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TRICHODERMA HARZIANUM AS GROWTH STIMULATOR AND BIOLOGICAL CONTROL AGENT AGAINST BACTERIAL LEAF BLIGHT (BLB) AND BLAST OF RICE

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A B S T R A C T

Rice blast and BLB diseases caused by *Pyricularia oryzae* and *Xanthomonas oryzae* respectively has a significant impact on rice yield and quality worldwide. Biological disease control has been shown to be one of the most effective ways to manage rice diseases. The present study was conducted at Rice Research program, CSI, NARC, Islamabad to evaluate the *in vitro* and *in vivo* management of BLB and rice blast diseases by *Trichoderma harzianum. T. harzianum* inhibits almost 58% mycelial growth of the *P. oryzae* and 50% colony growth of *X. oryzae.* Effect of *T. harzianum* on seed germination was observed on agar media at three different concentrations (10^6, 10^8, and 10^{10}). *T. harzianum* at concentration of 10^{10} showed enhancement of plant growth; the maximum root length augmented from 38 to 47% and the shoot length was 15 % in KSK-434 and 44% in other three varieties as compared with control. Plant growth were examined under the impact of Volatile Organic compounds of *T. harzianum*. Root and shoot length were increased 24 to 48% and 15 to 39%, respectively compared with control. In the *in vivo* management, *T. harzianum* applied on the rice plants sown in pots. The disease pathogens *Xanthomonas oryzae* and *Pyricularia oryzae* were inoculated in rice plants, *T. harzianum* controlled the diseases at two concentrations i.e., 10^8 and 10^{10}. The disease was controlled 70 to 77% as compared to control. It was concluded that the characters as plant height, panicle length, number of tillers, and number of grains per panicle and yield of rice also increased with the application of *T. harzianum. T. harzianum* may serve an efficient biological agent in rice crop to mitigate the diseases stress.

Keywords: Rice, Rice blast, *Pyricularia oryzae*, *Trichoderma harzianum*, *Xanthomonas oryzae*, VOCs.

INTRODUCTION

Rice (*Oryzae sativa* L.) is a staple food for about 63% of the world's population and accounts for 20% of global caloric intake (Fahad *et al.*, 2019). Foreign rice trading is very limited; only 7.1% of the total rice produced in the world is traded internationally (Otsuka and Fan, 2021). Fragrant rice, such as basmati and jasmine varieties, accounts for 15 to 18% of the global rice trade (Baldwin & Childs 2011; FAO, 2012; sj, 2013). Rice is a major cash crop of Pakistan, accounting for 10% of total cropped land (Shah *et al.*, 2020). Pakistan is well-known for its Basmati rice, an aromatic rice variety. According toWebster and Gunnell (1992), 50 diseases and disorders can affect rice crops, including 6 bacterial pathogens, 21 fungal pathogens, 4 nematodes, 12 viral diseases, and 7 other types. Rice blast, brown spot, bacterial leaf blight, leaf streak, sheath blight, sheath rot, *Fusarium wilt* or *Bakanae*, *stem rot, Tungro virus, false smut*, and post-harvest infections are severe problems, too. These diseases are thought to have contributed to a worldwide yield decrease of between 14 and 18% (Chowdhury *et al.*, 2015). Rice is vulnerable to a number of diseases, the most severe of which are rice blast and bacterial leaf blight (BLB) diseases. *Magnaporthe oryzae* (syn:

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Pyricularia oryzae) is a filamentous ascomycete fungus that causes the disease. It has been identified in over 85 countries across the world (Jamal-u-ddin Hajano *et al.*, 2011; Salimah et al., 2019). Significant crop damages are caused by the BLB disease, which affects crops at a rate of between 70 and 80% (Basso *et al.*, 2011; Sere *et al.*, 2005). After repeated cultivation, BLB on rice becomes prevalent in Asian countries (Mew *et al.*, 1993). It is a significant disease because numerous improved and high-yielding cultivars were handled with high nitrogen, close spacing, and insufficient pathogen resistance. In the recent years, a number of biological control agents have been described, and they may be a useful addition to existing disease management techniques (Ajouz *et al.*, 2011; Elmer and Reglinski, 2006). Biological control refers to the use of natural enemies to combat infection. Bio-control agents are made from organic substances including plants, animals, bacteria, fungi, and some minerals. The harm posed by industrial fungicides is eliminated by fungus that exhibits mycoparasitic behavior (Nega, 2014).

Plant pathogenic fungi's development and activity are constrained by this process. Since the 1930s, numerous efforts have been made to utilize these helpful bacteria to prevent plant diseases due to their antifungal properties. *Trichoderma harzianum*, a soil fungus, has demonstrated the ability to operate as a mycoparasite against a variety of economically significant soil and aerial-borne diseases (ElaCarsolio *et al.*, 1994). *T. harzianum* appears manufacture and secrete mycolytic enzymes that lysis the cell wall at the point of contact, according to microscopic examination (Carsolio *et al.*, 1994). Extracellularly produced 1, 3-ßglucanases, chitinases, and proteases by *T. harzianum* are all essential for bio-control (Elad and Kapat, 1999; Carsolio *et al.*, 1994).

The present study was conducted to evaluate the effect of *T. harzianum* to control the rice diseases (BLB and Blast) under *in vitro* conditions and also check the effect of different concentration of *T. harzianum* to control these diseases in field conditions and in seed germination of rice plants and the effect of volatile organic compounds of *T. harzianum* on rice plants.

MATERIALS AND METHODS

This research was conducted at Rice program Crop Science Institute, NARC. The two Pathogens *P. oryzae* and *X Oryzae* and biological control agent *T. harzianum* were available in Rice Molecular Lab in CSI, NARC. Four commercial rice varieties were selected for this study. (Super basmati, Basmati 2019, KSK 282, KSK 434).

