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Biological control of rice blast disease with *Trichoderma spp.* under upland rice System

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ABSTRACT

Rice blast caused by Magnaporthe oryzae *is one of the most devastating diseases of rice and account for yield loss up to 65% in susceptible cultivars of rice.* Trichoderma *spp. has emerged as a potential biological control agent against many plant pathogenic fungi and also improved plant growth parameters. An experiment was carried out to evaluate the efficacy of seed treatment with different* Trichoderma *spp. isolate against leaf blast in four rice varieties; Swarna, IR–64, Samba Mahsuri, and Sahbhagi Dhan under upland rice conditions at Almora and Hazaribag.* Trichoderma *spp. (isolate Th–3) treated seed of Samba Mahsuri (57%) showed maximum plant height percentage followed by Tv–12 isolate with Samba Mahsuri (44%) as compared to control. It also increases root length (51–93%), total number of leaves (6.–60.%), tillers (3.–41%), panicles (4–39%), flag leaf length (2.–30%) and panicle length (5–32%) as compared to untreated control. The* Trichoderma *treated seed showed low disease intensity at Almora and Hazaribag as compared to untreated seed. The present study showed that it reduces the disease intensity by 10–25%, suggested that* Trichoderma *spp. may be used as bio–inoculants for biological control of rice blast disease.*

Key words: *Magnaporthe oryzae, rice blast, Trichoderma, upland rice*

Rice is the most important staple food grain for more than two billion people living in the rural and urban areas of humid and sub–humid Asia. It accounts for 30 to 50% of agricultural production and 50–90% of the calories consumed by these people (Hossain and Fischer, 1995). A substantial portion of the yield potential of rice remains low as compared with world average due to the resurgence of pests, diseases, and weeds. *Magnaporthe oryzae* (anamorph of *Pyricularia oryzae* Cav.) causes rice blast disease in rice cultivation areas worldwide (Chin 1975; Kato 2001). It is estimated that each year rice blast causes harvest losses of 10–30% of the global rice yield (Talbot 2003), 1–100% in Japan (Kato 2001), 21–37% in Bali Indonesia (Suprapta and Khalimi 2012), and 30–50% in South America and Southeast Asia (Baker *et al.* 1997; Scardaci *et al.* 1997), leading to serious epidemics all over rice growing regions of the world, and contributing considerably to the cost of rice production. Disease severity has increased recently due to modern agricultural practices for increasing rice production *viz.*, use of high yielding rice varieties, excess nitrogen fertilization, exhaustive use of agrochemicals, increased plant population per unit area and continuous cropping with rice that favours the crop susceptibility to rice blast (Faria *et al.* 1982). Thus, minimizing the occurrence of disease epidemics and reducing year to year losses are essential to sustaining rice productivity so farmers often depends a lot on the use of fungicides to control the plant diseases. However, excessive use and misuse of synthetic fungicide pose definite problems such as environmental pollution, residual toxicity and fungicidal resistance and negatively influences the soil microbiota. (Gerhardson 2002). These challenges have lead to research into alternative sustainable agricultural strategies, with a strong focus on exploiting beneficial organisms, since studies have shown that various types of naturally occurring antagonistic microorganisms offer a practical

and economical alternative for the management of plant pathogens with a potential to emerge as an substitute to chemical control. This has been the focus of intense research throughout the world.

Numerous genera of fungi such as *Trichoderma, Penicillium, Gliocladium* and *Rhizopus* inhabiting the rhizosphere of crop plants have the ability to encourage plant growth by the production of phytohormones, degradation of complex substrates and suppression of pathogenic soil microbes and thus, considered to be an alternative to the use of chemicals in agriculture. Among this member of the fungal genus, *Trichoderma* has the potential for reducing existing reliance on the use of environmentally damaging and unsustainable chemicals necessary for disease control (Fantke *et al.* 2012). *Trichoderma* spp. is free-living fungi that have a wide spectrum of biotypes that ranges from efficient soil colonizers to non–plant symbionts (Cardona and Rodriguez 2006; Meraj–ul and Nandkar 2012) that live in the rhizosphere and are able to successfully colonize the plant epidermis. They have been used as biocontrol agents against different plant pathogens (Abeysingne 2007; Steindorff *et al.* 2012; Harman *et al.* 2004) and are supposed to antagonize plant pathogens through competition for the substrate, antibiosis and parasitism (Suarez *et al.* 2005). Therefore, it is well recognized as an effective biological control agent of plant diseases caused by soil borne fungi.

