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Effect of bioagents on the growth of rice against blast disease caused by *Pyricularia oryzae*

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Abstract
Rice (*Oryza sativa* L.) is the most important sources of food which provide diet more than 50% of the world population. Blast disease caused by *Pyricularia oryzae*, contributes a major reduction in rice production. The rampant use of fungicides causes deleterious effect on the environment. To minimise side effects of fungicides alternative means are opted. To concern about climate change, environment pollution and sustainable crop production disease management on sustainable basis is crucial. Thus, bioagents use as an alternative to chemical fungicides for the management of diseases. Effect of bio control agents (BCAs) evaluated against pathogen *Pyricularia oryzae* causing blast disease of rice *in vitro* and field condition. The result revealed that after three days of inoculation *Trichoderma harzianum* found most effective against *Pyricularia oryzae* which inhibited mycelial growth around 63%. Meanwhile *Trichoderma viride* inhibited 60% radial growth of *Pyricularia oryzae*. The best result shown by *Trichoderma harzianum* which inhibited pathogen by 63%, 56% and 41% at consecutive interval of three, five and seven days respectively. Dry shoot weight was found increased by *T. harzianum* (48.24%) followed by *T. viride* (45.98%) with respect to seed treated with pathogen. Among treatment of different bioagents the best yield was found 15.01 q/hac and 14.88 q/hac treatment with *T. harzianum* and *T. viride* against crop infected with pathogen. Seed treated with *T. harzianum* and *T. viride* the disease severity was found decreasing of 51.33% and 48.52% respectively. The application of bio control agents not only reduced the disease severity, promoted the plant growth and ultimately increased the grain yield significantly compared to control without any hazardous effect on environment.

Keywords: Rice, *Pyricularia oryzae*, bioagents, fungicides, environment

Introduction
Rice (*Oryza sativa* L.) is one of the most important sources of food for the world population. Around 3 billion people which about 50% of human population uses rice as food and nutrients source. Globally rice occupies an area of 166 million hectares with a production of 758.8 metric tonnes of paddy (FAO, 2017). Rice is subjected to the attack of over 30 fungi in country like India (Rangaswamy, 1992). Paddy is susceptible to several diseases; the blast disease of rice is most significant. The disease is caused by a filamentous, ascomycete fungus *Magnaporthe oryzae* (syn: *Pyricularia oryzae* Cav.) and is reported from more than 85 countries of the world (Gilbert *et al*., 2004 and Scardaci *et al*., 1997) [5, 9]. The leaf blast is characterized by the white to grey green lesions or spots with darker borders produced on the leaf. The old lesions are elliptical or spindle shaped and whitish to gray with necrotic border. The neck blast is characterized by the dark brown lesion on the basal of the panicle neck that make it cannot support the panicle. *P. oryzae* can infect the rice plant at various growth stage (Yolanda, 2013) [13]. Sometime the fungus can also infect the grain (Tebeest *et al*., 2007) [11]. Rice blast disease first reported in India in 1913 (Padmanabhan, 1965) [6]. The blast diseases of rice caused by *Pyricularia oryzae* which infect leaf, node, and panicle of the paddy (Correll *et al.* 2000) [3]. The bio agents inhibit the pathogen by competition, siderophore production, and antibiosis induced resistance or by plant growth promotion (Weller, 1988) [12] and have long lasting effect without environmental hazards. Biocontrol approach for managing these diseases is considered to be practical and economical alternative (Reddy *et al*., 2009) [7]. With the aim of controlling the rice blast by using biological control methods the present investigation was undertaken as *in vitro* and field experiments against the disease in order to find out suitable biological control for soil borne pathogens. The application of bio control agents not only reduced the disease severity, promoted the plant growth and ultimately increased the grain yield significantly compared to control without any hazardous effect on the environment.

Methods and Material
The commonly used bioagents *viz. Trichoderma harzianum, T. viride, T. hamatum, T. virens* and *T. longibractum* were evaluated the antagonistic activities against P. oryzae under *in vitro*

conditions through dual culture plate technique (Bell *et al*., 1982) [2]. Radial growth of the pathogen was measured and percent inhibition was calculated by the following formula I%=C-T/C x 100; where I= inhibition of mycelial growth, C= growth of pathogen in the control plate (mm) and T= growth of pathogen cultures (mm). Biocontrol agents (BCAs) *Trichoderma harzianum, T. viride, T. hamatum, T. virens* and *T. longibractum procure* from Indian type culture collection from ITCC, New Delhi. The experiment was done following Randomized Complete Block Design (RCBD) with three replications.