Dual culture Assay: Dual culture assay is used to check the antagonistic behavior of any microorganism under *in vitro* conditions. Fungal isolates were inoculated in potato dextrose agar (PDA) solution. *T. harzianum* disk from previously cultured plate was placed at the one edge of PDA plate and *P. oryzae* at the opposite side. For BLB, *T. harzianum* disk putted in the center of the Petri plate and liquid BLB culture poured on the four sides around the *T. harzianum*. Petrti-plates were sealed with a paraffin film. The Petri-plates were incubated at 25°C. Experiment was performed in 5 replicates. Petri-plates were observed after five days. Growth of *P. oryzae* and *X. Oryzae* towards the antagonistic *T. harzianum* in dual culture plates was measured using the following formula (Jagtap, 2022).

$$PI = \frac{C - T}{C} \times 100$$

PI = percent inhibition

C = growth of pathogen in control plates (cm)

T = growth of pathogen in dual culture.

Bio priming of Rice seeds with *T. harzianum* for seed germination: *T. harzianum* was grown on PDA for 7 days 28±2 °C, and then spores were harvested and brought up to a final concentration (10^6, 10^8, 10^{10}) colony forming unit (cfu) ml^{-1} through centrifugation at 10,000 rpm for 10 min (Mishra *et al.*, 2020b). The seeds of rice were surface sterilized with 1.5% sodium hypochlorite (NaOCl) for 5 minutes and then washed thrice with double distilled water. These seeds were dried under laminar air flow on sterilized filter paper (Jain *et al.*, 2012). The sterilized seeds were soaked in the spore suspension of *T. harzianum* and these seeds were dried in the laminar air flow (Singh *et al.*, 2013). The dried seeds were put in agar media plates. After 15 days of sowing, measured the root and shoot length and germination percentage of seeds (Khan *et al.*, 2005).

Effect of volatile organic compounds (VOC's) on growth of rice plant: This experiment was performed in rice molecular Lab CSI, NARC to evaluate the effect of *T. harzianum* on rice root length and shoot length. In this experiment, sterilized glass jars and pots were used. First glass jars were sterilized in the autoclave and media poured in these jars. Then the antagonistic agent *T. harzianum* was grown in these glass jars. On the other side rice seeds sown in pots. These pots were placed on the jars, sealed with paraffin tape and placed in the growth chamber. After 14 days, root and shoot length were noted (Tahir *et al.*, 2017). Experiment was performed in three treatments (*T. harzianum*, media, without media) with three replications for each treatment and also for varieties.

BIO-CONTROL ASSAY OF T. HARZIANUM AGAINST RICE DISEASES: The four rice varieties were cultivated in the pots at the experimental field of Rice Program, Crop Science Institute NARC. *T. harzianum* was grown on PDA for 7 days at 28±2 °C, and then spores were harvested and brought up to a final concentration (10^6, 10^8, 10^{10}) at 10,00 rpm for 10 min (Mishra *et al.*, 2020a). The conidial suspension of *P. oryzae* was adjusted at 10^6 and three concentration of *T. harzianum* at 10^6, 10^8, 10^{10} by using a serial dilutions method. Bacterial inoculum was prepared in 5ml of sterile distilled water @ 10^9 CFU/ml (Kauffman, 1973). To enhance the adherence of conidia to rice leaves, Tween 20 was added to gelatin (0.02% Tween 20 in 0.25% gelatin) to the suspension of *P. oryzae* and *T. harzianum* (Jia et al., 2003). After 24 hour of application of *T. harzianum, P. oryzae* was sprayed and *Xanthomonas Oryzae* was inoculated by clipping method (Kauffman, 1973; Bhusal *et al.*, 2018).

STATISTICAL ANALYSIS

The data were analyzed using analysis of variance (ANOVA) using statistical software SPSS version 17.0. All experiments were repeated three time.

RESULTS AND DISCUSSION

Dual Culture Assay: Evaluation of *T. harzianum* to control the rice blast and BLB diseases in the *in vitro* condition. *T. harzianum* significantly controlled the mycelial growth of *Pyricularia oryzae* by 58% and colony growth bacterial colony was reduced about 50%. (Kumar and Ashraf, 2019;Gangwar and Sharma, 2013). The visual result has been portrayed in Figure 1.