Several investigators have pointed out that *Trichoderma* species are appealing candidates for control of blast disease in rice (Abeysingne 2007; Lixuan *et al.* 2008; Steindorff *et al.* 2012). *Trichoderma* strains are able to grow in a wide range of pH and existing in all the soil types. They are capable of secreting hydrolytic enzymes and causing mycoparasitism of fungal pathogens of plants. In order to identify successful biocontrol agents, continuous screening of new isolates is desirable for the effective formulation of biocontrol agents against specific pathogens under different field condition is required before any biocontrol agent can be developed commercially (Ou 1985; Harman 2000; Baha 2002). Here, we present the field screening of three *Trichoderma* isolates already performed well in dual culture condition, against rice blast in blast–prone upland rice systems such as, ICAR–Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS) Almora and ICAR–Central Rainfed Upland Rice Research Station (CRURRS) Hazaribag. The effect of these *Trichoderma* isolates in field conditions was compared and data derived was statistically analyzed.

MATERIALS AND METHODS

Preparation of mass culture

The mass culture of *Trichoderma* spp. was prepared on clean and intact sorghum grains. The grains were pre-wetted by boiling them in water for 20–30 minutes so as to raise the moisture content of grains up to 40–45% and to make soft enough for the copious growth of the fungus. After boiling, the grains were spread on wire mesh so as to drains the excess water. The grains were mixed with gypsum (calcium sulfate 2%) and chalk powder (calcium carbonate 0.5%) on the dry weight basis to check the pH of the medium and prevent from sticking with each other. Clean glucose bottles were filled with such sorghum grains (100 g each) and then steam sterilized for 1–2 hr. The bottles were then allowed to cool at room temperature and inoculated with actively growing the culture of *Trichoderma* spp. then bottles were incubated at 25±2˚C for days. The bottles were shaken once or twice daily for quick and uniform colonization of the fungus and finally, *Trichoderma* culture was prepared as powder formulation.

Seed treatment

Seeds were surfaced sterilized with 0.1% sodium hypochlorite solution for 2 minutes, then washed thoroughly with sterile water. After surface sterilization, the seeds were treated with dried powder spore mass of *Trichoderma* spp. at 4g/kg and mixed comprehensively to ensure uniform coating. The coated seeds were kept in the refrigerator for 24 hr and next day used for direct sowing in the field at 150 seeds per plot. Each plot consisted of 5 rows of 3 m length with spacing (15 cm x 10 cm). All the recommended package of practice was followed to raise a good crop. Observation of plant height, root length, total number of tillers, total number of leaves, total number of panicles, flag leaf length, panicle length and disease incidence at 30, 60, 90 and 120 days after sowing (DAS) were recorded on ten randomly selected plants from each entry for both treated and untreated in each

replication, and their means were used for statistical analyses. There were three replications arranged in a randomized block design.

Disease assessment

For the assessment of rice blast, ten plants were selected randomly from both treated and untreated plots in each replication. Five leaves from the top of each culm were taken for observation. Now the disease area was calculated and scoring was done according to the rating scale 0–9 scale (Table 1) developed by International Rice Research Institute (1996) and then it was converted into percent disease intensity by using formula.

Disease intensity (%) = (area of disease score/9) x 100

Disease scoring area (%) = (area of leaf affected / total leaf area) x 100

Statistical analysis

The data were analyzed using the IRRISTAT v.92-1 programme developed by the Biometric Unit, International Rice Research Institute, the Philippines. Data were subjected to analysis of variance (ANOVA). The treatment means were compared by Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

