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Effect of bio control agents and botanicals against Blast of Paddy caused by *Pyricularia oryzae*

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Abstract
Rice blast caused by *Pyricularia oryzae* cave continues to be a major constraint in rice production. Since, the existing chemical control measures being costly and may favor development of resistance in pathogens, the potential alternative methods have been explored in the present studies. Bio control agents *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and botanicals neem oil and neem oil + neem leaf extract (*Azadirachta indica*) were evaluated their efficacy against blast of rice (Pusa Basmati 1121). Carbendazim was used for standard check fungicides for comparison. The results concluded that the *P. fluorescens* inhibit the blast (21.99%) as compared to treated and untreated control (18.57% and 34.15 %, respectively) when *T. viride* is best bio-control (24.53%) which was followed by *T. harzianum* (23.08%) neem oil (26.20%) and neem oil + neem leaf extract (24.15%) inhibit the blast respectively.

Keywords: Neem oil, *Pseudomonas fluorescens*, *Pyricularia oryzae*, *Trichoderma* spp

Introduction
Rice (*Oryza sativa* L.) is the most important staple food crop and grown in India providing of 43 per cent of calorie requirement for 70.0 per cent of the Indian population. Rice is the major source of food for more than half of the world population. More than 90 per cent of the world's rice is grown and consumed in Asia, known as rice bowl of the world where 60 per cent of the earth's people and two thirds poor live. To focus attention on the importance of rice in global food security and the necessity to increase rice production and productivity, United Nations General Assembly in 2002 declared to celebrate the year 2004 as "International year of rice (IYR, 2004)" with the name of "Rice is life".

World production of the rice is 751.0 million tones and area grown was 107.0 million tones according to the FAO first forecast (global paddy production in 2014). Rice crop is prominent cereal of U.P. in terms of area (5.34 million hectares) and production of 9.95 million tons with the productivity at 1.79 tonnes/ha which was very low as compared to international and national productivity levels (Anonymous, 2014) [3]. There are several biotic and abiotic factors behind the lower productivity compared to other major rice growing countries. Among the biotic factor fungal diseases *viz.*, blast (*Pyricularia oryzae*), brown leaf spot (*Bipolaris oryzae / Helminthosporium oryzae*), stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), bacterial disease such as bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and viral disease such as (rice tungro virus) are very important. Rice blast disease first reported in India in 1913 (Padmanabhan, 1965).

The fungus can infect rice plants at any growth stage. The mycelium may survive within the tissues of embryo, endosperm and glumes. Certain cultural practices encourage blast: excessive use of nitrogen (through chemical fertilizers) increases susceptibility of rice to the fungus, as does inadequate spacing (often practiced under rice intensification programs). The use of bio-control agents and botanicals, for plant protection, has assumed greater importance in recent years all over the world due to environmental pollution and health hazards associated with the indiscriminate use of synthetic fungicides.

Use of fungicides can be minimized by the integrated approach for management of plant diseases. This study aims to find a safe and cheap way to manage the plant diseases and to provide good and economically accessible methods of disease control with bio-control agents and botanicals compared to fungicides used by farmers. The biological method of plant disease management appears to be a better alternative to chemical fungicides in managing the blast

disease. With the aim of controlling the rice blast by using biological control methods the present investigation was undertaken as a field trail experiments against the disease in order to find out suitable biological control for soil borne pathogens.

Materials and Methods

Experimental site

The experiment was conducted in Laboratory and the Research Experimentation Field Department of Plant Pathology, Central Research Farm, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad during *kharif* season in the year 2014-2015.

The nursery

Seeds of rice cultivar Pusa basmati-1121 at the rate of 60 kg were soaked in water for 24 hours and incubated for 48 hours to hasten early germination. Pre-germinated seeds were uniformly broadcast in the nursery on 15th may in 2014 seasons.

Preparation of the experimental field

The selected field area was well prepared and plot marked as per the lay out plan of the experiment. The selected field was dug up, cleaned and the soil was pulverized after which the total area was divided in to sub-plots.

Maximum Relative Humidity (%) during the crop period (July- October 2014) was in the range of 71.43-89.85. Minimum Relative Humidity (%) during the crop period (July- October 2014) was in the range of 34.29-64.24. Maximum temperature was 37.82-32.24℃. Minimum temperature was 28.71-20.7937 ℃ and rainfall 00.01-16.94 mm.

