The Pharma Innovation Journal 2021; 10(11): 1685-1689

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 1685-1689

www.thepharmajournal.com
Received: 18-08-2021
Accepted: 20-10-2021

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Evaluation of bio-controlling agents against rice blast pathogen

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Abstract
Bio-controlling of rice blast pathogens could be an alternative and eco-friendly management. Therefore, in this trial, the antagonistic potential of some bio-controlling agents (BCAs) (*Trichoderma sp., Bacillus* spp., *Pseudomonas* sp.) were assessed against six rice blast pathogens through *in vitro* and *in vivo* trials. Maximum inhibition (72%) was produced by *Trichodermma* spp. Under glasshouse condition, the infection was best controlled with *Trichoderma sp.* soil treatment @ 10 g/kg + *Bacillus* spp. seed treatment @ 10 g/kg following foliar application of pathogen @ 45 days of planting. From this, it was concluded that BCAs could effectively be used for controlling the infection of rice blast diseases. Hence it is recommended for sustaining rice farming. Further study on validation of above findings through location specific field trials is recommended.

Keywords: Rice blast, Bio-control agents, foliar fungi, *Trichoderma* spp; *Bacillus* spp, *psedumonas* sp.

Introduction
Rice is a staple food for more than half of the world. To meet challenges of the demand of rice production supply must be double by 2050 to keep up with food demand for the rising population growth. One of the largest impediments to increased rice production is the presence of rice blast (*Magnaporthe oryzae*), which directly decreases rice yields and indirectly increases production costs. Rice blast is one of the most frequent and costly rice diseases in temperate rice-growing regions worldwide. The pathogen manifests itself at the seedling, tillering and flowering stages of crop growth causing losses on account of leaf, node- and neck-blast in the state.

The state of Tripura had large number of rice cultivars but mostly replaced by high yielding cultivars and they are grown both a *Kharif* rice or during in *Boro* season. In Tripura the poor productivity of rice has been identified as poor choice of rice cultivars, strongly acidic PH-4.5-5(49.20%) soil with low organic matter and higher phosphate fixation, poor adoption of modern agronomic package of practices and constraints due to pre-thora of pest and diseases and quickly growing weeds. Rice blast, brown spot and sheath blight are the major diseases in Tripura. After introduction of HYV, along with them, BLB, tungro and sheath blight have become major diseases. Recently diseases like sheath rot, false smut, stem rot and grain discolouration which were minor and occurring sporadically are emerging and causing considerable yield loss. This is primarily due to climate change, crop intensification and changes in practice. Out of the total yield loss due to diseases in rice, 35% is by blast, 25% by sheath blight, 20% by BLB and remaining 10% by other diseases. Due to high population growth emerging of new diseases and due to climate change and natural hazards like flood, drought and soil erosion pro-duction of rice in Tripura is a great challenge to meet the food demands for rapidly growing population. The farmers of Tripura have been complaining about the disease, which has wiped out almost half of their crop in particular area of some district. The disease is still threatening to drastically reduce yield.

Serious yield losses due to epiphytotic of blast diseases have been recorded in different regions in India, such as Tanjore delta, Nellore, Hyderabad, Bombay, parts of Orissa, Kashmir & Kerala. In India first recorded outbreak of blast in 1918 in Tanjore district of Tamil Nadu was reported by (MacRae, 1922) [16] who estimated the loss as 69%. In 1952, the crop was completely wiped out in Deras Farm in Orissa. In 1955-56 season the early rice was severely damaged by blast.

Biological control is another alternative and eco-friendly way to control diseases and reduce the use of agro-chemicals (Mishra and Singh, 2012) [17]. In biological control, new or resident

living organisms are purposefully used to suppress the activities and reproduction of pathogens (Pal and Gardener, 2006) [20]. The fundamental mechanism involves is reduction of disease incidence/severity by direct/indirect manipulation of microorganisms. As a result, understanding of bio-control of plant diseases through the interaction of bio-agent and pathogens, may allow us to manipulate the soil environment to make conditions favorable for successful bio-controlling/to improve bio-control strategies against the plant diseases (Chaur, 1998) [6]. Biological control is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which lead to serious ecological hazards. At present, synthetic pesticides are being used to manage plant diseases and microbial contamination in agricultural products. But, repeated and injudicious use of these agro-fungicides lends health hazards in animals/humans because of residual toxicity. In recent years, therefore, large numbers of synthetic fungicides have been banned in the western world because of its undesirable attributes. A number of bio-controlling agents (BCAs) are available for employing in agriculture production systems; however its adoption demands better understanding of the complex interaction among the plants, people and the environment. Although the value of eco-friendly pest (bacteria, fungi, insects, mites, nematodes, rodents, weeds, etc) management in sustainable agriculture has been well recognized, only very little is being adapted at field level. Fungi of genus *Trichoderma* and bacteria of *Bacillus* are the most promising bio-control agents against a range of plant pathogens under a variety of environmental conditions (Chen *et al*. 1983) [7]

Materials and Methods

The study was conducted in the Department of Plant Pathology, college of agriculture, Lembucherra Tripura. For routine phytopathological and analytical works, standard literatures were followed.