The present study revealed that *Trichoderma* spp. showed significant responses on plant height, root length, total number of tillers, total number of leaves, total number of panicles, flag leaf length, panicle length and disease incidence as compared to untreated control (Table 2–6). Similar results were also reported by other workers (Mathivanan *et al.* 2006, Singh *et al.* 2012). *Trichoderma* treated seed of rice varieties showed a range of mean performance of root length from 51.0 to 93.33%, at 30 DAS, the total number of tillers, the total number of leaves 0 to 41.1%, 0 to 41.23% and 6.06–60.70%, 7.18–39.68% at 60 DAS and 90 DAS respectively. The total number of panicles, flag leaf length and panicle length at 90 DAS shows 0–38.83%, 2.03–29.95% and 5.40–32.43%, respectively. Whereas plant height showed increase in performance for all the three stages such as 30 DAS, 60 DAS and 90 DAS as 11.0–57.66%, 9.90–43.75% and 9.17–24.4% respectively as compared to untreated control. *Trichoderma* spp. enhance plant height of all rice varieties, at 30 DAS highest for Samba Mahsuri with Th–3 isolate (57.66%) followed by Tv–12 isolate (44.33%) and lowest for Swarna (19.83%) with IRRI 3 isolate (Table 2). For 60 DAS, highest plant height was observed in Samba Mahsuri with Tv–12 isolate (43.75%) followed by Sahbhagi Dhan Th–3 isolate (32%) and lowest for Swarna with IRRI 3 isolates (9.9%) (Table 3). In case of 90 DAS highest for IR64 with Tv–12 isolate (24.4%) followed by Th–3 isolate (16.93%) and lowest for IRRI 3 isolate (9.17%) (Table 4).

Highest root length at 30 DAS was recorded for IR–64 with Tv–12 isolate (93.33%) followed by Samba Mahsuri with Tv–12 isolate (87.05%) and lowest for Samba Mahsuri with IRRI 3 isolate (46.36%) compared to untreated control (Table 2). For the total number of tillers at 60 DAS, the highest number of tillers were recorded for IR64 with Tv–12 isolate (41.1%) and lowest numbers of tillers for Samba Mahsuri with IRRI 3 isolate (5.8%). In 90 DAS, highest numbers of tillers for Sahbhagi Dhan with Tv–12 isolate (33.92%) and lowest for IR64 with no change in tillers as compared with untreated control (Table 4). For total number of leaves at 60 DAS the highest was recorded

Table 1. Scale for rating of blast disease (0–9)

Scale description	
0	No lesions observed
1	Small brown specks of pin–point size or larger brown specks without sporulating center
2	Small roundish to slightly elongated, necrotic gray spots, about 1–2 mm in diameter, with a distinct brown margin
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area
5	Typical blast lesions infecting 4–10% of the leaf area
6	Typical blast lesions infecting 11–25% of the leaf area
7	Typical blast lesions infecting 26–50% of the leaf area
8	Typical blast lesions infecting 51–75% of the leaf area and many leaves are dead
9	More than 75% leaf area affected

Table 2. Effect of three *Trichoderma* isolates on plant growth of four rice varieties under 30 days after sowing

Rice varieties	Treatments	Plant height (cm)	Root length (cm)
Swarna	IRRI 3	44.7	15.1
	Th–3	51.3	16.3
	Tv–12	50.3	17.7
	Control	37.3	10.1
IR64	IRRI 3	43.3	16.2
	Th–3	44.7	17.2
	Tv–12	34.4	17.2
	Control	35.7	9.4
Samba Mahsuri	IRRI 3	33.3	14.1
	Th–3	47.3	16.6
	Tv–12	53.3	17.6
	Control	30.0	9.7
Sahbhagi Dhan	IRRI 3	40.3	13.9
	Th–3	44.7	15.1
	Tv–12	39.0	14.2
	Control	31.3	8.5

in IR64 with Tv–12 isolate (60.07%) and lowest for Samba Mahsuri (6.06%) with IRRI 3 isolate (Table 3). The same results were recorded for 90 DAS with 39.68% and 7.18% respectively. For a total number of panicles, flag leaf length and panicle length at 90 DAS, the highest number of panicles was found in Sahbhagi Dhan with Tv–12 isolate (37.58%) and lowest in IR–64 with no change in panicles compared with control (Table 4). For flag leaf length among the varieties, Samba Mahsuri showed the highest with Tv–12 isolate (29.95%) and lowest for IR–64 with IRRI 3 isolate (2.03%). The highest panicle length was recorded for Sahbhagi Dhan with Tv–12 isolate (32.43%) and lowest for Swarna with IRRI 3 isolate (5.40%). The results indicated that *Trichoderma* spp. promoted the plant growth in rice. The observations recorded in the present study are corresponding to the results of several other workers (Mishra *et al*. 2000 and Khan *et al*. 2005).