Method of application

Time of the application of the *Trichoderma* is also important. Therefore, it may be used strictly as a preventive measure, it can't cure infection. *Trichoderma* spp. is least effective against the systematic disease than against more superficial one. It cannot control the existing disease. A combination of a chemical treatment with *Trichoderma* spp. will be highly effective. A single strain of *Trichoderma* spp. may not be sufficient to be effective under all conditions and agents are effective against the disease.

Count the colony forming unit of *T. viride* and *T. harzianum* formulation:

One gram of bio agents powder respectively *Trichoderma* and *Pseudomonas fluorescens* 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. and Kings B Agar for *Pseudomonas fluorescens* was poured into plates. The plates were incubated in an inverted position at 25 ± 2 °C. After 3 days, average numbers of colonies were counted per plates of both 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) [2].

$$\text{C. f. u} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution factor}}$$

Use of Neem Botanical

Preparation of neem leaf extracts

The collected plant leaves were chopped after cleaning in running tap water three times to remove soil materials. The dried leaves of each plant species were made into powder separately using a sterilized mortar and pestle and then sieved with one millimeter sieve. The extracts were filtered through cheese cloth. The powder of neem leaf extracts was packed in water proof plastic bags and labeled appropriately as described by Akinbode and Ikotun (2008) [1] and stored at 4 ˚C until used. Crude plant extracts were obtained by infusing 50 g of plant material in 100 ml SDW to give 50% w/v in a 500 ml conical flask and the mixture was incubated at 25˚C – 28 ˚C for 20 hours. The infusion was filtered separately through sterile double-layered cheese cloth into a sterile 400 ml beaker and the resulting stock solution was collected and stored at 25 ˚C – 28 ˚C until used.

Application of spray solution

Plant extracts, bio-control agents and chemicals were sprayed as solution into the experimental plots as per treatments. Spraying was done for 3 times with 10 days interval at 65, 75 and 85 DAT respectively. Adequate precautions were taken to avoid drifting of spray materials from one plot to the neighbouring ones**.**

Assessment of the disease incidence in the field

Each plot was visited for recording the incidence. The disease incidence was recorded in the three growth stage of the plant namely flowering stage, milking stage and maturity stage.

Assessment of the disease severity in the field five plants from each unit plot were randomly selected and tagged for grading the severity of diseases. Disease severity of leaf blast (*Pyricularia oryzae*) of rice was recorded by Singh (2000) used a 0-9 scale as follow: 0 = no lesion observed; 1 = 1% leaf area covered; 3 = 10% leaf area covered; 5 = 25% leaf area covered; 7 = 50% leaf area covered and 9 = more than 50% leaf area covered. Disease severity of leaf blast (*Pyricularia oryzae*) of rice was recorded by Singh (2000) used a 0-9 scale as follow: 0 = no lesion observed; 1 = 1% leaf area covered; 3 = 10% leaf area covered; 5 = 25% leaf area covered; 7 = 50% leaf area covered and 9 = more than 50% leaf area covered by using the disease rating scale of 0-9 developed by International Rice Research Institute (IRRI1996) [5] and then converting into percent disease by using the formulas.

$$\text{Disease severity \%} = \frac{\text{Sum of disease grade} \times \text{No. of infected tiller}}{\text{No. of tiller} \times \text{maximum disease grade} \times \text{No. of tiller asses}}$$

Measurement of Shoot and Root weight

After the assessment of disease when the rice plants were 60 days after transplanting. The plants were uprooted carefully from the field. The root region was cut separated from the plants and washed thoroughly to remove adhered soil particles with much care and the fresh shoot weight, dry shoot weight, fresh root weight and dry root weight of the plants of each treatment were measured in gram.

Experimental design and statistical analysis

The experiment was done following Randomized Complete Block Design (RCBD) with three replications. The experimental field was primarily divided into 7 blocks. Each block was further divided into 3 plots. Total number of plots was 21.

Result and discussion

Effects of biotic and botanicals inducers on rice crop

Number of tiller of paddy as affected by different treatments at 60 DAT

Among the bio-agents and botanicals used the maximum number of tiller was recorded in T_1-*Pseudomonas fluorescens* (46.43) as compared to treated and untreated control (38.46 and 25.93, respectively). The second best treatment was T_2-*Trichoderma harzianum* (19.53), which was followed by T_3-*T. viride* (44.13), T_4 – neem oil (42.66) and T_5 – neem oil + neem leaf extract (40.50) as compared to T_0 - untreated control (36.86). Among the treatments most effective was T_1-*Pseudomonas fluorescens* (46.43).

