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Rhizosphere *Trichoderma* isolates as potential biocontrol agent for maydis leaf blight pathogen (*Bipolaris maydis*) in fodder maize

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ABSTRACT

Two indigenous isolates of *Trichoderma* isolated from rhizosphere soils of maize plants were identified as *T. harzianum* and assessed for antagonistic activity against *Bipolaris maydis* causing maydis leaf blight in fodder maize *in vitro* and under field conditions for two consecutive kharif seasons of 2016 and 2017. Dual culture assay of *T. harzianum* isolates showed significantly higher degree of mycelial inhibition (74.35%) against *B. maydis*. Similarly, under field conditions, seed treatment and foliar spray of *T. harzianum* -I provided highest reduction in leaf blight severity (54.86%) along with 19.03 percent increase in green fodder yield in comparison to control. The efficacy of *T. harzianum* isolates to boost defence responses against maydis leaf blight disease in maize was also evaluated in bioagents treated leaves. Plants treated with biocontrol agents showed significantly higher activities of antioxidative defence enzymes like peroxidase (POX), superoxide dismutase (SOD) and catalase (CAT). In treated leaves, the activities of POX and SOD reached maximum at 24 h and activity of CAT reached the highest at 36 h after inoculation of pathogen *B. maydis*. Enzyme activities induced by *T. harzianum* isolates were more obvious than that induced by pathogen only. This implies that biocontrol agent induced defence responses against maydis leaf blight pathogen in fodder maize.

Keywords: Maize; *Bipolaris maydis*; biocontrol; defence enzymes; *Trichoderma harzianum*; maydis leaf blight.

1. Introduction

Maize is the third major food grain crop after rice and wheat worldwide and is also used as feed, specialty corn and starch. In Indian agriculture, maize has special significance due to its wide diversity as food and fodder crop under varied agro-climatic conditions. It can be successfully grown in rainy (*Kharif*), winter (*Rabi*) and spring summer (*Zaid*) crop seasons. It has the potential to supply large amounts of energy-rich forage for dairy animals and can be safely fed at all growth stages. In recent years, forage maize has become a major constituent of dairy cow diets which increases animal palatability (Bhagat *et al.*, 2017).

Maize is affected by several diseases such as leaf blight, stalk rot, root rot and ear rot during different stages of growth. Among these, maydis leaf blight caused by *Bipolaris maydis* is a serious fungal disease worldwide (White, 1999). The disease appears in more severe form in warm, humid, temperate to tropical areas where temperature ranges from 20-30ºC during the cropping period (Singh and Srivastava, 2012).

This disease has great significance in the history of agriculture due to its epidemic in 1970s and caused huge yield losses to the extent of 28 to 91 percent (Reddy *et al.,* 2013). Thus, maydis leaf blight (MLB) is a major obstacle in the successful cultivation of maize.

Traditionally, various fungicides have been used to control the disease. However, over use of chemical fungicides causes health hazards to humans and animals; environmental pollution, soil residue and fungicide resistance in pathogens (Bajwa *et al.,* 2003). Thus, there is need for alternative methods that would reduce reliance on fungicides. The use of microorganisms as biological control agents (BCA) therefore, offers an alternative ecofriendly approach to manage plant diseases which can reduce many ill effects of chemical fungicides on animal health and environment.

Plant growth promoting microbes are soil bacteria that stimulate plant growth by various means, often in association with plant roots, sometimes on leaves and/or within plant tissues (Glick, 2012).These rhizosphere microorganisms enhance the growth of plants either directly by fixing atmospheric nitrogen, production of siderophores, solubilization of minerals such as phosphorus and synthesis of phytohormones or indirectly by competing with pathogens for niches and nutrients, production of antifungal metabolites, secretion of enzymes and induction of systemic resistance (Nadeem *et al.,*2014). *Trichoderma* is a group of fungi that belongs to ascomycetes widely distributed in soil. Numerous studies have been reported for the control of foliar and soil borne pathogens in maize plants against *B. maydis* by *Trichoderma* spp. and generate resistance in maize plants (Harman, 2011; Jie *et al.,* 2014).