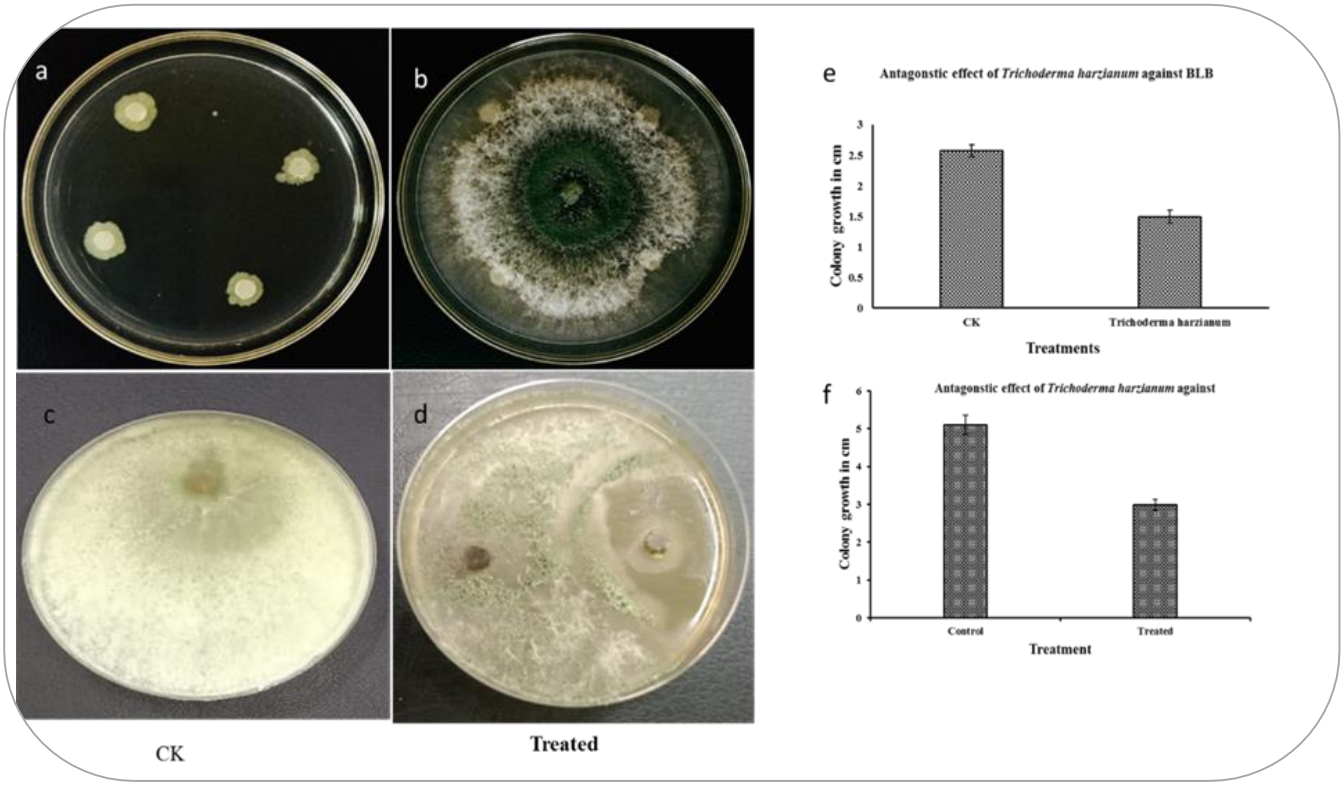

Figure 1. Control of rice blast and BLB in dual culture assay. Colony growth of BLB in control (a) Inhabitation of *Xanthomonas Oryzae* (b) Mycelial growth of *Pyricularia Oryzae* (c) Inhabitation of mycelial growth of *Pyricularia oryzae* (d) Graphical representation of inhabitation of BLB (e) and blast (f).

Bio priming of rice seeds with *T. Harzianum* for seed germination: In germination test, effect of *T. harzianum* on rice seeds showed significant growth as in root and shoot length as compared to the untreated seeds. The statistical analysis showed the significant difference in varieties and among the concentrations while performing the germination test. As our result depicted that in Basmati-2019, increase in root and shoot length was 55.59% and 44.81% respectively at concentration 10^{10}. While at concentration 10^8, 47.13% and 39.12% increase in root and shoot length. In other three varieties same results were observed. Super basmati, there was increase in root length was 30% at 10^8 and 45.17% at 10^{10}. Shoot length at concentration 10^8 was 35.68% and at concentration 10^{10} was 44.98%. KSK-282, increase in root length at 10^{8} concentration was 30.00% and at concentration 10^{10} was 45.17%. In KSK-434, root length was 27.50% at 10^{8} and 38.62 % at 10^{10} and there was increase in shoot length at 10^{8} 19.34% and at 10^{10} 15.70 %. Increase in root and shoot length at 10^{8} was also significant. Our results have similar finding of (Khan *et al.*, 2005). His finding showed that rice seed treatment with *Trichoderma harzianum* increase the root length and shoot length of rice seeds. The results indicated that *T harzianum* promoted the growth of rice seedlings (Fig)(Devi *et al.*, 2020)(Harman, 2006)(Sellal *et al.*, 2020), reported that the treated seeds had a higher rate of seedling emergence than control seeds. Additionally, the treatments improved the growth of the roots and shoots length in the agar media. The highest rate of *T. harzianum* concentration was most successful in enhancing plant vigor and growth. *Trichoderma* spp. can promote plant development, and this is because they produce growth regulating substances. The antagonist's enzymes may increase the breakdown of organic materials and the release of nutrients that promote plant development. Additionally, it has been found that certain metabolites and antibiotics produced by bio-agents both directly and indirectly encourage plant growth.