The rice blast pathogen namely blast *magnaporthe grisea* was isolated from rice leaves having the disease symptoms through tissue segment method (Rangaswami, 1958) [23]. The morphological identities of the isolated fungi were confirmed using the text of Booth and Sutton (1984) [3] and Chowdhry *et al.* (2000) [8]. Reproducibility of disease reaction/virulence by the isolates was confirmed following the detached leaflet technique (Foolad *et al*. 2000) [10] on rice cultivars.

BCAs used were *Trichoderma sp., Bacillus* spp and pseudomonas spp. *Trichoderma* spp. were sub-cultured in PDA and preserved at 5^0C. *Bacillus* spp.were sub-cultured in NAS following the aseptic technique. The cultures were renewed at 10 days interval to maintain the purity and potency.

The antagonistic potential of *Trichoderma* spp. against the test pathogen was assessed through the dual culture technique (Morton and Straube, 1955) [18]. Both pathogen and *Trichoderma spp.* were belonging to same age while testing. 6 mm diameter blocks of the pathogen and *Trichoderma spp*. were inoculated at the same time on the opposite sides of the PDA in petriplates (9 cm dia.). Then, the plates were incubated at 28 ± 1^0 C for 8 days. In each test, a control plate was maintained to compare the result. The antagonistic ability of *Trichoderma* sp was assessed on the modified Bell's scale (Bell *et al*. 1982) [2]. The hyphal interactions were assessed by growing them on the cellophane membrane placed over the solidified PDA (Dennis and Webster, 1971) [9]. Both the fungi when came into contact to each other, the contact zone was cut using sterile scalpel and taken out along with the cellophane. Then, it was gently washed with sterile distilled water, mounted under 0.1% lactophenol cotton blue over a clean glass slide and observed under a microscope. The hyphal interaction was photographed.

For *in-vitro* assessment of *Bacillus* spp., sterile PDA was poured into the sterilized petri-plates. After solidification of the medium, a loop of 24-48 hrs. old culture was taken from slants and streaked on one side of the plate. Fungal plugs were carefully placed on the opposite side of the bacterial streak. Both the bacteria and fungi of same age were used. Incubation was done in a BOD incubator at 30 ± 2^0C for 3-4 days. The length of fungal and bacterial growth and zone of inhibition was measured using a scale (mm). In each test, one control plate was maintained for comparison.

After *in vitro* assessment, the BCAs were evaluated under glasshouse condition in polythene bags (30 x15 cm) against blast pathogen following Thilagavathi *et al.* (2007) [26]. Briefly, a talc-based formulation was first prepared. For seed treatment, mixed with the formulation (@10 g/kg of seed) and shed-dried (Nandakumar *et al*. 2001) [19]. For soil treatment, the talc-based formulation was mixed with soil (@10 g/kg). And then seeds are hand dipped into each polythene bag. The plants were watered daily @ 50 ml/ bag. The design of experiment followed was completely randomized block design (CRBD) with two replicates for each combination. The percent disease index (PDI) was calculated following Mayee and Datar (1986) [15].

Results and Discussion

Antagonistic potential of bio-control agent

Antagonistic potential of *Trichoderma* spp.

The *Trichodermma* spp. has showed inhibitory effect on p. oryzae. The inhibition rate 72% in case of p.oryzae. (Table-1, Figure-1). The direct mycoparasitic activity of *Trichoderma* is one of the major mechanisms involved in this inhibition (Bruce *et al*. 1995 [4]; Haran *et al*. 1996) [12]. Maximum inhibition (72%) was produced by *Trichodermma* spp. which corroborated the finding of Pandey (2010) [21].

Table 1: Antagonistic potential of *Trichoderma* spp. against s*p. Oryzea*

Sl. No.	Bio-control agents	Point of contact (DAI)	Distance covered (cm) at final day of observation by		Antagonistic potential on modified Bell's scale (at final day of observation)	Percentage inhibition (%)
			Pathogen	Antagonist		
1.	*T.sp (Th)*	2 days	0.7	5.05	S_2	72.0

Table 2*:* Antagonistic activity of *Bacillus spp. against p. oryzea*

Sl. No.	Bio-control agents	Inhibition zone (cm)	Distance covered (cm) by pathogen in		Percentage inhibition (%)
			Dual culture	Control	
1.	*Bacillus.sp (Bs)*	0.80	2.60	5.7	53.38

Table 3: Antagonistic activity of Pseudomonas *sp. against p. oryzea*

Sl. No.	Bio-control agents	Inhibition zone (cm)	Distance covered (cm) by pathogen in		Percentage inhibition (%)
			Dual culture	Control	
1.	***Pseudomonas sp.***	0.80	2.12	5.9	64.06

Antagonistic potential of *Bacillus* spp.