In case of disease intensity at both the places, ICAR–VPKAS, Almora and ICAR– CRURRS Hazaribag, disease intensity increased continuously in IR–64 and Sahbhagi Dhan up to 90 days after sowing and then became constant between 90–120 days after sowing, because these are medium duration maturity varieties, and at that time crops attained maturity, whereas varieties like Swarna and Samba Mahsuri showed continuous increase in disease intensity up to 120 days after sowing and then become constant because these varieties are long duration maturity. At Almora, the maximum reduction in disease intensity was recorded in Samba Mahsuri with Th–3 isolate (43.74%), followed by Swarna with Th–3 isolate (39.14%) and lowest reduction in Samba Mahsuri (13.46%) with IRRI 3 isolate (Table 5). Whereas at Hazaribag, the maximum reduction in disease intensity was recorded for Swarna with Tv–12 isolate (43.77%) followed by Sahbhagi Dhan with Tv–12 isolate (33.65%) and the lowest reduction was recorded for IR64 with IRRI 3 isolate (8.18%) compared to untreated control (Table 6). However, *Trichoderma* treated seed showed 24.85% to 43.50% disease intensity compared to untreated seed 40.40% to 50.37% in Almora, whereas in Hazaribag,

Table 3. Effect of three *Trichoderma* isolates on plant growth of four rice varieties under 60 days after sowing

Rice varieties	Treatments	Plant height (cm)	Total no. of tillers	Total no. of leaves
Swarna	IRRI 3	54.1	11.0	45.0
	Th–3	56.2	11.3	38.3
	Tv–12	60.5	12.7	47.3
	Control	49.2	9.3	33.7
IR64	IRRI 3	55.3	9.3	36.0
	Th–3	59.7	11.7	42.7
	Tv–12	63.0	12.7	45.3
	Control	49.0	9.0	28.3
Samba Mahsuri	IRRI 3	58.3	12.0	31.0
	Th–3	57.3	13.0	41.7
	Tv–12	69.0	14.7	45.0
	Control	48.0	12.0	33.0
Sahbhagi Dhan	IRRI 3	63.0	10.7	35.7
	Th–3	69.3	12.0	42.0
	Tv–12	61.7	13.3	45.0
	Control	52.5	10.0	31.7

Table 4. Effect of three *Trichoderma* isolates on plant growth of four rice varieties under 90 days after sowing

Rice varieties	Treatments	Plant height (cm)	Total no. of tillers	Total no. of leaves	Total no. of panicles	Flag leaf length (cm)	Panicle length (cm)
Swarna	IRRI 3	94.5	13.3	61.3	10.2	29.22	29.19
	Th–3	92.7	12.3	51.7	11.0	25.11	29.37
	Tv–12	98.3	14.3	63.3	11.5	30.00	33.22
	Control	85.0	11.3	47.0	9.0	23.56	27.63
IR64	IRRI 3	85.3	9.7	54.7	9.0	20.11	28.93
	Th–3	87.7	12.0	58.7	10.7	21.11	31.15
	Tv–12	93.3	12.7	63.0	12.3	23.00	33.11
	Control	75.0	9.7	45.0	9.0	19.78	25.26
Samba Mahsuri	IRRI 3	94.0	13.7	50.7	12.7	25.67	29.67
	Th–3	91.0	14.7	58.7	12.7	29.67	34.67
	Tv–12	96.0	16.0	61.3	15.3	30.89	35.52
	Control	86.1	12.7	47.3	11.3	23.78	27.48
Sahbhagi Dhan	IRRI 3	89.7	12.7	52.7	10.7	24.67	30.67
	Th–3	89.0	13.7	59.8	12.3	25.33	34.56
	Tv–12	92.3	15.0	63.7	14.3	27.10	36.78
	Control	82.0	11.2	46.3	10.3	22.67	27.56

Table 5. Effect of three *Trichoderma* isolates on disease intensity of four rice varieties at ICAR–VPKAS Almora