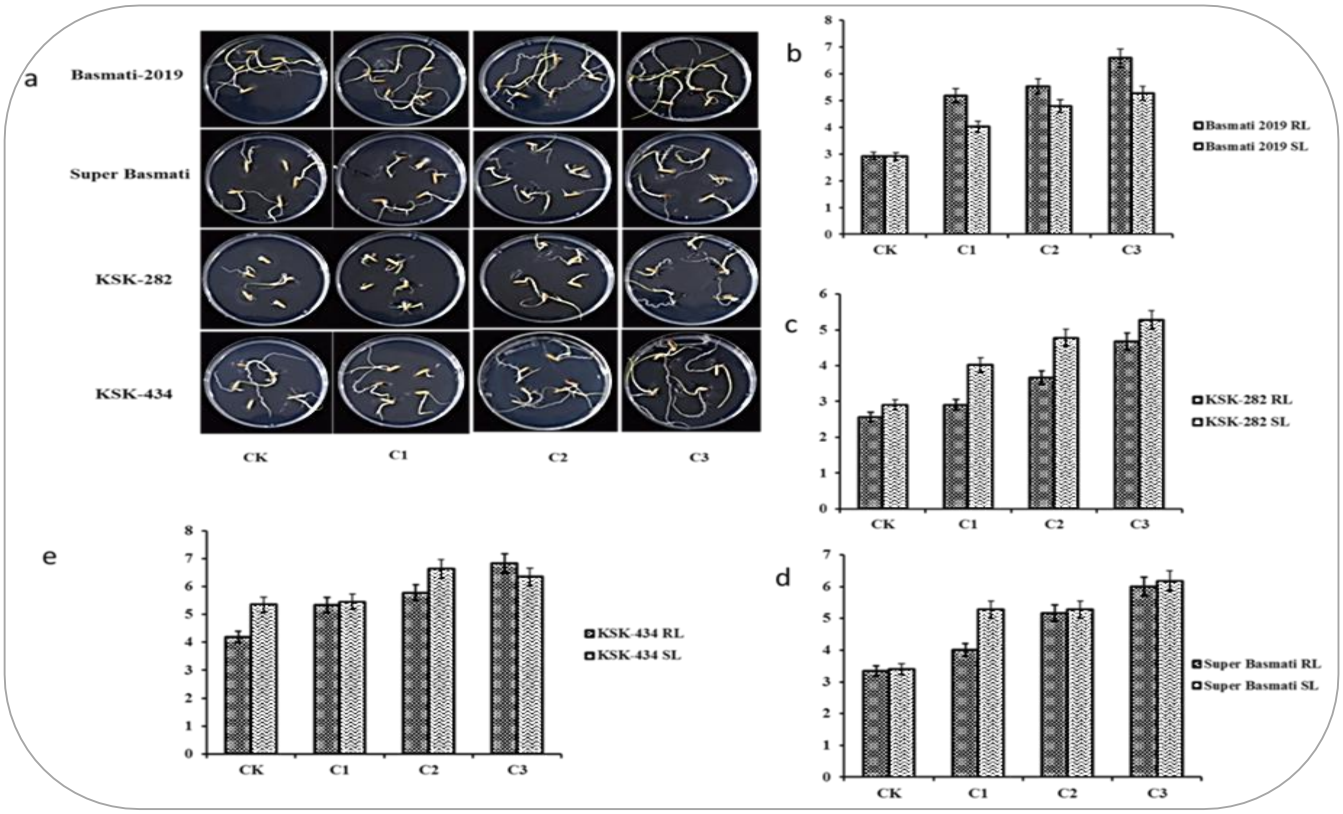

Figure 2. Bio priming of rice seeds with *Trichoderma harzianum* for seed germination. Different concentration of *Trichoderma harzianum* on seed germination of four commercial varieties of rice (a). Graphical representation of effect of different concentration of *Trichoderma harzianum* on root and shoot length of Basmati-2019 (b) KSK-282 (c) Super basmati (d) KSK-434 (e).

Effect of volatile organic compounds (voc's) of *T. Harzianum* on growth of rice plant: This experiment was performed to check the effect of *T. harzianum* on rice root length and shoot length. VOS's (volatile organic compounds) of *T. harzianum* showed the significant effect on root and shoot length of rice plants. The rice plants that were treated with *Trichoderma harzianum* show significant results as compared to control. The increase in shoot length of Basmati 2019 in treated was 15.17 % and in root length was 48.22 %. In super basmati the increase in shoot length and root length was 27.06 % and 24.20 % respectively. The increase in shoot length and root length in KSK-282 about 37.77% and 34.77 % respectively. In KSK-434, the increase in shoot length and root length 39.28% and 36.19% respectively. The rice seeds with media and without media showed the same results. Our results were similar to the finding of (Lee *et al.*, 2016). They reported that *Trichoderma* showed a variety of outcomes, such as larger plants, neutral effects, and, less frequently, growth suppression. Exposure of plants to

Trichoderma strains were often *Trichoderma* showed a variety of outcomes, such as larger plants, neutral effects, and, less frequently, growth suppression. Exposure of plants to *Trichoderma* strains were often longer and brighter in color longer and brighter in color. (Bailly and Weisskopf, 2012) said that despite the fact that we identified several VOCs that have been investigated as substances that aid plant development, none of these substances alone can explain the variances in growth that different *Trichoderma* strains produce. Fungi produce a lot of volatile organic compounds (VOCs), and as they develop, their volatile profile changes.