Bacillus spp. showed inhibitory effect on *p.oryzea* (Plate-2.). The inhibition rate was 53.38% in case of *p.oryzea* (Table-2, Figure-2), which is similar to the finding of Souja *et al.* (2014) [24]. This inhibition was due to the secretion of many kinds of antibiotics, including mycosubtilin, and zwittermicin by the bacteria (Pal and Gardener, 2006) [20]. Inhibition was produced by *Bacillus sp* was due to secretion of Fengycin and bacillomycin (Cao *et al.* 2011) [5].

In case of *Pseudomonas sp*., inhibition rate was found 64.06 %. (Table-3, Figure-3), which is similar to the finding of Souja *et al.* (2014) [24] and Abdallah *et al.* (2015) [1]. This inhibition was due to secretion of hydrolytic enzyme (Fujimoto and Kupper, 2016.) [11], peptide antibiotics (Mannanov and Sattarova, 2001) [14], volatile extracellular metabolites (Podile *et al.* 1987) [22], mycosubtilin, and zwittermicin by the bacteria (Pal and Gardener, 2006) [20].

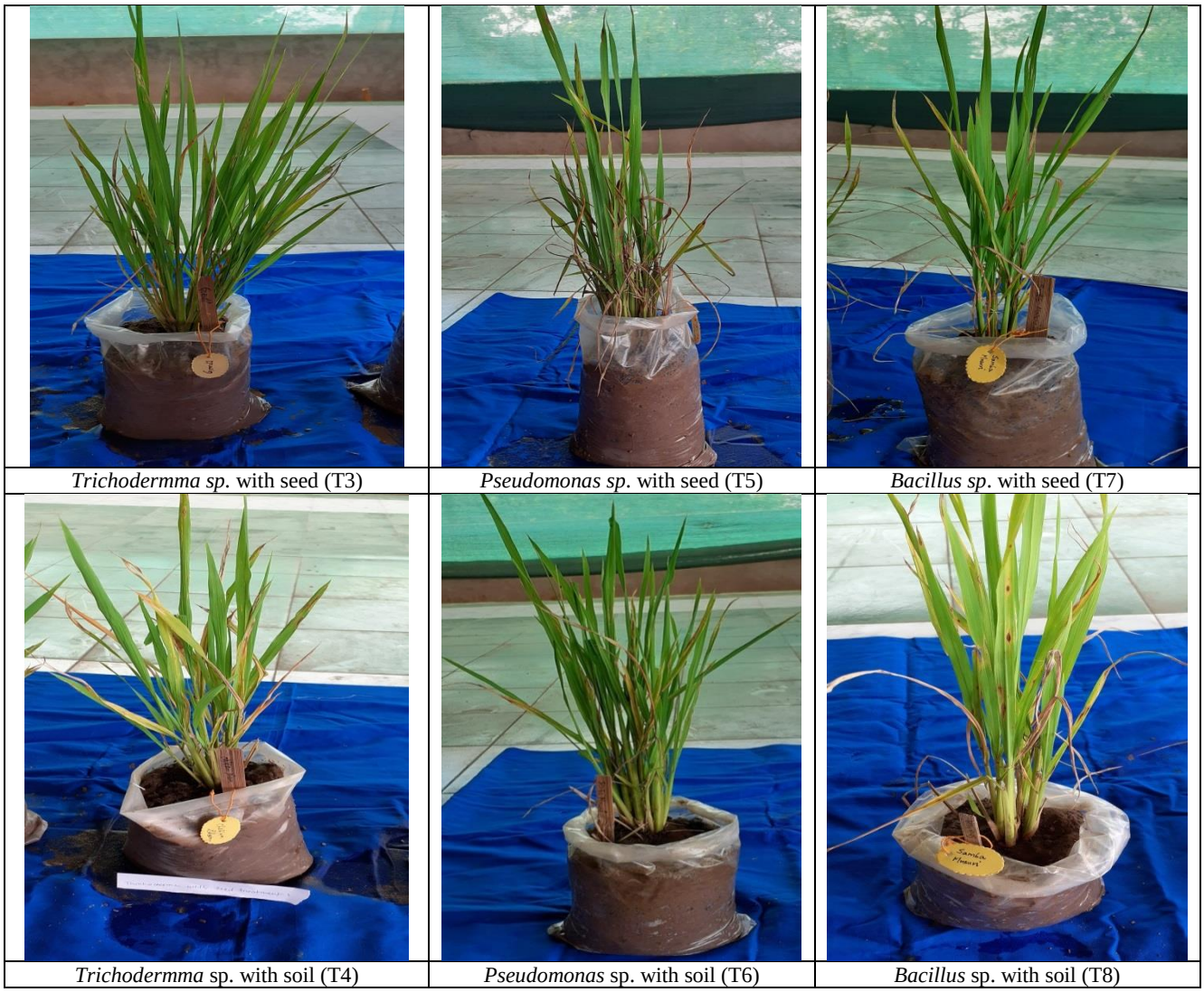
Fig 1: *Trichodemma sp*

Fig 2: *Bacillus sp*

Fig 3: *Pseudomonas sp.*

Healthy plant (T1)

Healthy plant with diseasesd inoculation (T2)



Trichodermma sp. with seed (T3) | *Pseudomonas sp.* with seed (T5) | *Bacillus sp.* with seed (T7)

Trichodermma sp. with soil (T4) | *Pseudomonas* sp. with soil (T6) | *Bacillus* sp. with soil (T8)

Table 4: Effect of bio-control agents and its consortia on the PDI and rice yield during pottrial (2 kg soil/pot) under net house condition

Treatments	Combinations	PDI (%)	Decrease in PDI over disease control (%)
T1	Healthy plant + No treatment (Negative control)	12.2	40.77
T2	Healthy plant + Disease inoculation (positive control)	20.6	-
T3	Seed treatment with *Trichodermma sp.* @10g/kg + foliar application of pathogen at 45 DAP	15.5	24.74
T4	Soil treatment with *Trichodermma sp.* @10 g/kg + foliar application of pathogen at 45 DAP	12.0	41.74
T5	Seed treatment with *Bacillus sp* 10 g/kg + foliar application of pathogen at 45 DAP	13.4	34.95
T6	*Bacillus sp* soil treatment @ 10 g/kg + foliar application of pathogen at 45 DAP	16.4	20.38
T7	Seed treatment with *Pseudomonas sp.* 10 g/kg + foliar application of pathogen at 45 DAP	11.1	46.11
T8	*Pseudomonas sp.* soil treatment @ 10 g/kg + foliar application of pathogen at 45 DAP	8.5	58.73

DAP (Days after planting)