Among various biochemical responses to pathogen attack, the antioxidative response is important due to production of large number of reactive oxygen species. These antioxidative responses include release of enzymes such as peroxidase (POX), superoxide dismutase (SOD) and catalase (CAT), which are induced by oxidative injury caused by plant pathogens (Chugh *et al*., 2011). POX is a key enzyme that is involved in the synthesis of lignin and phytoalexins (Liao *et al.*, 2009). SOD scavenges oxygen free radicals and active oxygen to protect plant membrane and key enzymes in salicyclic acid pathway. CAT participates in plant disease resistance (Polidoros*et al*., 2001). In the current study, isolates of *Trichoderma* were isolated from rhizosphere soil of maize plants grown for fodder purpose and investigated under controlled and field conditions for the reduction in disease severity of maydis leaf blight infested by *B. maydis* and biochemical responses in terms of defence enzymes such as POX, SOD and CAT in maize.

2. Materials and methods

2.1. Source of pathogen: Fodder maize variety 'J 1006' susceptible to MLB pathogen was obtained from the Forage and Millet Section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, India and was used throughout the study. *Bipolaris maydis*, a causal agent of MLB was isolated from infected leaves of maize plant collected from the fields of fodder section. Pathogen was isolated, purified and maintained on potato dextrose agar (PDA) and used for further evaluation. It showed same symptoms on maize leaves when reinoculated after isolation.

2.2. Isolation of rhizosphere Trichoderma isolates: Soil samples were collected from the rhizosphere of maize plants growing in the fields of PAU, Ludhiana. The soil was loamy sand; low in organic carbon; and high in available nitrogen and potassium. For sampling, root system was dug out and rhizosphere soil was carefully taken in plastic bags to the laboratory for the isolation of rhizosphere fungi. Fungi were isolated by serial dilution and spread plate technique (Sundaraand Sinha,1963) on potato dextrose agar and Trichoderma selective medium (TSM) (Askew and Laing, 1993). Plates were incubated at 28 ± 2°C for

48 - 72 h. Colonies of *Trichoderma* were picked and purified by hyphal tip method and maintained in TSM medium. *T. harzianum* isolates were identified upto species level on the basis of morphological keys described by Barnett and Hunter (1972) and stocked for further use.

2.3. Biocontrol assay in vitro: Antifungal activity of two isolates of *Trichoderma harzianum* was evaluated against *B. maydis*, on PDA medium using dual culture technique (Utkhede and Rahe, 1983). Five millimetre discs of actively growing seven day- old cultures of test pathogen as well as biocontrol agents were taken with the help of a cork borer. Two discs, one each of pathogen and biocontrol agent, were placed equidistantly (60 mm) apart in each of the 90 mm petriplates containing PDA under aseptic conditions. The plates containing PDA medium inoculated with pathogen alone served as control. The plates were incubated at 25 + 1ºC. The radial growth of the bioagents and the pathogens from the centre of disc towards the centre of the plate was recorded after the control plates were completely covered by pathogens. Each treatment was replicated four times. The experiment was repeated twice. Observations on the growth of biocontrol agent and pathogen were recorded after 10 days of incubation and percent mycelial inhibition was determined by following I = (C-T/C) x100 [I =Percent inhibition of mycelium, C = Growth of mycelium in control (mm), T = Growth of mycelium in treatment (mm)].

