Biological Forum – An International Journal **14(1): 1424-1431(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Biochemical Validation of Rice Blast Incidence Reduction due to *Trichoderma* Application

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(Received 30 November 2021, Accepted 09 February, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Rice crop is extremely sensitive to attack by different pathogens at all phases of development, affecting both quality and quantity of its yields, which results in lower productivity. Among the different diseases, blast disease is considered the principal fungal disease of rice and is the major threat to rice production due to its cosmopolite presence. Rice plants are also impacted by abiotic stresses such as drought, salinity, submergence, and so on, of which, drought plays a significant role in reducing yield as well as growth and development of the plant. Bio-control agents, such like *Trichoderma* spp. are hailed as effective, eco-friendly, and cheap sources of stress beaters. Drought aversion using morphological adaptations, drought tolerance through physiological and biochemical adaptations, and accelerated drought recovery are all claimed to be mechanisms used by *Trichoderma* spp., a well-known beneficial fungus. Hence it was planned to mitigate blast and drought simultaneously using *Trichoderma harzianum* isolates to enhance the productivity of rice crops. The current study involved five different isolates of *Trichoderma harzianum viz.* IRRI-2, IRRI-3, IRRI-4, IRRI-5 & IRRI-6 were 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-fungal activities against blast pathogen *in vitro*. The study also revealed that *Trichodermaharzianum* intervention increased the growth of roots which in turn improved tolerance against drought. Among the different isolates *Trichoderma harzianum* isolate IRRI-5 was most effective one after showing longer roots. The leaf rolling which is an indicator of water stress condition was measured using a 0 to 4 scorecard. It was observed that *Trichoderma* treated plants had fewer rolling leaves in comparison to untreated plants. Biochemical characters like MSI (membrane stability index), proline, phenol, SOD (Superoxide dismutase), MDA (Malondialdehyade), lignin, APX (Ascorbate peroxidase) were also recorded. Various biotic and abiotic stresses increased the levels of many stress-induced metabolites and ROS (Reactive Oxygen Species) in rice leaves while there was a decrease in membrane stability index. *Trichoderma harzianum* colonization resulted in a decrease in proline, MDA as well as the increase in SOD, APX, lignin, and phenol content which justifies the lower incidence of diseases and less stress due to drought. *T. harzianum* isolate IRRI-5 was found to be the best multiple stress abater followed by IRRI-2, IRRI-4, IRRI-3, and IRRI-6 respectively.

Keywords: *Trichoderma harzianum*, drought, Biochemical characters, proline, ROS (Reactive Oxygen Species), SOD (Superoxide dismutase), MDA (Malondialdehyade), lignin, APX (Ascorbate peroxidase).

INTRODUCTION

Rice is a vital cereal crop belonging to the family Poaceae. The theme of International Year of Rice 2004 (IYR, http://www.fao.org/rice2004/ index_en.htm) - *"Rice is Life- reflects the importance of rice as a primary food source, and is drawn from an understanding that rice-based systems are essential for food security, poverty alleviation, and improved livelihood"*. Rice crop is subjected to attack by 50 diseases and disorders including 6 bacterial, 21 fungal, 4 nematodes, 12 viral, and 7 miscellaneous diseases and disorders (Hollier *et al.*, 1993; Webster & Gunnell, 1992; Javeen *et al.*, 2011 a & b). Among the various diseases, blast malady is taken into account as the principal fungal disease of rice and is the major threat to rice production due to its cosmopolite presence and its catastrophic congenital condition. Besides the biotic