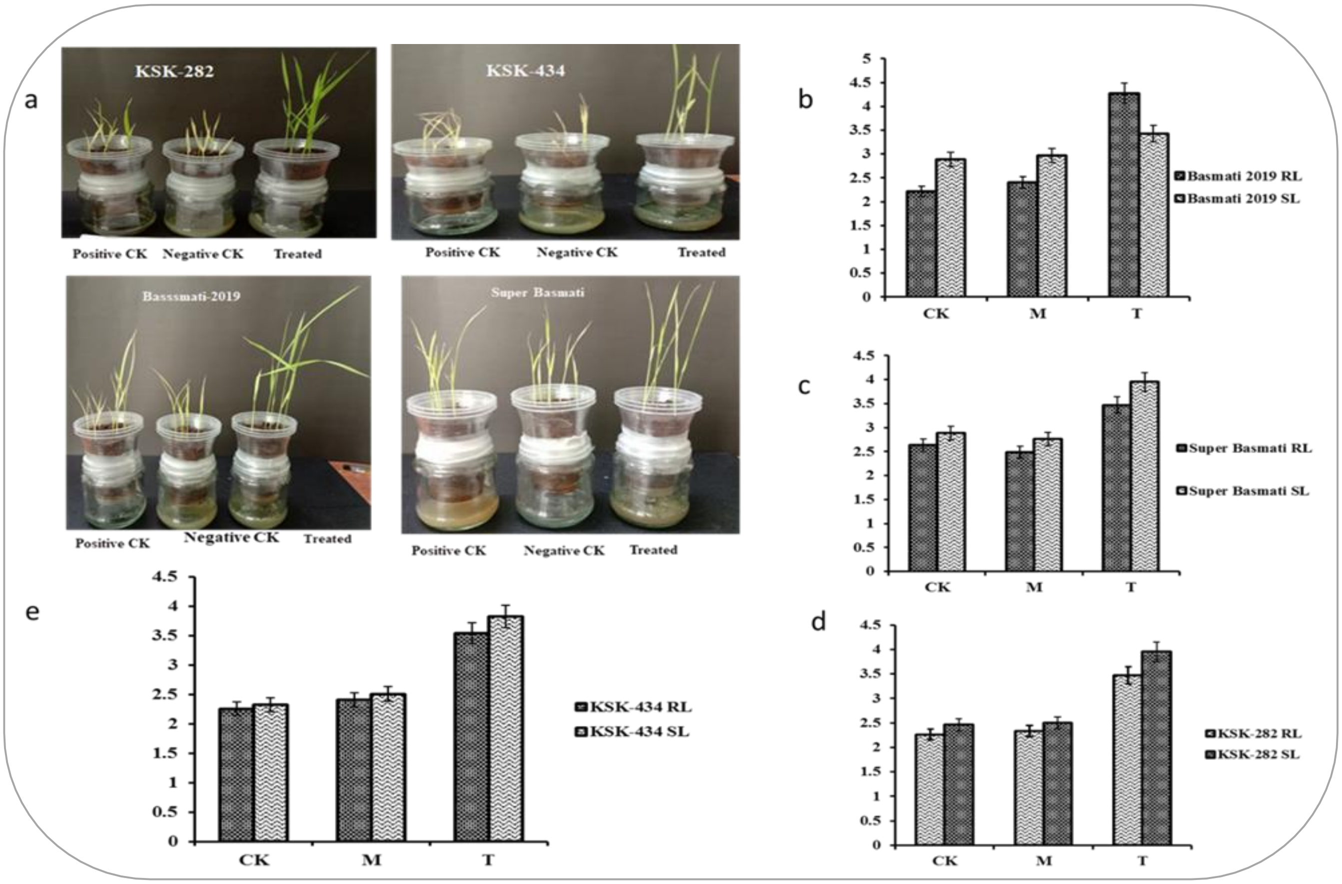

Figure 3. Effect of volatile organic compounds (VOCs) of *Trichoderma harzianum* on growth of rice plant. Effect of VOC's of *Trichoderma harzianum* on growth of four different varieties of rice plant (a). Graphical representation of VOC's of *Trichoderma harzianum* on Basmati-2019 (b), Super basmati (c), KSK-282 (d), KSK-434 (f).

Effect of *Trichoderma Harzianum* on Disease Intensity: The results show that *T. harzianum* have significant effect to control rice blast disease through foliar application of *T. harzianum*. Disease percentages in all concentrations significantly different. The disease intensity of BLB and Blast was around 25% and 20% at 10^{10} concentration respectively. While disease intensity in control of BLB and Blast was about 57% and 71% respectively. The disease percentage observed in 10^{10} was lowest as compared to the other 10^{8} and 10^{6}. About 75% and 80% disease reduction in BLB and blast at highest concentration of *Trichoderma harzianum.* At 10^{8} And 10^{6} the disease reduction was around 65% and 54% in BLB respectively. While in Blast the disease reduction was 72% and 46% at 10^{8} and 10^{6} respectively. In control the disease percentage were observed highest in all rice varieties. The rice plants that were sprayed with *T. harzianum* showed the diseases percentage lower than in control. Analysis of variance for the effect of *Trichoderma harzianum* to control the rice blast and BLB diseases showed the significant difference in concentrations and among the varieties. The findings of this study were almost relate to (Singh *et al.*, 2012). According to Gohel and Chauhan (2015), the *Trichoderma* foliar application technique was successful in decreasing the incidence of rice leaf blast and neck blast while increasing yield factors. Application of *T. harzianum* strain CEPA A-34 in a safe setting resulted in a drop in AULBPC of up to 67.5%(Pérez-Torres *et al.*, 2018).

