minimum yield of 23.67q/ha. The untreated plots in Basmati-370 showed a yield of 22.87q/ha. In the variety Jaya, the maximum yield (39.72q/ha) was recorded with application of *T. harzianum*, followed by *T. viride* (38.29q/ha), whereas, the minimum increase in yield was observed by *P. fluorescens* (37.41q/ha) as against the untreated plots which showed a yield of 36.87q/ha. In PC-19 the maximum increase in the yield was shown by *T. harzianum* (42.00q/ha) followed by *T. viride* (39.82q/ha), whereas, the minimum increase in yield was shown by *P. fluorescens* (37.96q/ha) as against the untreated plots which showed a yield of 34.65q/ha. Maximum per cent enhancement in yield of 11.30, 7.00 and 17.00 were recorded by foliar application of *T. harzianum* in Basmati-370, Jaya and PC-19, respectively (Fig. 2). Foliar application of *T. harzianum* has been shown to reduce the disease intensity and significantly improve grain yield, total grain carbohydrates and proteins in addition to a significant improvement in the total photosynthetic pigment in the rice leaves (Abdel-Fattah *et al.*, 2007). Baker (1988) attributed the plant growth response induced by *Trichoderma* species due to the production of a growth regulating factor and induction of systemic response in the host.

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