The result of pot culture trial is presented in Table 4. The magnitudes of PDI and yield were varied from treatment to treatment. The PDI was 8.5% with *pseudomonas sp.* soil treatment @ 10 g/kg with foliar application of pathogen, 11.1% with seed treatment with *Trichoderma. sp.* @ 10 g/kg with foliar application of pathogen,12.0% with soil treatment with *Trichoderma sp* @ 10 g/kg + foliar application of pathogen, 12.2% in healthy plant with no treatment (Negative control), 13.4% with seed treatment with *Bacillus sp* @ 10 g/kg + foliar application of pathogen, 15.5% with seed treatment with *Trichodermma sp* @10g/kg + foliar application of pathogen and 16.4% with *Bacillus sp.* soil treatment @ 10 g/kg + foliar application of pathogen in comparison with 20.4% in healthy plant with disease inoculation (positive control).

This indicated that *pseudomonas sp* soil treatment @ 10 g/kg + foliar application of pathogen at 45 DAP is best among the treatment combinations tried. This might be due to its higher capability to inhibit the pathogen and to promote crop growth and yield through increased nutrients uptake stimulated by the growth of the promoting factors such as IAA and GA_3 and decreased levels of enzymes owing to colonization of roots (Idris *et al.* 2007) [13]. Seed treatment with the BCA (*B. subtilis*) has reduced the outbreak of disease in the crop during the pot trial. It might be due to microbial competition, antibiosis, hyperparasitism and induction of systemic acquired resistance in the host plants. BCAs have remarkable capacity of multiplication; thus, when the seeds treated with them, it might be multiplied in the exponential ratio and formed thick-walled spores around the seed to overcome with the stress

caused by the pathogens. Suleiman *et al.* (2016) [25] also reported PDI 30.76% and yield 2.78 q/ha with the use of *T. viridae* @ 2x10^7 CFU/g and *B. thuringiensis* @ 2x10^8 CFU/g.

Conclusion

In conclusion, out of three bio-control agents. (*Trichodermma sp*, *Bacillus sp* and *Pseudomonas sp*), *Trichodermma sp* (inhibition rate-72%) was the best bio-controlling agent against the blast pathogen. The result of pot culture trial, The magnitudes of PDI and yield were varied from treatment to treatment). From this study, it is clear that combination of *Trichodermma sp* soil treatment @ 10 gm/kg + seed treatment with *Bacillus* spp. @ 10 gm/kg + foliar application of pathogen at 45 DAP is best against the potato foliar disease.

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