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Efficacy of *T harzianum* isolates in plant growth promotion and BLB management in rice

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Abstract

Rice crop is highly vulnerable at all stages of growth to different pathogens that affect the quality and quantity of its yield. Among the different diseases, Bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*), a deadly bacterial disease is among the most destructive affliction of cultivated rice (*Oryza sativa* and *O. glaberrima*). In severe epidemics, crop loss may be as high as 75 percent, and millions of hectares of rice are infected annually. Biological control based on antagonism is a potential, non-chemical and eco-friendly approach for managing plant diseases. Biocontrol agents like Trichoderma spp. are acclaimed as effective, eco-friendly and cheap, nullifying the ill effects of chemicals. The present study involved five different isolates of *Trichoderma harzianum* viz. IRRI-2, IRRI-3, IRRI-4, IRRI-5, IRRI-6 obtained from International Rice Research Institute. Variations were observed while comparing the efficacy of different *Trichoderma harzianum* isolates, IRRI-5 and IRRI-2 isolates exhibited superior anti-bacterial activities in vitro. Rice crop was raised from Trichoderma treated seeds and further challenge inoculates with Xoo to induce disease in plants. All the *T. harzianum* treated plants had lower incidence of diseases and higher yield than the untreated plant. Yield attributing characters like plant height, days of active tillering, panicle initiation and maturity, total number of grains per panicle, number of filled grains per panicle was positively influenced by *Trichoderma harzianum* application. However, there were variations among the treatment, isolate IRRI-5 was found most effective followed by IRRI-2, IRRI-4, IRRI-3 and IRRI-6 respectively.

Keywords: *T harzianum*; Bio-control agents; Eco-friendly approaches; Yield attributing characters; *Xanthomonas oryzae* pv. *oryzae*

1. Introduction

Rice is central to the lives of billions of individuals round the world. Rice contributes 21% of world's per capita energy and 15% of per capita protein [1]. It is one of the most widely cultivated food crops of the world but the diseases caused by fungi, bacteria and viruses declining its yield. Among all the diseases infecting rice, bacterial leaf blight (BLB) or bacterial blight (BB) which is a vascular disease and systemic in nature, caused by *Xanthomonas oryzae* pv. *oryzae*. It is one of the most destructive diseases of rice in both irrigated and rainfed ecosystem. Infection occurs in nursery seedling, following transplanting and subsequently at booting or heading stage [2]. It is most serious and devastating at its kresek phase [3]. Some of the chemicals are effective against this disease but they leave harmful residues in soil and plants and also not cost effective. Lately accentuation has been given on eco-friendly management practices. Bio-control agents aid in the reduction of agricultural losses caused by plant pathogens by natural processes including a variety of modes of action such as myco-parasitism, antibiosis, competition, [4], and induced resistance [5]. Bio-control agents like

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Trichoderma spp. are acclaimed as effective, eco-friendly and cheap sources of stress beaters. They showed a high inhibitory effect against both biotic and abiotic stresses by means of different mechanisms like morphological and biochemical adaptations etc. exceeded the effect of chemical pesticides [6]. An experiment was conducted by G.P. Gangwar in 2013[7] showed that the fungal bio-agents mainly the *Trichoderma* isolates take a major role in reduction of bacterial leaf blight severity. In this investigation the impact of *Trichoderma harzianum* against *Xanthomonas oryzae* pv. *oryzae* was assessed and the variations among different isolates were quantified. Again the influence of *Trichoderma harzianum* isolates on morphological characters of plant was assessed and their variations were studied.

2. Material and methods

The present investigation was carried out from the month of March to July in 2019 at Department of Plant Pathology, OUAT, and Bhubaneswar. The analysis was led including five *Trichoderma harzianum* isolates obtained from International Rice Research Institute (IRRI) in the year 2019. This experiment was carried out which is based on CRD (Completely randomized design) with four replications in clay pots having a diameter of 12 inches. Desired quantities of seeds of Swarna variety were collected from OUAT and seedlings were raised during Kharif season after seed treatment with different strains of *T. harzianum*@10g/kg of seed and seedling root dip with spore suspension @10^6 cfu/ml for 2 hours before transplanting. The treatment comprised of *T. harzianum* isolates IRRI-2, *T. harzianum* isolate IRRI-3, *T. harzianum* isolate IRRI-4, *T. harzianum* isolate IRRI-5, *T. harzianum* isolate IRRI-6and a control (without any *Trichoderma* application).

2.1. Collection and Isolation of pathogen

The causal agent of bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae (*Xoo*)* was isolated on the symptoms basis, from nearer rice fields of the campus. A portion of affected along with of healthy tissue was cut from the diseased leaf and surface sterilized with 1% sodium hypochlorite (NaOCl) solution for one minute followed by washing with three changes of sterile distilled water. After drying on sterile blotting paper, small cut pieces (6-7) of rice leaves were transferred to nutrient agar (NA) medium and incubated at room temperature (25-27°C) for 48hrs. To get pure culture of the pathogen, the emerging colonies were sub-cultured onto NA medium plates.