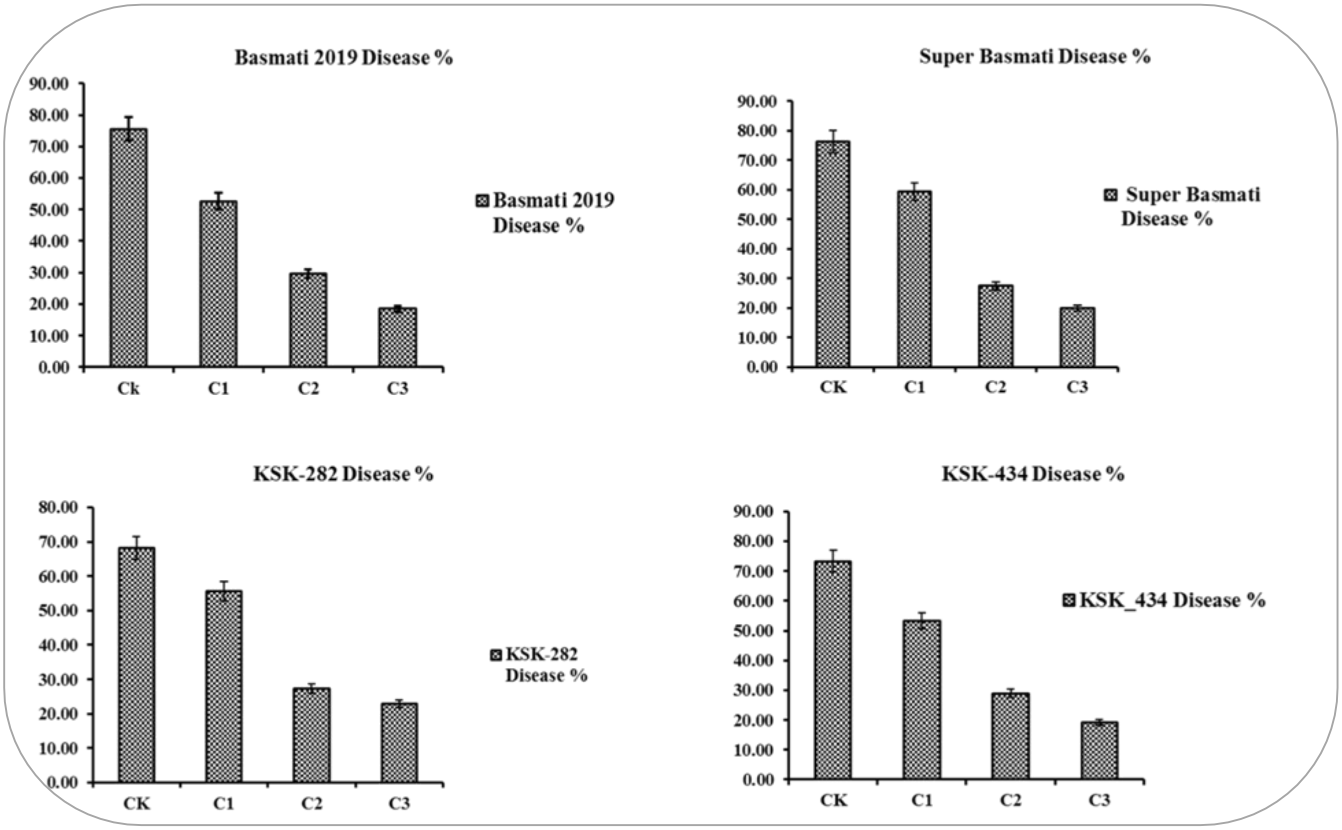

Figure 4. Graphical representation of effect of different concentration *Trichoderma harzianum* to control the rice Blast disease (*Pyricularia Oryzae*) in Basmati-2019 (a), Super basmati (b), KSK-282 (c), KSK-434 (d).

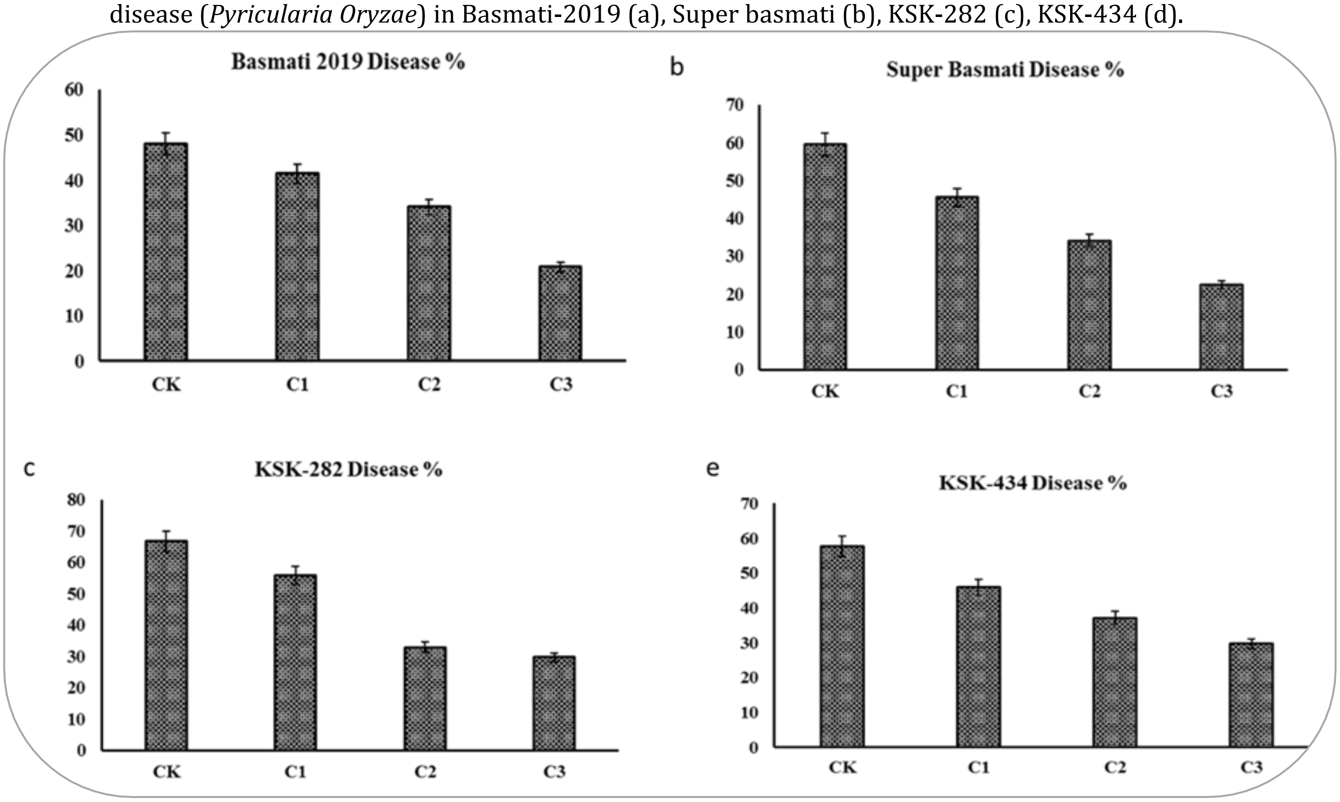

Figure 5. Graphical representation of effect of different concentration *Trichoderma harzianum* to control the rice BLB disease (*XanthomonasOryzae*) in Basmati-2019 (a), Super basmati (b), KSK-282 (c), KSK-434 (d).