stress mentioned above, rice plants are also subjected to abiotic stress such as drought, which has a significant impact on plant growth yield. Bio-control agents, such like *Trichoderma* spp. are hailed as effective, eco-friendly, and cheap sources of stress beaters. They showed a high inhibitory impact against both biotic and abiotic stresses that exceeded the effect of chemical pesticides. Different biotic and abiotic stresses led to a rise in many stress elicited metabolites and ROS (Reactive oxygen species) in rice leaves and decreasing MSI (membrane stability index) (Hasanuzzama *et al.,* 2021). Plants do possess a mechanism for scavenging ROS, the potency of which determines the ability of plants to tolerate the abiotic stress (Foyer *et al*., 1994). A variety of compounds are released by *Trichoderma harzianum*, that induce resistance response against different biotic and abiotic stresses. (Harman *et al.,* 2004). By increasing different antioxidant enzymes, secondary metabolites, and plant hormones, the *Trichoderma* association helps plants deal with abiotic stress like drought (Pandey *et al*., 2016; Mona *et al.,* 2017). Numerous examinations have indicated the root colonization of *Trichoderma harzianum* winds up in accumulated levels of plant proteins including various chitinase, peroxidase, lipoxygenase, hydroperoxides lyases, and compounds like phenols, phytoalexins and enhance cell membrane stability to give sturdy resistance against stresses (Nicolás *et al*., 2014; Brandão *et al*., 2019; Rawat, *et al*., 2011). Biochemical analysis of *Trichoderma*-inoculated plant samples reveals increased levels of SOD, peroxidase, glutathione reductase, glutathione –S- transferase (GST), and diverse detoxifying catalysts in the leaves. In the case of drought-induced plants, there is an increase in MDA content in both treated and untreated plants though the increase was less evident in *Trichoderma* treated rice plants (Shukla *et al.,* 2012). Among numerous compatible solutes, proline is one of the important molecules that have been proven to protect plants against singlet oxygen and free radical-induced damages (Sarker & Oba 2018). In this investigation the impact of *Trichoderma harzianum* against *Pyricularia grisea* and drought was assessed and also the variations among different isolates were quantified. Again the impact of *Trichoderma harzianum* isolates on biochemical responses and their variations were studied.

MATERIALS AND METHODS

The present investigation was carried out from the month of March to July in 2019 at Department of Plant Pathology, OUAT, Bhubaneswar. Five isolates of *Trichoderma harzianum* obtained from International Rice Research Institute (IRRI) in the year 2019 *were* used in this experiment. 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).

Collection and Isolation of pathogen. Rice plants affected by Blast disease was located in near paddy fields and leaf samples bearing the symptoms were collected for isolation of pathogen. 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. In a sterile Petri plate the leaf bits were inoculated containing oat meal agar medium, incubated at 27 ±2ºC, and the fungus development and growth was monitored on regular basis.

Pathogenicity Test. The pathogenicity of *P. grisea* which causes rice blast disease was confirmed by proving Koch's postulates. To make the conidial suspension, a 15-day-old single conidial culture of the isolate grown on oat meal agar was washed with 10ml distilled water. Inoculation was carried out soon after the panicle emergence and the spore suspension was injected 2 cm below the panicle base and the plants were covered with polythene bags moistened inside for 24hr to provide appropriate humid conditions during the initial stages of infection. Following incubation, the appearance and development of symptoms were monitored on regular basis. The infected leaves were collected and the pathogen was re-isolated when symptoms appeared after panicle base injection. When injected plants showed typical blast symptoms, the fungus' identification was established by comparing it to the original *P. grisea* culture.

***In vitro* efficacy of *Trichoderma* isolates against *Pyricularia grisea* through dual culture technique**

By placing a 5mm diameter cutting disc of *Trichoderma harzianum* isolates and the target pathogen opposite to each other in a Petri plate, the colony diameter of *P grisea* was measured and the percent inhibition was calculated. For control, a disc of *P. grisea* alone was placed in one Petri plate.

Drought tolerance study

Drought was instigated artificially by withholding water application for five consecutive days at the time of the flowering stage of the rice crop and cover by a thatch to keep away the entry of rainwater into the pots. The efficacy of *Trichoderma harzianum* isolates was observed on the drought-induced plant by taking different morphological attributes like root length and leaf rolling score. The pathogen inoculated plants and drought induced plants were kept separately in each treatments. Several plants were assessed and given a mean rolling score ranging from 0-4 (IRRI, 1976). (Swapna and Shylaraj 2017).

Scale	Rolling type
0	Not rolled means leaves are healthy
1	Leaves start to be folded
2	Leaves are folded (V shaped)
3	Leaves are fully cupped (U shaped)
4	Leaves are tightly rolled

Calculation and scoring of disease severity. After appearance of the disease symptoms percentage disease index (PDI) was calculated and scoring of disease severity was done by using the 0 to 9 scale (IRRI, 2002)

Biochemical analysis: After 14 days, three of the pathogen inoculated and water-stressed plants from each treatment were carefully uprooted separately. The collected leaves were washed properly and these samples were subjected to various biochemical analyses.