2.2. Pathogenecity Test

Pathogenicity of Xoo was done by Inoculating of rice plants by leaf clipping method. Approximately 1-2 cm of the leaf tip is cut with scissors previously dipped in bacterial suspension just before tillering stage and covered with plastic polythene sheets for 72 hrs. Later stage (after 5-6 days) disease symptoms were observed.

2.3. In vitro efficacy of *Trichoderma* against *Xanthomonas oryzae* pv. *oryzae* (Xoo)

Inhibition zone method was used to evaluate the antibacterial activity of different of *Trichoderma* isolates against Xoo in vitro. For this 30ml of PDA medium was poured on a sterilized Petri plate and allowing it to solidify. Then by using sterilized spreader, a loop full of Xoo was spread over the Petri plate. After 24 hours, a 5 mm diameter of well was created in the center of the Petri plate containing bacterial lawn with the help of sterilized cork borer and 2 ml of broth containing bio-agents were poured into that well. In control sterilized distilled water was poured in the well. Observations regarding inhibition zone formed by bio-control agents against Xoo were recorded 5-7 days after inoculation by incubating at 28±2^{0}C.

2.4. Calculation of disease severity

Severity of disease was measured as percentage of tissue area infected out of the total leaf area examined. In each field, disease severity was determined by calculating the percentage average lesion area of 15 leaves collected. In the field, Bacterial blight severity was scored by using following scale [8]. It is a quantitative, which measures the amount of disease on a plant in terms of intensity of symptoms or damage. Disease severity (DS) can be calculated by using formula.

$$\frac{\text{Area of plant tissue infected}}{\text{Total area}} \times 100$$

$$\text{Now, Percent Disease index} = \frac{\text{Sum of all disease rating}}{\text{Total no of rating} \times \text{maximum disease grade}} \times 100$$

2.5. Observations of morphological characters

Different morphological attributes such as plant height (cm), number of tillers per pot, days to active tillering, panicle initiation and 50 % flowering, days to maturity, grains per panicle, fertility percentage and grain yield was observed.

2.6. Statistical analysis

The above experiment was carried out by using CRD with six treatments and each treatment comprised of four replications. The data obtained were subjected to factorial analysis of variation (ANOVA). Here SE(m)± & C.D.($p \leq 0.05$) values were maintained.

3. Results and discussion

3.1. In vitro efficacy of *Trichoderma* against *Xanthomonas oryzae* pv. *oryzae*

The five different isolates of *Trichoderma* tested for their antibacterial activity against *Xanthomonas oryzae* pv. *oryzae* revealed that all the isolates exhibited some degree of growth inhibition of test bacteria. However, there were variations among the isolates in their zone of inhibition. The maximum inhibition of *Xanthomonas oryzae* pv. *oryzae was* observed in case of T_1 (5.9mm) and it was followed by T_4 (5.5 mm) and the difference among them was statistically significant. Hence, they are superior over other isolates. The minimum zone of inhibition of *Xanthomonas oryzae* pv. *oryzae* was recorded in case of T5 (4.6 mm) followed by T2 & T3 with inhibition zone of 4.9 mm and 5.1 mm respectively. This suggests that fungal bioagents have anti-bacterial properties against the test pathogen that restrict the growth of the pathogen *in vitro*. It has been known that *Trichoderma* spp. restricts the bacterial development, through different mechanisms like competition for nutrient and space against the pathogens, parasitism by withdrawing nutrients from the harmful pathogens, and through antibiosis by production of different inhibitory metabolite products [9][10]. This kind of inhibition was probably due to nutritional competition and parasitism [11] or by activity of different defence mechanisms induced by the *Trichoderma* against the bacteria [12]. Similar kind of results were observed by G.P. Gnagwar in 2013[13] in which the efficacy of fungal bio-agents tested against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight of rice.

Table 1 Antibacterial activity of *Trichoderma* against *Xanthomonas oryzae* pv. *oryzae*

Treatments	*Trichoderma harzianum* isolates	Zone of inhibition (mm)
T1	IRRI-2	5.9
T2	IRRI-3	4.9
T3	IRRI-4	5.1
T4	IRRI-5	5.5
T5	IRRI-6	4.6
Control		0
SEm (±)		0.07
C.D.($p \leq 0.05$)		0.23