Effect of *Trichoderma Harzianum* on Yeild: Foliar applications of *T. harzianum* showed the significant effect on growth parameters of rice plants and also control the diseases of rice diseases (blast and BLB). In this study we studied the effect of *Trichoderma harzianum* on rice growth such as plant height, panicle length, number of tillers, and number of grains and yield of rice plants per pots. This experiment was performed in three replications with three different concentrations of *T. harzianum*. Results showed significant difference as compared with control. In Basmati-2019 the increase in plant height, numbers of tillers, panicle length, grain per panicle and yield was about 27%, 45%, 28%, 18%, 38% in blast and BLB35%, 25%, 25%,27%,46% respectively. Super basmati showed the increase in plant height, numbers of tillers, panicle length, grain per panicle and yield was about 29%, 26%, 28%, 30%, 41% in blast and BLB 37%, 21%, 28%, 32%, 40% respectively. While the course varieties (KSK-282 AND KSK434) significant increase also observed. In KSK-282, the increase in plant height, numbers of tillers, panicle length, grain per panicle and yield was about 27%, 51%, 20%, 29%, and 18% in blast and in BLB 36%, 28%, 29%, 35% and 17% respectively. In KSK-434, there was increase in plant height, numbers of tillers, panicle length, grain per panicle and yield was about 19%, 41%, 28%, 37%, and 20% in blast and BLB 33%, 35%, 26%,63%, and 35% respectively. This increase at the highest concentration of *Trichoderma harzianum* (10^{10}). In the growth parameters significant results was observed at two concentrations 10^{10} and 10^{8}. The highest increase at 10^{10} concentration was found and at 10^{8} the increase also significant as compared to control and concentration of 10^{6}.The results of this experiment similar to the finding of this(Chou *et al.*, 2020).*Trichoderma* has successfully been shown to have the ability to stimulate growth response and show antagonistic actions against rice sheath blight disease(Chaube and Sharma, 2002).

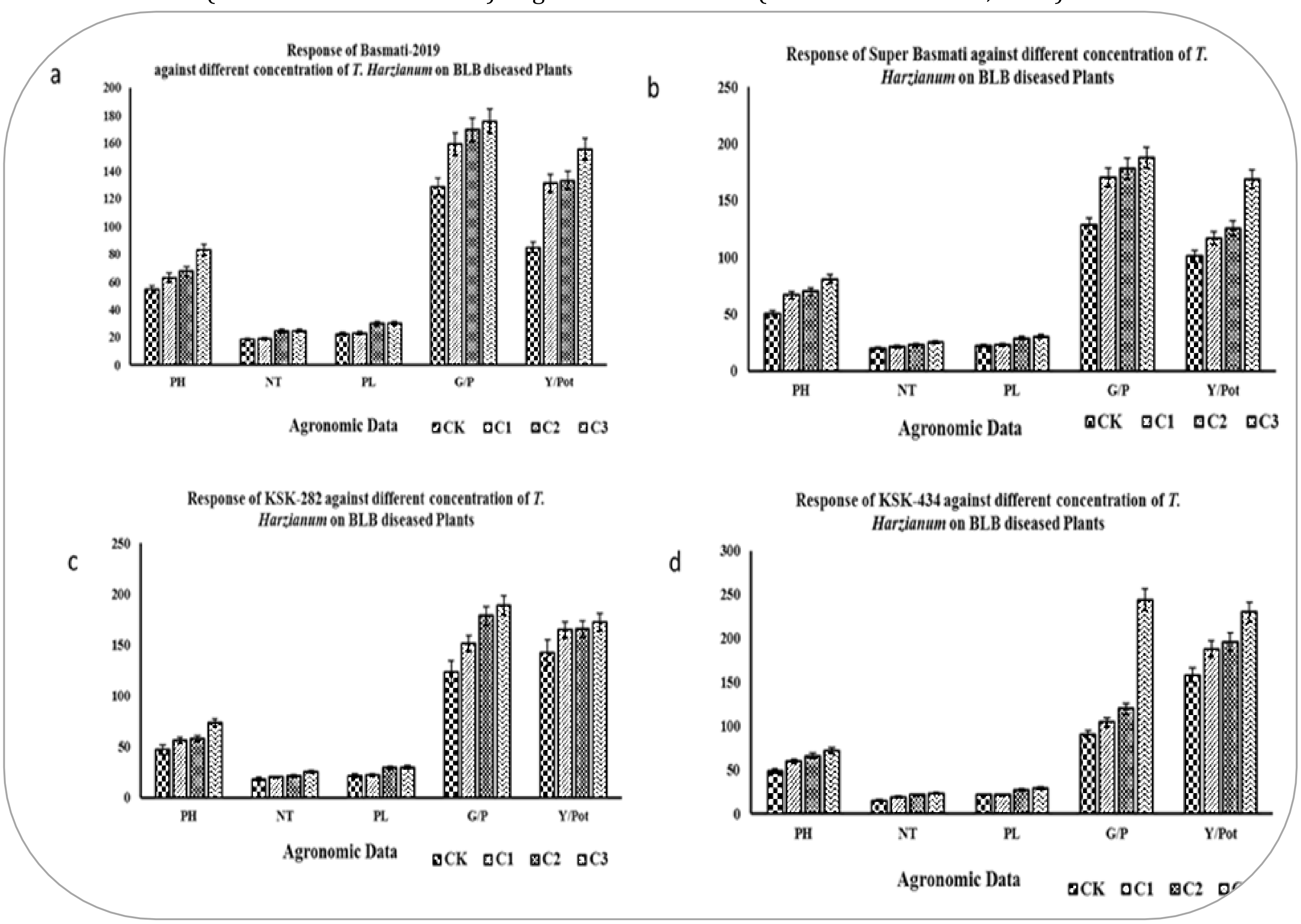

Figure 6. Graphical representation of effect of different concentration *Trichoderma harzianum* on growth parameters of plants (plant height, numbers of tillers, panicle length, grain per panicle, and yield per pots) of BLB (*Xanthomonas oryzae*) diseased plants in Basmati-2019 (a), Super basmati (b), KSK-282 (c), KSK-434 (d).