Shoot length (cm) of paddy as affected by different treatments 90 DAT

Among the bio-agents and botanicals used the maximum shoot length (cm) was recorded in T_1-*Pseudomonas fluorescens* (74.70) as compared to treated and untreated control (66.00 and 62.23, respectively). The second best treatment T_2- *Trichoderma harzianum* (62.23), which was followed by T_3- *T. viride* (70.16), T_4 – neem oil (69.56) and T_5 – neem oil + neem leaf extract (67.93) as compared to T_0 - control (62.23). Among the treatments most effective was T_1-*Pseudomonas fluorescens* (74.70). Similar findings were reported by Karpagavalli (2001) [8] under field condition all the treatments tested in this study gave satisfactory result against blast of paddy (*Pyricularia oryzae*) Malleswari and Bagyanarayana (2013) [11] and suggested that growth promotion by bio-agents might be due to direct involvement of some plant hormones such as auxins, cytokinins etc.

Table 1: Effect of biotic inducers on Shoot length, number of tiller, shoot and root weight of paddy and severity of blast disease of rice

Treatments		Shoot length (cm) 60 DAT	No. of tiller 60 DAT	Fresh shoot wt. (g) 60 DAT	Fresh shoot wt. (g) 60 DAT	Fresh root weight (g) 60 DAT	Dry root weight (g) 60 DAT	Disease severity 90 DAT	Yield (q/ha)
T_0	Untreated control	62.23	36.86	120.40	25.66	26.46	5.80	34.15	35.43
T_1	*Pseudomonas fluorescens* @ 8g/kg (ST)*Pseudomonas fluorescens* @ 0.2% (FS)	74.70	46.43	126.16	30.63	32.20	8.53	21.90	38.22
T_2	*Trichoderma harzianum* @ 8g/kg (ST)*Trichoderma harzianum* @ 10g/l(FS)	72.23	42.83	124.76	29.26	30.46	7.63	23.08	37.72
T_3	*Trichoderma viride*@ 8g/kg (ST)*Trichoderma viride*@ 10g/l(FS)	70.16	44.13	124.43	28.50	29.06	7.33	24.53	37.26
T_4	Neem oil @ 1 ml/kg (ST). Neem oil @ 0.5 ml/l(FS)	69.56	42.66	122.33	26.96	28.60	7.30	24.20	36.81
T_5	Neem oil @1 ml/kg (ST). Neem leaf extract @20ml/l(FS)	67.93	40.50	123.40	27.80	28.03	6.70	24.15	36.15
T_6	Carbandazim 50 WP @ 2g/kg (ST)Carbandazim 50 WP @0.2% (FS)(Treated control)	66.00	38.46	121.80	26.53	27.46	6.50	18.57	39.90
F test		S	S	S	S	S	S	S	S
S. Ed. (±)		2.430	0.566	0.252	2.566	2.556	4.126	0.47	2.566
CD(P=0.05)		2.976	1.335	0.553	1.268	1.268	0.523	3.014	0.636