2.4. Evaluation under field conditions: Field trials were conducted to evaluate the efficacy of rhizosphere *T. harzianum* isolates against MLB pathogen in the fields of PAU, Ludhiana, during *Kharif* of 2016 and 2017. The experiment was laid out in a complete randomized block design (RBD) with three replications in unsterilized soil. There were eight treatments that are T_1 (Seed dressing with *T. harzianum*- I @ 20 g per kg seed), T_2 (Seed dressing with *T. harzianum*- II), T_3 (Foliar spray of *T. harzianum*- I @ 20 g per litre), T_4 (T_1+ foliar spray of *T. harzianum- I*), T_5 (Foliar spray of *T. harzianum*- II), T_6 (T_2 + foliar spray of *T. harzianum- II*), T_7 (foliar spray of fungicide (Indofil M-45) as chemical check) and T_8 (untreated control). Susceptible variety J 1006 was sown in rows following recommended agronomic practices. The plot size was 5 x 5 m^2 and distance between row to row and plant to plant was 45 and 10 cm respectively. Thinning was done to maintain the proper distance between rows and plants. Plots of maize were irrigated when necessary. The inoculation of *B. maydis* was performed by culturing test pathogen on sorghum [*Sorghum bicolor* (Linn.) Moench] seeds following the method of Lim (1975). The plants were inoculated twice by placing a pinch of powdered inoculum in the whorl, first 15 days after sowing and second 15 days thereafter. After 20 days of sowing, first spray of spore suspension of biocontrol agent (1×10^8 conidia ml^{-1}) was done on both leaf surfaces whereas the second spray was done after 30 days of sowing. Spraying with sterile water served as the control. The disease severity and bio control efficacy were evaluated at 35, 45 and 55 days after sowing. Disease severity was calculated by the formula {Σ (nv) / (NG)} x100, where Σ (n x v) = sum of the score, N = total number of leaves counted and G = highest score. A scale of 0-5 was used to estimate the disease severity of corn leaf blight (Mir *et al*. 2015). Increase in green fodder yield was calculated by the formula (T-C)/C x 100, where T = green fodder yield (q/ha) of treatment, C = green fodder yield (q/ha) of control

2.5. Defense enzyme activity assay:

2.5.1. Sample collection: Leaves were treated with two isolates of *T. harzianum* at 1×10^8 conidia mL−1 concentration and subsequently with *B. maydis* at the same concentration. Samples of maize leaves were collected at 0, 24, 36, 48, and 72 h after inoculation with

test pathogen. Maize plants inoculated with pathogen only served as control. All the samples were immediately frozen in liquid nitrogen, ground into powder and stored at –80°C for enzyme activity detection. Total protein content of all enzyme extracts was determined by the method of Lowry *et al.* (1951). Each treatment was studied in three replicates.

2.5.2. Catalase (CAT): Activity of CAT was determined by taking 1.8 ml of 50 mM sodium phosphate buffer (pH 7.5) to which 0.2 ml of enzyme extract was added. The reaction was initiated by adding 1 ml H_2O_2 and utilization of H_2O_2 was recorded at an interval of 30 sec for 3 min by measuring the decrease in absorbance at 290 nm (Chance and Machly, 1955). Extinction coefficient for H_2O_2 was 0.0394 $mM^{-1}cm^{-1}$. The enzyme activity was expressed in U mg^{-1} protein.

2.5.3. Superoxide dismutase (SOD): Assay system of SOD contained 1.4 ml of 100 mM Tris HCl buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1 ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract (Marklund and Marklund, 1974). Change in absorbance was recorded at 420 nm after an interval of 30 sec up to 3 min. A unit of enzyme activity was expressed as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank. SOD activity is expressed in U mg^{-1} protein.

2.5.4. Peroxidase (POX): Assay system of POX contained 3 ml of 0.05 M guaiacol in 100 mM phosphate buffer (pH 6.5), 0.1 ml of enzyme extract and 0.1 ml of 0.8 M H_2O_2 (Hammerschmidt *et al.*, 1982). The reaction mixture without H_2O_2 was taken as a blank. The reaction was initiated by adding H_2O_2 and rate of change in absorbance was recorded at 470 nm for 3 min at an interval of 30 sec. POX activity was expressed as change in absorbance min^{-1} mg^{-1} of protein.

2.6 Data analysis: Statistical analyses were conducted using Duncan's Multiple Range Test (DMRT). Data on percentages were transformed to arcsine and analysis of variance (ANOVA) was carried out with transformed values. The means were compared for significance using DMRT ($p<0.05$).