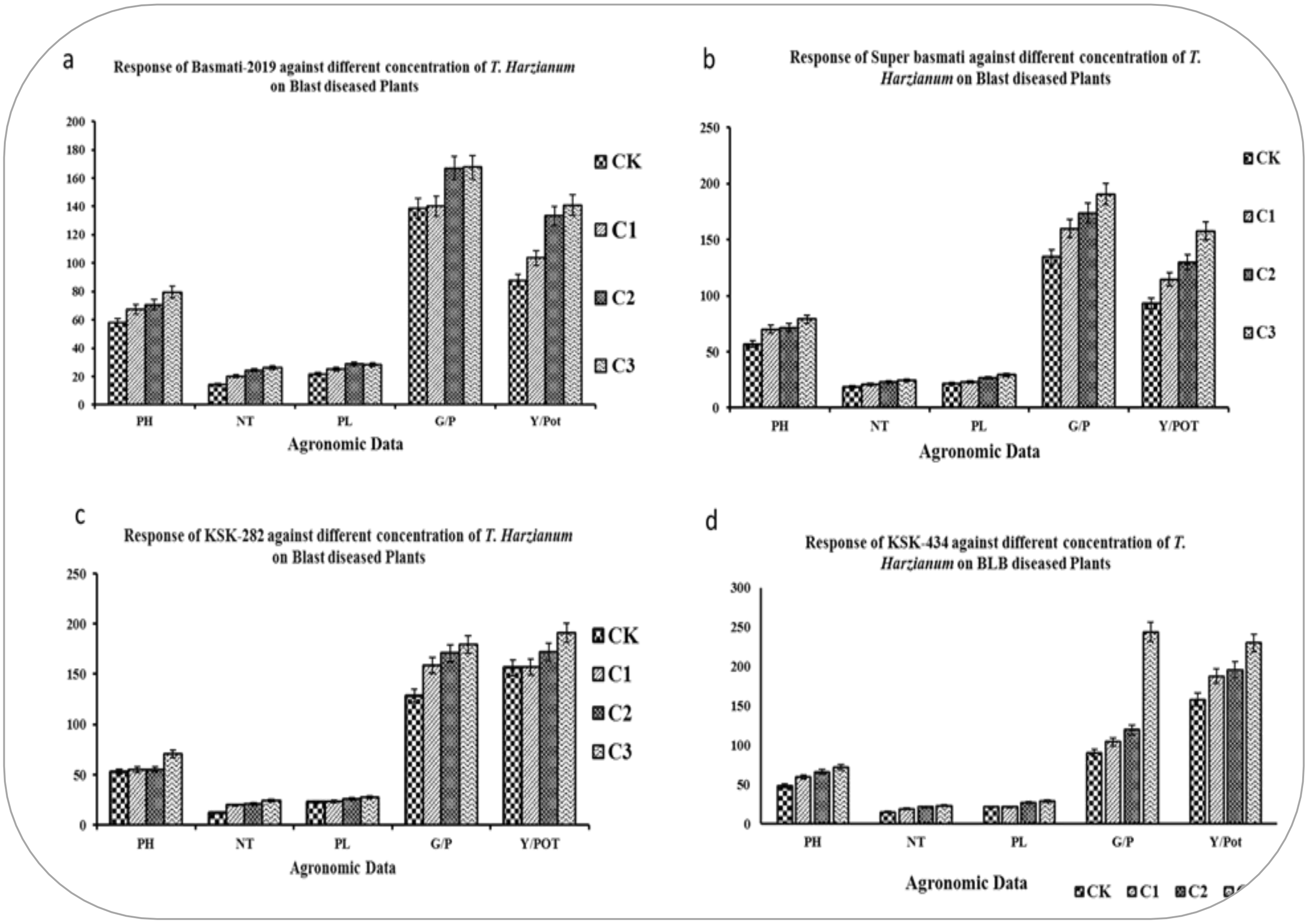

Figure 7. Graphical representation of effect of different concentration *Trichoderma harzianum* on growth parameters of blast affected plants (plant height, numbers of tillers, panicle length, grain per panicle, and yield per pots) in Basmati-2019 (a), Super basmati (b), KSK-282 (c), KSK-434 (d).

CONCLUSION

This study demonstrated that *Trichoderma harzianum* is an effective biological control agent. *T. harzianum* inhibits mycelial growth of *Pyricularia oryzae* and colony growth of *Xanthomonas Oryzae* in dual culture analysis. In bio-control assay, it reduces disease intensity by 60% to 70%. Our study revealed that concentrations 10^{10} and 10^{8} showed the best results in field conditions. *Trichoderma harzianum* not only inhibit the rice diseases by antagonistic action, it also stimulates the plant growth by direct or indirect means i.e. seed priming and volatile organic compounds (VOC's).

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Faiza Siddique	;	Helped in field experiments
Ammara Rehman	;	Helped in writing the manuscript
Alvina Hanif	;	Helped in designing Research experiments