Rice varieties	Treatment	Seedling stage 30 DAS	Tillering stage 60 DAS	Flowering stage 90 DAS	Harvesting stage 120 DAS	Reduction in disease intensity(%)
Swarna	IRRI 3	14.84^{c}	28.73^{c}	37.16^{c}	38.99^{c}	16.59
	Th–3	12.49^{b}	22.60^{b}	33.44^{b}	36.77^{b}	39.14
	Tv–12	10.53^{a}	16.48^{a}	22.44^{a}	24.85^{a}	22.81
	Control	16.68^{d}	31.71^{d}	41.77^{d}	44.67^{d}	0.00
IR64	IRRI 3	12.23^{c}	23.73^{c}	35.37^{c}	36.06^{c}	14.9
	Th–3	10.68^{b}	19.62^{b}	34.77^{b}	35.77^{b}	15.6
	Tv–12	9.50^{a}	17.33^{a}	24.19^{a}	26.59^{a}	37.30
	Control	14.84^{d}	31.59^{d}	38.87^{d}	42.40^{d}	0.00
Samba Mahsuri	IRRI 3	13.55^{b}	27.82^{c}	41.19^{c}	43.59^{c}	13.46
	Th–3	11.35^{a}	20.33^{a}	28.71^{a}	28.34^{a}	43.74
	Tv–12	13.28^{b}	25.54^{b}	35.32^{b}	40.45^{b}	19.69
	Control	17.90^{c}	36.03^{d}	47.29^{d}	50.37^{d}	0.00
Sahbhagi Dhan	IRRI 3	16.23^{c}	24.65^{c}	34.87^{c}	35.32^{c}	13.58
	Th–3	10.98^{b}	23.34^{b}	27.23^{b}	30.65^{b}	25.02
	Tv–12	9.88^{a}	16.12^{a}	24.43^{a}	28.45^{a}	30.39
	Control	14.34^{d}	25.34^{d}	38.88^{d}	40.87^{d}	0.00

*Values are the mean of three replications. Means followed by a common letter are not significantly different at 5% level by DMRT.

Trichoderma treated seed showed 25.98% to 37.78% disease intensity compared to untreated seed 37.76% to 49.67%, therefore it reduced approximately 10–25% disease intensity. Similar results were reported by other workers (Singh *et al*. 2012; Khan and Sinha 2007; Biswas *et al*. 2010; Ha 2010). Baker (1986) reported that increased plant growth by *Trichoderma* spp. as an added advantage and is ascribed to the pathogens and production of growth regulatory factors. Enzymes produced by antagonists might have accelerated the degradation of organic materials and the release of nutrients leading the improved plant growth (Baby and Manibhusanrao 1993). It has also been demonstrated that bioagents produced different metabolites and antibiotics which encouraged plant growth directly or indirectly (Kloepper *et al.* 1991). Fungal bioagents have been used successfully as seed or rhizosphere inoculants for the biocontrol of several plant pathogens (Mishra and Sinha 1997). The results of present findings suggest that there has been a positive response on the enhancement of plant growth and also reduced the intensity of blast disease of rice. Therefore, it can be used as effective biocontrol measure for rice blast besides increasing the yield of the rice crop.

Table 6. Effect of three *Trichoderma* isolates on disease intensity of four rice varieties at ICAR– CRURRS Hazaribag

Rice varieties	Treatment	Seedling stage 30 DAS	Tillering stage 60 DAS	Flowering stage 90 DAS	Harvesting stage 120 DAS	Reduction in disease intensity(%)
Swarna	IRRI 3	15.94[c]	30.13[c]	34.65[c]	37.78[c]	18.24
	Th–3	13.29[b]	23.20[b]	32.63[b]	35.87[b]	22.37
	Tv–12	12.34[a]	16.73[a]	23.62[a]	25.98[a]	43.77
	Control	18.23[d]	34.32[d]	42.45[d]	46.21[d]	0.00
IR–64	IRRI 3	11.34[c]	20.56[c]	33.38[c]	34.67[c]	8.18
	Th–3	12.54[b]	18.76[b]	32.34[b]	35.23[b]	6.70
	Tv–12	8.98[a]	16.44[a]	23.65[a]	25.78[a]	31.72
	Control	15.63[d]	30.76[d]	36.99[d]	37.76[d]	0.00
Samba Mahsuri	IRRI 3	15.76[b]	29.56[c]	40.16[c]	41.43[c]	16.59
	Th–3	12.65[a]	22.22[a]	26.45[a]	30.23[a]	39.14
	Tv–12	14.23[b]	23.67[b]	30.78[b]	38.34[b]	22.81
	Control	18.45[c]	34.24[d]	41.98[d]	49.67[d]	0.00
Sahbhagi Dhan	IRRI 3	16.23[c]	24.65[c]	34.87[c]	35.32[c]	11.41
	Th–3	10.98[b]	23.34[b]	27.23[b]	28.65[b]	28.14
	Tv–12	9.88[a]	16.12[a]	24.43[a]	26.45[a]	33.65
	Control	14.34[d]	25.34[d]	38.88[d]	39.87[d]	0.00

*Values are the mean of three replications. Means followed by a common letter are not significantly different at 5% level by DMRT.

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