3. Results

3.1. Isolation of rhizosphere Trichoderma: Trichoderma colonies grown on TSM medium (Askew and Laing, 1993) were isolated and evaluated for antifungal activity through dual culture technique. Total four colonies of *Trichoderma* were obtained and purified by hyphal tip method. Among them, two isolates showed more than 60 percent mycelial inhibition of *B. maydis* and rest were not effective against the test pathogen. Antifungal isolates of *Trichoderma* were identified on the basis of colour of the colony, formation of chlamydospores, conidiophores and phialides characters, shape of conidia as the main characters to identify the species as described by Barnett *et al.* (1972). These isolates were maintained on PDA slants for further studies.

3.2. Efficacy of biocontrol agents in vitro: T. harzianum isolates showed strong antifungal activity against MLB in dual culture method (Table 1). *T. harzianum*-I provided maximum mycelial inhibition of *B. maydis* that is 74.35 percent followed by that of *T. harzianum*-II (63.41%) as compared to control. This showed that both the tested isolates had strong antagonistic properties against the maize blight pathogen. All the *T. harzianum* isolates were found statistically at par with each other and *T. harzianum*-I significantly reduced the mycelial growth of test pathogen.

3.3. Biocontrol assay in field conditions: Isolates of *T. harzianum* were tested under field conditions against MLB in maize during 2016 and 2017.The observations showed reduction in disease severity and increase in green fodder yield. Maximum disease severity observed during two seasons was 56.00 and 67.33 percent respectively (Table 2). Seed treatment + foliar spray with *T. harzianum*-I provided highest 54.86 percent mean reduction in leaf blight severity followed by *T. harzianum*-II with 48.11 percent disease reduction. Foliar spray with *T. harzianum*-I and *T. harzianum*-II showed 41.08 and 37.03 percent reduction in disease severity respectively as compared to untreated control. These isolates also gave better control of leaf blight than chemical check Indofil M-45 (0.25%) which exhibited only 31.63 percent reduction in leaf blight severity . Rest of the treatments exhibited reduction in disease severity in the range of 23.24 to 27.03 percent. It was observed that both the isolates of *T. harzianum* significantly reduced the leaf blight severity in maize. Treatment T_4 (Seed treatment + foliar spray with *T. harzianum*-I) was found statistically significant than rest of the treatments and provided least per cent disease severity. Other treatments were at par with each other during 2016 and 2017.

With reduction in disease severity, effective treatments significantly enhanced the green fodder yield of maize with the application of both the isolates of *T. harzianum*. Application of *T. harzianum*-I as seed treatment + foliar spray provided 416.93 quintal per hectare of green fodder yield followed by that of *T. harzianum*-II, (407.41 quintal per hectare) as compared to chemical and untreated control (384.13 and 350.27 quintal per hectare) respectively (Table 3). Results revealed that maximum 19.03 and 16.31 percent increase in green fodder yield was provided by both the *Trichoderma* isolates. Other treatments (T_1, T_2, T_3 and T_5) showed 6.34 to 13.59 percent increase in yield than chemical (9.67%) and untreated check. Treatment T_4 (Seed treatment + foliar spray with *T. harzianum*-I) was found statistically significant than the rest of the treatments and provided highest green fodder yield during both the years.

3.4. Effect of Trichoderma isolates on defence enzyme activity: Activity of defence enzymes *viz;* POX, SOD and CAT was observed in seedlings of maize when subsequently inoculated with spore suspension of two *Trichoderma* isolates and leaf blight pathogen at four to five leaf stage.

3.4.1. Peroxidase (POX)

POX activity was higher in treated leaves than untreated leaves during the sampling period of 0-72 h (Table 4). Increase in POX enzyme activity was recorded at 0-24 h after inoculation with *T. harzianum*-I, and maximum was 4.95 U mg^{-1} at 24 h after inoculation, after which the activity started decreasing. Whereas, *T. harzianum*-II showed enzyme activity of 4.31 Umg^{-1} at 24 h after inoculation, as compared to the chemical check and control treatment which generated an enzyme activity of 3.15 and 2.13 U mg^{-1} respectively. These results indicate that POX in maize leaves was induced by *T. harzianum* isolates.