3.2. Effect of *Trichoderma harzianum* on incidence of disease and yield

The data depicted in Table 2 regarding the influence of *Trichoderma* treatment on the incidence of bacterial blight and grain yield of the cultivar. It revealed that all the five different isolates of *Trichoderma* had some degree of influence on disease management and yield of the plant. They all were significantly better than the control where no *Trichoderma* was used. The isolate IRRI-5 recorded least disease incidence (25.42%) and yielded highest amount of grain (48.74 g per plant) and the least effective among the *Trichoderma* treatments was isolate IRRI-6 which recorded as high as (44.33%) disease incidence and yielded only 35.11 g per plant. The isolate IRRI-5 which recorded least incidence of disease and highest yield was followed by IRRI-2 and IRRI-4 with a disease incidence and yield of 29.51%, 45.16 g per plant and 35.38% and 41.30 g per plant respectively. The difference between the two in respect of disease incidence as well as yield was highly significant. Similar findings were also reported by Singh *et al.,* 2012[14] in which *Trichoderma*

harzianum isolates have positive responses on the seed germination and yield of the rice crop and also reduced the intensity of rice blast disease.

Table 2 Incidence of disease (BLB) and grain yield as influenced by *Trichoderma harzianum* isolates

Treatment	*Trichoderma harzianum* isolates	Percent disease index	Yield (Gram/plant)
T1	IRRI-2	29.41	45.16
T2	IRRI-3	39.43	37.41
T3	IRRI-4	35.38	41.30
T4	IRRI-5	25.42	48.74
T5	IRRI-6	44.33	35.11
CONTROL		56.11	29.67
SE(m)±		0.44	0.42
C.D. (p ≤0.05)		1.34	1.28

3.3. Influence of *Trichoderma* treatment on morphological characters of rice plants

Table 3 Influence of *Trichoderma harzianum* on morphological characters of rice plant

Treatments	*Trichoderma isolates*	Plant height(cm)	Days to active tillering	Panicle initiation (days)	Days of maturity	Total no of grains per panicle	Number of filled grains per panicle	Fertility percentage (%)
T_1	IRRI-2	95.32	43	89	147.75	178.75	147.25	82.37
T_2	IRRI-3	90.42	45	90	148.50	172.25	133.00	77.21
T_3	IRRI-4	92.37	45	90	148.50	170.50	136.25	79.91
T_4	IRRI-5	95.33	43	89	146.25	184.75	156.50	84.70
T_5	IRRI-6	88.51	46	91	148.75	166.25	125.25	75.33
Control	-	87.41	47	93	151.25	154.50	113.25	73.30
SE(m)±		0.43	0.40	0.64	0.85	4.97	6.84	
C.D.(p≤0.05)		1.29	1.32	1.93	2.56	1.66	2.28	

Intervention of *Trichoderma* in the plants leads to plant growth promotion through different morphological adaptation like increasing in root length, increasing in plant height, and inhibition of deleterious root microflora, that enhance the water and nutrients uptake of the plant [15]. *Trichoderma harzianum* treatment resulted in significant increase in plant height over the untreated plants. A variation was observed among the *Trichoderma* isolates in increasing the rice plant height. Among the different isolates used, *Trichoderma harzianum* IRRI-5 resulted in maximum increase in plant height. In the present investigation *Trichoderma* promotes plant growth by enhancing nutrient uptake, which resulted in early tillering. There was a considerable advancement in time taken for active tiller production in comparison to untreated plants. There was considerable advancement which was evident from early tillering, panicle initiation and maturity. The intervention by *Trichoderma* influenced the total number of grains per panicle and number of filled grains per panicle. All the *Trichoderma* isolate treated plants recorded higher number of filled grains per panicle over control. However, variations were noticed among the isolates of *Trichoderma* indicate of their efficacy. *Trichoderma's* role in better nutrient absorption, root and shoot development, plant vigour, and biotic/abiotic stress tolerance has been thoroughly reported [16]. Auxins, which are crucial for plant growth and root development, are produced by

Trichoderma spp [17]. Auxins are key hormones effecting plant growth and it has a positive impact on different morphological attributes of rice plant. In this present study *Trichoderma* treated plants showed enhancement of growth promotion as well as higher yield of the crop.

4. Conclusion

The study revealed that *Trichoderma harzianum* intervention in the present investigation resulted in significant increase in plant height. The tillering and panicle initiation stages in the crop were considerably advanced in plants treated with *Trichoderma*. Despite of all these advancement, the time required for maturity showed marginal variation. The intervention by *Trichoderma* also influenced the total number of grains per panicle and total number of filled grains per panicle that means high fertility percentage. All the *Trichoderma* treated plants, recorded higher number of grains per panicle and total number of filled grains per panicle over control plants. However, there were variations among the treatment, isolate IRRI-5 was found most effective followed by IRRI-2, IRRI-4, IRRI-3 and IRRI-6 respectively. It can be concluded from the current study that *Trichoderma harzianum* is not only a bioagent but also has multiple benefits in plant. The use of different isolates and record of variations among them in rendering benefit is a new concept which needs to be further investigated.

Compliance with ethical standards

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Disclosure of conflict of interest

No actual or potential conflict of interest in relation to this article.

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