Cell Membrane stability index (CMSI). CMSI was calculated by using the formula given by Vincent (1947).

$$CMSI = \left(1 - \frac{C1}{C2}\right) \times 100$$

Where C_1- Conductivity of leaf samples after keeping it at 10°C for 24 h, followed by warming at 25°C

C_2- Conductivity of leaf samples after autoclaving for 15 min.

Ascorbate Peroxidase (APX). The Ascorbate Peroxidase (APX) level was assayed after purification and the enzyme extracts prepared from leaf samples and the absorbance was measured at 290 nm under UV–visible spectrophotometer (Sharma, and Dubey, 2004).

Proline content. Proline content of leaf sample was estimated using the method given by Bates *et al.*, (1973). The chromatophore development was quantified and OD was measured at 540 nm. A standard curve of proline was used for calibration and expressed as mg g^{-1} FW.

Lignin content. The lignin content of leaf samples was estimated by using the method given by Moubasher *et al*. (1982). Here 0.25 g dry material was ground with 4 ml of ether and centrifuge at 2000 rpm for 5 min and the sediments were washed with water, re-centrifuged and the supernatant was discarded. After repeated washing, 2 ml of 0.5N NaOH was added to the residue and extracted at 70-80°C after 12-16 hours. After cooling, 0.45ml of 2N HCl was added and the pH was adjusted to 7 – 8 with NaOH. Volume was maintained by adding 3ml of water, then again centrifuged at 2000g for 5min. 0.8ml of supernatant was taken to which 0.8ml of 0.1M sodium phosphate buffer was added and another aliquot of 0.8 ml of 0.1N NaOH was added to it and the pH was maintained to 12.3. The absorbance was measured at 245 nm and 350 nm. Finally, the lignin concentration was derived from the difference between A_{245} and A_{350} on pH 7.0 and 12.3 with buffer and NaOH respectively.

Superoxide Dismutase (SOD). The SOD assay was based on the capacity of the extracts to inhibit the photochemical reduction of nitro-blue tetrazolium(NBT) in the presence of the riboflavin – light – NBT system (Beauchamp & Frodovich, 1971) 50Mm. Potassium Phosphate buffer, 2µM riboflavin, 0.1Mm EDTA, 75µM nitro-blue tetrazolium (NBT), 50µM Methionine, 50µM enzyme extracts was mixed to prepare 0.3 ml of reaction cocktail and the pH was maintained to 7.8 and the volume was adjusted by adding distilled water. To calibrate the spectrophotometer, a blank was created that was devoid of enzyme and nitro-blue tetrazolium (NBT). NBT was used as reference in which but no enzyme was added. The reaction was started by adding 0.1 ml of riboflavin to the tube stand then placing them under two 15 W fluorescent lamps for 15 minutes. The reaction was halted when the lights were turned off and the tubes were placed in the dark. The absorbance was immediately measured at 560nm. The enzyme activity was measured in units per milligram of fresh weight of sample.

Malondialdehyde (MDA) content. The malondialdehyde (MDA) was measured in terms of lipid peroxidation in the leaf based on reaction with Thiobarbituric acid (TBA) and Trichloroaceticacid (TCA) (Draper, and Hadley, 1990). Fresh samples of the leaf (500mg) were homogenized in 10 ml of 0.1 % trichloroacetic acid (TCA). Then homogenates were centrifuged at 15000g for 5 min. Then 2ml aliquot of supernatant was taken and 0.5 % thiobarbituric acid (TBA) in 20% TCA was beaded into it. The mixture was heated at 95ºC for 30 minutes and then quickly cooled in ice bath followed by centrifugation at 10000g for 10 minutes. The turbidity that was suspended in the water was removed. The absorbance of the supernatant was measured using a UV visible spectrophotometer at 520 nm. The absorption coefficient of 155 $mmol^{-1}$ cm^{-1} was used to calculate the MDA content.