3.4.2. Superoxide dismutase (SOD)

Similarly, increase in SOD enzyme activity was observed during 0–24 h after inoculation in maize leaves treated with both the isolates of *Trichoderma* and *B. maydis* and decreased at 36 h after inoculation (Table 5). However, at 48 h after inoculation, increase in the SOD activity was detected for the second time, that gradually declined again. Maximum SOD activity was exhibited by *T. harzianum*-I treated leaves at 24 h after inoculation (39.05U

mg^{-1}) and then again at 48 h after inoculation (35.27U mg^{-1}). *T. harzianum*-II also showed increased activity of SOD (31.09U mg^{-1}) as compared to both the checks. This suggests a positive relationship of *T. harzianum* isolates in the induction of resistance to *B. maydis*.

3.4.3. Catalase (CAT)

Both the isolates of *T. harzianum* induced strong activity of CAT enzyme in treated maize leaves. CAT activity was highest at 36 h after inoculation (Table 6) after which it declined. *T. harzianum* isolates elicited the production of CAT enzyme and were effective in the induction of maize leaf resistance to *B. maydis*. The CAT activity was 29.91U mg^{-1} as compared to the control treatment (22.16U mg^{-1}). *T. harzianum* isolates significantly triggered the enzymatic activity which suppressed the growth of maize blight pathogen.

3. Discussion

Plants have well organized defence system of biochemical reactions that are induced by invading phytopathogens and biocontrol agents. This can be one of the novel management strategies in agriculture (Kashyap and Dhiman, 2009). *Trichoderma* species especially *T. harzianum, T. viride* and *T. atroviride* exhibited variability with respect to their antifungal property against plant pathogens of different crops (Surekha *et al*., 2014). The growth inhibition in the presence of *Trichoderma* species could be attributed to all the three modes of antagonism *in vitro viz.,* competition, antibiosis and mycoparasitism; and also due to its fast growing nature, rapid sporulation, secretion of gliotoxin and cell wall lytic enzymes (Shalini and Kotasthane, 2007).

In the present study, two isolates of *T. harzianum* isolated from rhizosphere soil of host plant exhibited potential biocontrol activity against MLB caused by *B. maydis in vitro* and under field conditions. Antagonistic activity of bioagents in dual culture may be ascribed to its ability to produce diffusible and volatile metabolites (Khamari and Beura, 2014; Naglot *et al*., 2015). The results obtained from present investigation indicate the strong antagonistic activity of bioagents when applied to crop as seed treatment, foliar spray and as both. These tested bioagents significantly reduced the leaf blight severity and increased the green fodder yield over untreated control. Present findings are in confirmation with observations of Malik *et al*. (2018) who have tested *T. harzianum* and *T. viride* against MLB and banded leaf and sheath blight under field conditions. It can be therefore assumed that the antifungal role of *Trichoderma* may be due to combination of hydrolytic enzymes and secondary antifungal metabolites such as trichodermin, trichodermal, etc. which play important role in the inhibition of pathogens (Dickinson *et al*., 1995).

BCAs isolated from native sources have ability to induce systemic defence responses (Glick, 2014). Defence responses are also triggered by plant pathogens which induced the production of defence related antioxidant enzymes like POX, SOD and CAT which suppress the formation of free radicals and convert them into less harmful species (Gill and Tuteja, 2010). In the present study, inoculation of biocontrol agents on maize leaves induced antioxidant defence enzyme activity. Leaves treated with *T. harzianum* showed higher activities of POX, SOD and CAT enzymes, which further increases after *B. maydis* inoculation. These results suggested that *T. harzianum* triggered enzymatic activity which might have suppressed the growth and spread of maize blight pathogen.