Isolation of fungal pathogen

The infected leaves of paddy plants were washed thoroughly with tap water and were cut into small pieces of 2-4 mm in size using sterilized blades. These pieces were surface sterilized by dipping in 1% sodium hypochlorite for one minute and were washed by sterilized water for several times. The sterilized pieces were placed in the petriplates containing 20 ml solidified potato dextrose agar (PDA).

Count the colony forming unit of *Trichoderma* spp. formulation

One gram of bio agents powder respectively *Trichoderma* spp. was weighed and the volume was made up to 10 ml with sterilized distilled water, shaken well (1:10) inside laminar flow hood. Out of this suspension 1 ml was taken out and transferred to 9 ml of sterilized distilled water in a test tube (1:100). Serial dilution was made similarly by transferring 1 ml of each suspension to the subsequent tubes to get 10^{-7} and 10^{-8} dilution respectively. 1 ml of each suspension (10^{-7} & 10^{-8}) was transferred to sterilized Petri plates. 15 ml of each medium such as PDA for *Trichoderma* spp. was poured into plates. The plates were incubated in an inverted position at 25 ± 2 ^{0}C. After 3 days, average numbers of colonies were counted per plates of bio agents. Colonies were observed per plate and the number of colony forming unit present in 1 g was calculated by the formula (Aneja, 2004) [1].

Cfu=Number of colonies Amount plated × dilution factor

Seed treatment with biofungicide formulations of *Trichoderma harzianum, T. viride, T. hamatum, T. virens* and *T. longibractum* was done @ 4 g / kg seed.

Analysis of data

The data of the experiments have been analysed statistically using R Studio statistical computing version 3.0.1. Three replicates were maintained for each set of experiment. The data on colony diameter, blast incidence, phenological characters and yield were analysed for single factor ANOVA. To measure specific differences between pairs of means Duncan's Multiple Range test (DMRT) has used. Least significance difference (LSD) was calculated at $P<0.05$ for all variables to compare individual treatments.

Results and Discussion

The effect of bioagents activity on mycelial growth of *Pyricularia oryzae* presented in Table 1. The result revealed that after three days of inoculation *Trichoderma harzianum* found most effective against *Pyricularia oryzae* which inhibited mycelial growth around 63%. Meanwhile, *Trichoderma viride* inhibited 60% radial growth of *Pyricularia oryzae*. However, *T. hamatum, T. virens, T. longibractum* found equally antagonised the mycelial growth of *P. oryzae*. The advancement of inoculation days the affectivity of bioagents found less effective. *T. harzianum* and *T. viride* antagonised the radial growth of *P. oryzae* around 56% and 53%, 41% and 39% with respect to inoculation of 5 days and 7 days respectively. The efficacy of various biocontrol agents against *Pyricularia oryzae* through seed treatment under field condition has been demonstrated in Table 2. It was evident that all the bio control agents (BCAs) were effective to increase the yield significantly over control plots. The yield was found 15.01 q/hac treated with *T. harzianum* against crop infected with pathogen. Meanwhile, 14.88 q/hac treated with *T. viride*. Treatment with *T. hamatum, T. virens* and *T. logibractum* yield was found 14.32 q/hac, 13.51 q/hac and 13.88 q/hac respectively. Dry shoot was found increasing by 48.24% and 45.98% with treatment of *T. harzianum* and *T. viride* respectively. Fresh root weight was also found increasing by 41% and 39% treated with *T. harzianum* and *T. viride* respectively. However, dry root weight and fresh shoot weight found almost equal l 47.40gm and 2.90gm treatment of *T. harzianum* and *T. viride* respectively against seed treated with pathogen. Severity of disease was found 38%, 37% and 37% of seed treated with *T. hamatum, T. virens*, and *T. longibractum* respectively. While, Disease severity was found decreasing of 51.33% and 48.52% treatment of seed with *T. harzianum* and *T. viride* respectively. Similar findings were reported by (Singh and Sinha 2009) [10] (Kumar *et al*. 2017) [7] (Dennis and Webster 1971) [4]. They evaluated the efficacy of bio control agents (BCAs) used against blast of paddy incidence and promoting plant growth of paddy in field conditions.