Estimation of Total phenol content. For estimation of Total phenol content (TPC), leaf tissue extract was prepared by grinding 1.0 g of fresh tissues into fine powder. To this, 20 ml of 0.1% TCA (Trichloro acetic acid) was added and the content was centrifuged at 12,000 rpm for 10 min at room temperature. The supernatant thus obtained was collected and used for estimation of Total phenol content (TPC). 2.5 ml of 10% Folin-Ciocalteu reagent (v/v) and 2 ml of 7.5% sodium carbonate were mixed with 0.5 ml of aqueous extract. The reaction mixture was incubated for 40 min at 45°C, and the absorbance was measured using spectrophotometer at 765 nm. As a standard phenol, Gallic acid was used (Singleton *et al*., 1999). The TPC was calculated as milligrams of Gallic acid equivalents per gram tissue extract.

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.

RESULTS AND DISCUSSION

***In vitro* efficacy of *Trichoderma* isolates against *Pyricularia grisea* through dual culture technique.**

The efficacy of different *Trichoderma harzianum* isolates were tested against *Pyriculariagrisea*at in vitro conditions. The radial growth of test fungus was significantly less as than the control indicating substantial antifungal activity of *Trichoderma harzianum* against the test fungus. The result presented in Table 1 revealed that among the different isolates of *T. harzianum*, minimum radial growth was obtained in case of isolates IRRI-5 (31.27mm), which implied maximum growth inhibition (59.12%) of test fungus. In case of isolates IRRI-2 the radial growth was obtained (31.80) followed by isolates IRRI-4(33.63), isolates IRRI-3(36.39) and IRRI-6 (39.27) respectively. All the isolates of *Trichoderma harzianum* inhibited blast pathogen to varied extent but the isolates IRRI-2 and IRRI-5 were superior among other isolates. An experiment was conducted by Kulmitra *et al.* (2017) where the bio-control potential of different *Trichoderma* species was evaluated against *Pyricularia grisea* and the effectiveness was evaluated showing 67-70% growth inhibition .Similar kind of studies on antifungal activity of *Trichoderma harzianum, Pseudomonas fluorescence and Bacillus substilis* were conducted by Ali *et al.* (2006); Gangwar *et al.* (2013) suggesting aggressiveness of the *Trichoderma* isolates against the phyto-pathogens with its antifungal and antibacterial activities.

Table 1: Influence of *Trichodermaharzianum* on growth of *Pyriculariagrisea.*

Treatments	*Trichoderma harzianum* isolates	Radial growth of P grisea in 90 mm plates(mm)	Percent Inhibition(%)
T_1	IRRI-2	31.80	58.42
T_2	IRRI-3	36.39	52.42
T_3	IRRI-4	33.63	56.03
T_4	IRRI-5	31.27	59.12
T_5	IRRI-6	39.27	48.65
Control		76.5	
SE(m)±		0.56	
C.D.(p≤0.05)		1.74	

Effect of *Trichoderma harzianum*isolates on incidence of disease and yield. In pot culture trials, *Trichoderma* intervention by seed treatment, seedling root deep and foliar spray resulted in the reduction of disease incidence and increased yield of rice plants. The result presented in Table 2 revealed that all the isolates recorded significantly better results than the control (without *Trichoderma* isolates inoculation) however there were variations among the isolates. The blast disease was reduced most by IRRI-5 isolates followed by IRRI-2 isolates. The treatment with isolate IRRI-5 recorded the least incidence of disease (22.19 %) and highest grain yield (46.32 g/plant). It is followed by isolates IRRI-2 with a disease incidence of (24.35 %) and grain yield (43.83 g/plant) and isolates IRRI-4 with disease incidence (26.71 %) and grain yield (40.13 g/plant). Among the isolates, the highest disease incidence and lowest grain yield were observed in isolates IRRI-6 which is (31.24 %) and grain yield (35.26 g/plant) followed by isolate IRRI-3 (29.66 % PDI) and (38.47 g/plant) grain yield respectively. For many years, it has been known that *Trichoderma* spp. restricts the fungal development, mainly through three mechanisms: 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 (Harman *et al*., 2004). According to recent studies, *Trichoderma* spp. can also induce systemic and localized resistances, in addition, to directly attacking or limiting the growth of the plant pathogens (Harman *et al*; 2004; Lo *et al*., 2000). In addition, certain *Trichoderma* strains have a significant impact on plant growth and development (Hedge *et al*., 1962). Their ability to boost plant development has been recognised for a long time, and they may be found in natural field soils. Here in this experiment, disease incidence was significantly higher in control plants than all the treated pots and correspondingly the grain yield was lowest among all the treatments due to aforementioned mechanisms.