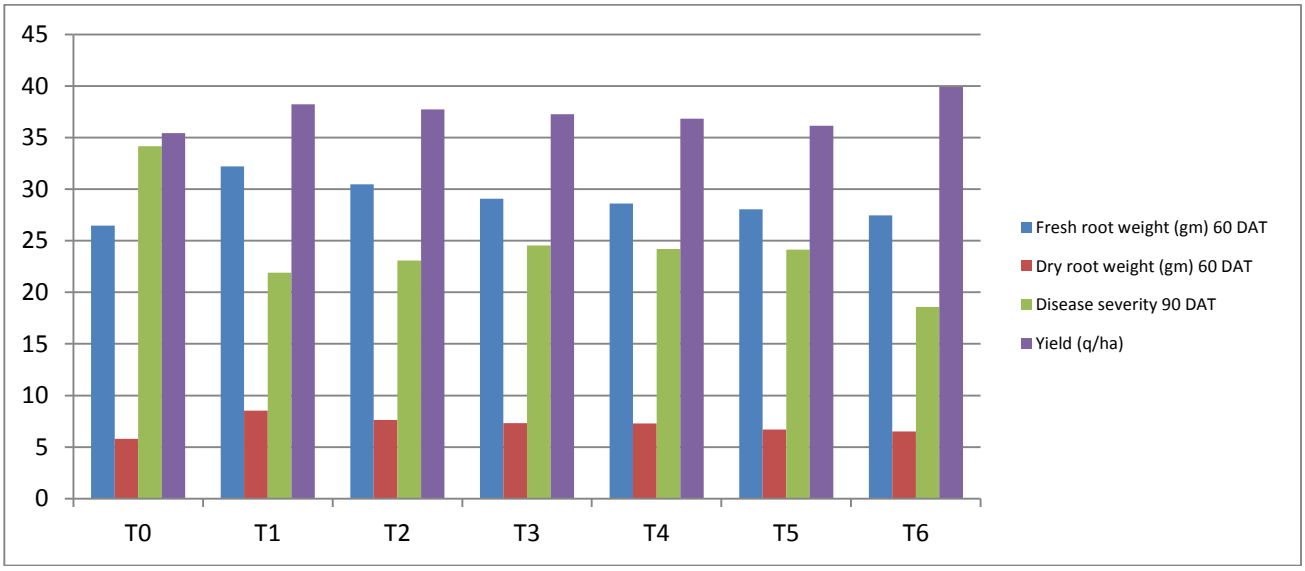

Fig 1: Effect of bio-agents and botanicals on fresh root weight, dry root weight Disease severity percent and yield(q/ha)

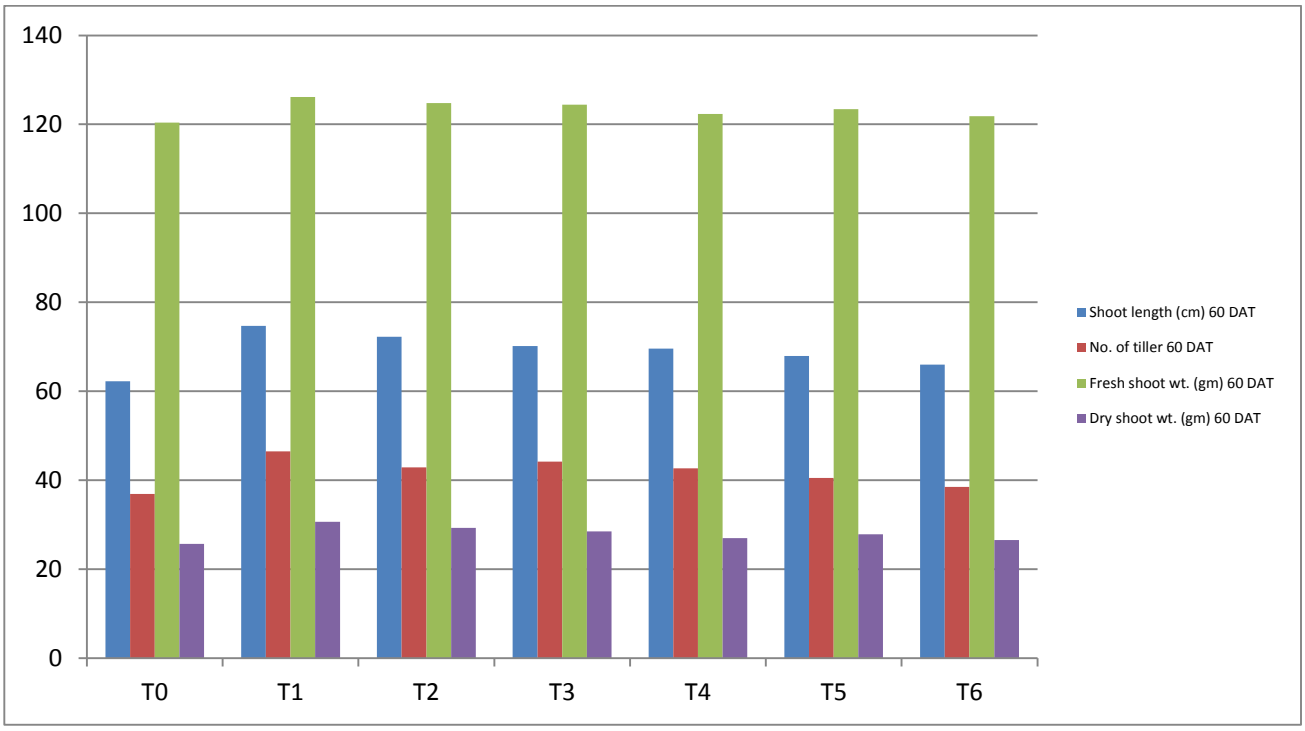

Fig 2: Effect of bio-agents and botanicals on shoot length, number of tiller, fresh shoot weight and dry shoot weight