Table 1: Effect of bio control agents (BCAs) against radial growth of *Pyricularia oryzae* (*in vitro*)

Biocontrol agents (BCAs)	3 Days of inoculation		5 Days of inoculation		7 Days of inoculation	
	Growth (mm)	Percent inhibition (%)	Growth (mm)	Percent inhibition (%)	Growth (mm)	Percent inhibition (%)
Control	**26.50a**		**40.7a**		**90.00a**	
Trichoderma harzianum	9.70e	63.40	17.65e	56.64	53.03d	41.08
Trichoderma viride	10.50d	60.38	18.97d	53.39	54.38c	39.58
Trichoderma hamatum	13.80c	47.92	23.40c	42.51	62.46b	30.60
Trichoderma virens	13.99b	47.21	24.73b	39.25	62.46b	30.60
Trichoderma longibractum	13.87bc	47.66	24.73b	39.25	62.57b	30.48

Table 2: Efficacy of various biocontrol agents (BCAs) against *Pyricularia oryzae* through treatment of seed (field experiment)

Treatments	Shoot length (cm)	Weight (Fresh) (g)		Weight (Dry) (g)		Yield (q/hac)	Disease severity
		Shoot	Root	Shoot	Root		
Control	**60.21a**	**121.33a**	**24.44a**	**23.66a**	**5.12a**	**50.55a**	
Pyricularia oryzae	23.12d	33.28d	7.70e	6.01e	2.01e	10.12g	75.22a
Trichoderma harzianum	31.21b	47.44b	10.86b	9.00b	2.94b	15.01b	36.46f
Trichoderma viride	30.68b	47.22b	10.69bc	8.80b	2.89bc	14.88c	38.72e

Trichoderma hamatum	29.36bc	46.00bc	10.25cd	8.50c	2.78cd	14.32d	46.24d
Trichoderma virens	27.90c	44.11c	9.76d	8.05d	2.65d	13.51f	47.52b
Trichoderma longibractum	28.16c	44.50c	9.85d	8.12d	2.67d	13.88e	47.00c

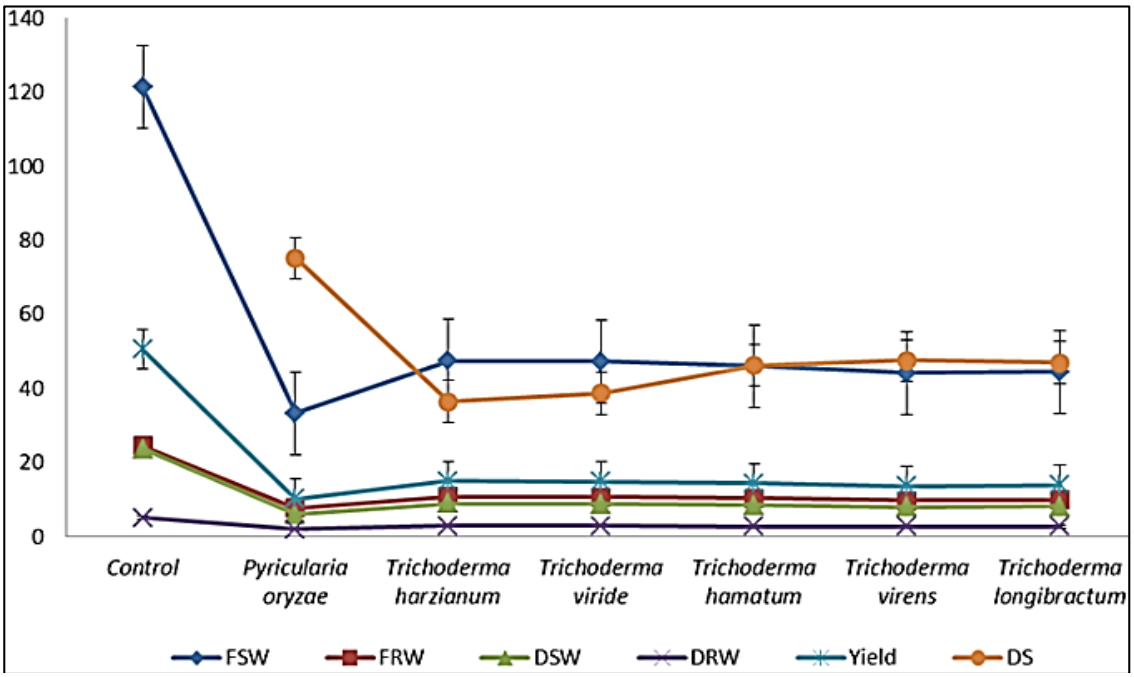

Fig 1: Effect of bioagents on fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, yield and disease severity

Conclusion

Plant diseases caused by pathogenic fungi may results significant yield losses of agricultural crops. The misuse of these chemicals May causes serious environmental and health problems. Microbial antagonists are potent bio control agents (BCAs) that can be explored to provide effective and safe means to manage plant diseases. For sustainable management of blast disease of rice, proper seed treatment with BCAs used in the field conditions to manage diseases results increasing the yield of rice grains. This combination could be used as eco-friendly management of the disease as a component of integrated disease management. The treatment will be easy to apply in the field by the farmers and further it will also promote plant vigour and thus increasing the yield of the rice crop. This method alternate to the injudious chemical fungicide.

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