Table 2: Incidence of disease (BLAST) and grain yield as influenced by *Trichoderma harzianum*.

Treatments	*Trichoderma* Isolates	Percent Disease Index (PDI)	Yield(gram per plant)
T_1	IRRI-2	24.35	43.83
T_2	IRRI-3	29.66	38.47
T_3	IRRI-4	26.71	40.13
T_4	IRRI-5	22.19	46.32
T_5	IRRI-6	31.24	35.26
Control	-	41.22	28.94
SE(m)±		0.56	0.41
C.D. (p ≤0.05)		1.70	1.23

Effect of *Trichoderma harzianum isolates* in rice under water stress condition. In the present study, the data recorded on root length under the influence of different *Trichoderma* isolates presented in Table 3 revealed an increase in the root length. The longest root (18.05 cm) was observed in rice plants treated with *T. harzianum* isolate IRRI-2 followed by isolate IRRI-5 (17.33 cm). Among the rest of the treatments, isolate IRRI-4 treated plants recorded 15.65 cm long root followed by isolate IRRI-3 and IRRI-6 treated plants with root length of 15.23 cm and 15.01 cm respectively. According to Zaidi *et al.* (2014), the application of *Trichoderma* strains in plants increases root length, which aids in increased water acquisition and thus increases the plant's ability to resist abiotic stresses and nutrients uptake. The current study's findings support the aforementioned phenomenon. The primary direct effect of *Trichoderma* colonization was to promote root growth, regardless of water availability, which delayed rice plant drought responses. Similar findings were reported by Pandey *et al.* (2016), who made a report that, since the interaction between the plant and the *Trichoderma* fungus happens largely at the rhizosphere; therefore, showed better tolerance to drought as compared to untreated plants. Again during the water-stressed condition, the leaves showed pronounced rolling. The reaction of the plant was assessed by recording the extent of leaf rolling with the help of a 0 to 4 scorecard given by IRRI in 1976. Similarly, Bashyal *et al.* (2020) reported that priming seeds with *T. harzianum* delayed drought stress by 3 - 5 days. The data revealed that leaf rolling decreased with the efficacy of *Trichoderma* spp. More effective the *Trichoderma* spp. less was the leaf rolling and less was the drought stress on the plant (Table 3).

Table 3: Influence of *Trichoderma harzianum* isolates on root length and leaf rolling.

Treatments	*Trichoderma harzianum* isolates	Root length(cm)	Leaf rolling score
T_1	IRRI-2	18.05	2.25
T_2	IRRI-3	15.23	3.25
T_3	IRRI-4	15.65	3.00
T_4	IRRI-5	17.33	2.75
T_5	IRRI-6	15.01	3.5
Control		13.07	3.75
SEM (±)		0.15	0.28
CD(≤0.05)		0.46	0.86

Influence of *Trichoderma* treatment on biochemical changes in rice plants

Cell membrane stability index (CMSI). Because the untreated plants in this study had substantial nutrient loss, the CMSI value in control plants was much lower, at 25.33 percent. T4 performed best among the isolates, with reduced leakage and a higher CMSI value of 39.80 percent, followed by T1, T3, T2, and T5 correspondingly (Table 4). Both disease and drought stress disrupted membrane stability manifested as an increase in solute leakage. CMSI results showed a decreasing trend as time stress increased. Untreated plants had more leakage than *Trichoderma* treated plants (Shukla *et al.*, 2012). *Trichoderma harzianum* improves membrane stability by a process that involves raising the concentration of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, and others that serve as ROS scavengers and therefore aid in cell membrane stability (Hashem, *et al.*, 2014).