Fresh shoot weight and dry shoot weight (gm) of paddy as affected by different treatments at 60 DAT

Among the bio-agents and botanicals used the maximum fresh shoot weight (gm) and dry shoot weight was recorded T_1- *Pseudomonas fluorescens* (126.16) and (30.63) respectively as compared to control (120.40 and 25.66 respectively). The second best treatment was T_2- *Trichoderma harzianum* (19.53, 29.26), which was followed by T_3- *T. viride* (124.73, 28.50), T_4 –neem oil (122.33, 26.90) and T_5 – neem oil + neem leaf extract (123.40, 27.80) as compared to T_0 control (120.40, 25.66). Maximum fresh shoot weight in *Pseudomonas fluorescens* may be due to activation plant growth permuting indole acetic acid (IAA) of an array of host defense mechanism including induced activity of enzymes accompanied by a significant increase in the growth .*P. fluorescens* has also been reported to enhance the crop growth and yield in rice Pathak *et al.*, 2004) [12]. Arshad and Frankenberger (1991) [4], Khalimi *et al.* (2012) [9].

Fresh root weight and dry root weight (gm) of paddy as affected by different treatments at 60 DAT

Among the bio-agents and botanicals used the maximum fresh root weight (gm) was recorded T_1-*Pseudomonas fluorescens* (32.20, 8.53) as compared to treated control (26.46, and 5.80 respectively). The second best treatment was T_2- *Trichoderma harzianum* (19.53, 19.53), which was followed by T_3- *T. viride* (29.06, 7.33), T_4 – neem oil (28.60, 7.30) and T_5 – neem oil + neem leaf extract (28.03, 6.70) as compared to T_0 - control (26.46, 5.80).

Disease severity per cent at 90 DAT

It is clearly shown from the Table No 1 that among the bio-agents and botanicals used the minimum disease severity per cent was in T_1-*Pseudomonas fluorescens* (21.99%) as compared to treated and untreated control (18.57% and 34.15 %, respectively). The second best treatment was T_2- *Trichoderma harzianum* (23.08%), which was followed by T_3- *T. viride* (24.53%), T_4 –neem oil (26.20%) and T_5 – neem oil + neem leaf extract (24.15%) as compared to T_0 - control (34.15 %).

Yield of paddy as influenced by different treatments.

Among the bio-agents and botanicals used yield was recorded in T_1-*Pseudomonas fluorescens* (38.22) as compared to treated and untreated control (39.90 and 35.43, respectively). The second best treatment wasT_2- *Trichoderma harzianum* (19.53), which was followed by T_3- *T. viride* (37.26), T_4 – neem oil (36.81) and T_5 –neem oil + neem leaf extract (36.15) as compared to T_0 -untreated control (35.43). Among the treatments most effective was T_1-*Pseudomonas fluorescens* (38.22).Similar findings were reported by Islam and Faruq (2012) [6] and (Razul and Hossain 2015) [14] (Jha and Subramanian (2013) [7] and Sharma (2013) [17] Ramezanpour (2010) [13] (Khorshidi *et al.* (2011) [10]. They evaluated the efficacy of bio control agents used against blast of paddy incidence and promoting plant growth of paddy in field conditions.

Conclusions

Plant diseases caused by pathogenic fungi may result in significant yield losses of agricultural crops. Farmers, in general still rely on the use of synthetic fungicides to control plant diseases. However, the misuse of these chemicals may cause serious environmental and health problems. Microbial antagonists are potential agents that can be explored to provide effective and safe means to manage plant diseases. Several microorganisms have been tested and proven to possess antagonistic properties against plant pathogenic fungi. Our recent study showed that the bio control agent *Pseudomonas* suppressed the growth of *Pyricularia oryzae*, the cause of rice blast disease. At the end of study *Pseudomonas fluorescens* has proved its potential as a bio-control for the management of rice blast.

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