Ascorbate Peroxidase (APX). Here, the APX level is highest in the isolate IRRI-5 (297.33 moles ascorbate oxidized min^{-1} mg^{-1} protein) and lowest in control (221.70 moles ascorbate oxidized min^{-1} mg^{-1} protein). Among other isolates IRRI-2 showing the highest APX level (253.73) followed by IRRI-4 (244.19), IRRI-3 (236.26), and IRRI-6 (230.25) respectively (Table 4). The enzyme ascorbate peroxidase (APX) is considered as a scavenger of hydrogen peroxide which is formed in plant cells under normal as well as stressed conditions (Das and Roy Choudhury 2014). The present study revealed a significant increase in the ascorbate peroxidase level in *Trichoderma-treated* plants. *Trichoderma* inoculation improves plant stress resistance by increasing ascorbate peroxidase (APX), activity in the early stages after being faced with challenges. In this case the APX level is directly proportional to the efficacy of different *Trichoderma harzianum* isolates. The results obtained suggested that *T. harzianum*, mediated protection against *both diseases and drought stress* may be associated with a reduction of the oxidative burst in host cells. Similar findings have been demonstrated by Behera *et al.* (2018) in the past.

Superoxide Dismutase (SOD). Superoxide dismutase (SOD) is another enzymatic antioxidant that detoxifies the reactive oxygen species (Saed-Moucheshi, *et al.*, 2014). The present study observed a significant increase in SOD activity in *Trichoderma-treated* plants under disease and drought stress compared to untreated plants. However, the SOD activity varied with the *Trichoderma* isolates. Among the *Trichoderma* treated plants, isolates (IRRI-5) was found to be more effective in the context of SOD (5.12 U mg^{-1} fresh weight) followed by IRRI-2 (4.34 U mg^{-1} FW), IRRI-3 (3.65 U mg^{-1} FW), IRRI-4(3.63 U mg^{-1} FW) and IRRI-6 (3.27 U mg^{-1} FW) indicating the efficacy of *Trichoderma* isolates in inducing the production of SOD. SOD is one of the most efficient components of the antioxidant defence mechanism in plant cells against Reactive Oxygen Species (ROS) damage, according to Gill and Tuteja (2010).

Proline content. The data presented in Table 4 revealed that plants treated with isolate IRRI-5 (152.42 µg g^{-1} of FW) showed the least increase in proline content as

compared to control plants (201.10 µg g^{-1} of FW). A significant difference was found in proline accumulation in different isolates. Among other isolates the proline content was recorded as 156.63 µg g^{-1} of FW in IRRI-2, 166.22 µg g^{-1} of FW in isolate IRRI-4, 169.5 µg g^{-1} of FW in isolate IRRI-3, and 173.00 µg$g^{-1}$of FW in isolate IRRI-6 respectively. In our study, however, drought inducing metabolites accumulation such as proline increased considerably in drought stress conditions, but in the case of *Trichoderma* treated plants the increase was less evident. Here *Trichoderma harzianum* isolates were found to significantly reduce stress levels by regulating various physiological pathways. Shukla *et al.* (2012) reported that proline content was increased in rice plants amid drought stress, but that the rise was lower in *Trichoderma*-treated plants. The current study's findings are consistent with those of Shukla *et al.* (2012).

Malondialdehyde (MDA). In the present investigation, the MDA content was highest (6.94 µmol g^{-1} of FW) in untreated (control) plants. The accumulation of MDA was lowest (3.09 mol g^{-1} of FW) in T_4(IRRI-5) treated plants followed by T_1 (3.57 µmol g^{-1} of FW), T_3 (4.10 µmol g^{-1} of FW),T_2 (4.31 µmol g^{-1} of FW), T_6 (4.65 µmol g^{-1} of FW) respectively (Table 4). The malondialdehyde (MDA), which is indicative of oxidative stress, increases with the advancement of drought. The findings showed that MDA levels increased in both treated and untreated rice plants, though the rise was less evident in *Trichoderma*-treated rice plants. Additionally, according to Beggs *et al.* (1986), when plant development is inhibited by a stress factor, additional repair processes like as photo-reactivation, excision repair, quenching, and free radical scavenging may be triggered to attenuate the stress and prevent damage before it becomes lethal.

Phenol content. The *Trichoderma* treated plants recorded an increased level of phenol than the untreated plants. In this present investigation, the highest phenol content (3.03 mg g^{-1} of FW) under disease and drought stress was observed in plants treated with *T. harzianum* isolate IRRI-5. It was followed by isolate IRRI-2 (2.82 mg g^{-1} of FW), IRRI-4 (2.61 mg g^{-1} of FW), IRRI-3 (2.47 mg g^{-1} of FW), and IRRI-6 (2.11 mg g^{-1} of FW) respectively. All the *Trichoderma* treated plants showed a significant increase in the phenol content over control (1.14 mg g^{-1} of FW. Shukla *et al.* (2012) also demonstrated the beneficial effects of *Trichoderma* treatment on plant phenol content under stress conditions, stating that root colonisation by *Trichoderma* resulted in an increase in defense-related plant enzymes such as peroxidases, chitinases, 1,3-glucanases, lipoxygenase, and hydroperoxidelyase, which cause changes in plant metabolism, resulting in the accumulation of anti-microbial compounds such as phytoalexin. In an experiment was conducted by Surekha, *et al* in 2014 reported that the *Vigna mungo* plants pre-treated with *Trichoderma* species alone showed more accumulation of total phenol than controls and plants infested with pathogens.

Lignin content

When disease and drought were induced in plants the *Trichoderma*-treated plants showed a higher rate of lignifications than the control plants. The highest lignin content was observed in T1 (309.87 ml^{-1} g^{-1} dry weight) followed by T4, T2, T3, T6 with lignin content 301.38, 283.45, 272.50, 265.33 mg g^{-1} dry weight respectively (Table 4). The results were comparable to those of Vidhyasekaran *et al.* 2001, who found an increase in lignification after treating rice plants with *Pseudomonas fluorescens* and inoculating them with *Xanthomonas oryzae pv. oryzae.*

Table 4: Impact of *Trichoderma* on biochemical constituents of rice plant.

Treatment	*Trichoderma* isolates	Ascorbate Peroxidase (APX)(n moles ascorbates oxidized min^{-1} mg^{-1} protein)	CMSI %	MDA (µ mol g^{-1} FW)	Proline Content (µg g^{-1} of FW)	Phenol Content (mg g^{-1} of FW)	Superoxide Dismutase (SOD) (U mg^{-1} Of FW)	Lignin (mg gm^{-1} dry weight)
T_1	IRRI-2	253.73	37.50	3.57	156.63	2.82	4.34	309.87
T_2	IRRI-3	236.26	31.85	4.31	169.50	2.47	3.65	283.45
T_3	IRRI-4	244.19	35.35	4.10	166.22	2.61	3.63	272.50
T_4	IRRI-5	297.33	39.80	3.09	152.42	3.03	5.12	301.38
T_5	IRRI-6	230.25	31.23	4.65	173.00	2.11	3.27	265.33
Control		221.70	25.23	6.94	201.10	1.14	2.79	251.83
SE(m)±		1.62	0.69	0.309	0.93	0.09	0.13	1.24
C.D. (p ≤ 0.05)		4.86	2.07	0.103	2.80	0.28	0.43	3.73

CONCLUSION

In the present investigation, it was employed to mitigate the biotic stress due to blast and abiotic stress such as drought. Separate studies have shown that they help to reduce disease and drought stress. It was concluded that *Trichoderma harzianum* has numerous advantages in the plant and its effects on disease incidence, drought stress, and yield was linked with morphological and biochemical features, substantiating the findings. The utilization of several isolates and records of differences among them in providing advantages is a novel idea that should be studied further.

FUTURE SCOPE

Due to the side-effects of chemical pesticides, the sustainable crop production through eco-friendly pest management is essentially required in recent scenario. Evidence from the different sources *Trichoderma* has been regarded as a potent bio-agent and an adoptable technique in eco-friendly disease management strategy. The utilization of several isolates of different *Trichoderma* species and their efficacy against different biotic and abiotic stresses should be studied further.

Acknowledgements. I am inexpressibly ecstatic to extend my deep sense of my gratitude to luminous educationalist and esteemed chairperson of my advisory committee Dr. Akshaya Kumar Senapati, Professor, Department of Plant Pathology, OUAT, for his dexterous guidance, illuminating suggestions and unremitting assistance throughout the period of study, research and in completion of this thesis. I express my deep sense of gratitude and indebtedness to Dr. R. K. Panda, Assistant professor, Department of Plant Physiology for extending the necessary laboratory facilities for the partial fulfilment of my research work

Conflict of Interest. None.

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How to cite this article: Biswajit Jena, A.K. Senapati, Lipilipsa Priyadarshinee and Sandhyarani Nanda (2022). Biochemical Validation of Rice Blast Incidence Reduction due to Trichoderma Application. *Biological Forum – An International Journal, 14*(1): 1424-1431.