According to Siddikee *et al*. (2012), the elevated activity of POX and CAT enzymes might be associated with the production of cell wall strengthening constituents acting as mechanical barrier for invading pathogens. During pathogen invasion, plant is protected from oxidative stress through degeneration, balancing and scavenging of ROS by enhanced SOD enzyme activity (Dixit *et al*., 2016). Our results are in accordance with the

results reported by earlier researchers who have observed enhanced activities of POX, SOD, CAT, PAL, polyphenol oxidase (PPO) and cinnamyl alcohol dehydrogenase (CAD) enzymes against *Rhizoctonia solani* seedling blight in sunflower (Singh *et al*., 2014) and *Sclerospora graminicola* in pearl millet (Siddaiah *et al*., 2017) induced by *Trichoderma* species. Similarly, after inoculation with *T. atroviride* in maize and *T. viride* in black gram against *Cochliobolus heterostrophus*, *Fusarium oxysporum* and *Alternaria alternate* significantly enhanced the production of defence enzymes (POX, SOD & CAT) respectively (Wang *et al*., 2015; Surekha *et al*., 2014). Therefore, the present studies showed decrease in disease severity of leaf blight due to seed and foliar application of fungal biocontrol agents.

Thus, the rhizosphere competent, biocontrol agents with antagonistic activity toward plant pathogens by inducing enzymes, hormones or antifungal compounds are considered as potential tools to develop commercial formulations. The use of natural antagonistic microorganisms is considered as the alternative ecofriendly approach to manage the diseases of plants. This strategy may be an effective complementary option for maydis leaf blight management in fodder maize.

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Table 1: Antagonistic activity of *T. harzianum* isolates against *Bipolaris maydis* in dual culture

Bio-agent	Mycelial Growth (mm)*	Mycelial Inhibition (%)
T. harzianum I	1.29±0.088[a**]	74.35
T. harzianum II	1.84±0.025[b]	63.41
Control	5.03±0.284[c]	0.00
CD (0.05%)	1.83	-

* The values are the mean ±Standard Error.
**The treatment means in a column with different lowercase alphabets show statistically significant difference among treatments at $p<0.05$.

Table 2: Field evaluation of *T. harzianum* isolates against maydis leaf blight

	Treatment	Disease Severity (%)*	Disease

		2016	2017	Mean	reduction (%)
T_1	Seed dressing with *Trichoderma harzianum*- I	43.00±0.577^{b**}	47.00±0.577^{c}	45.00±0.577^{c}	27.03
T_2	Seed dressing with *Trichoderma harzianum*- II	44.67±0.882^{b}	50.00±0.577^{b}	47.34±0.726^{b}	23.24
T_3	Foliar spray of *T. harzianum*- I	34.67±0.333^{e}	38.00±0.576^{f}	36.34±0.167^{f}	41.08
T_4	T_1+ foliar spray of *T. harzianum- I*	25.00±0.000^{g}	30.67±0.333^{h}	27.84±0.166^{h}	54.86
T_5	Foliar spray of *T. harzianum*- II	37.67±0.333^{d}	40.00±0.577^{e}	38.84±0.333^{e}	37.03
T_6	T_2 + foliar spray of *Trichoderma harzianum- II*	29.67±0.882^{f}	34.33±0.333^{g}	32.00±0.288^{g}	48.11
T_7	Foliar spray Indofil M-45 @ 0.25%	40.00±0.577^{c}	44.33±0.333^{d}	42.17±0.441^{d}	31.63
T_8	Control	56.00±0.577^{a}	67.33±0.881^{a}	61.67±0.167^{a}	0.00
	CD (0.05%)	1.384	1.643	0.943	

*The values are the mean ±Standard Error of experiment repeated in 2016 and 2017.

**The treatment means in a column with different lowercase alphabets show statistically significant difference among treatments at $p<0.05$.

The treatment means in a column with common lowercase alphabets are statistically at par with each other at $p<0.05$.

Table 3: Effect of *T. harzianum* isolates on green fodder yield of maize

	Treatment	Green fodder yield (q/ha)*			Increase in yield (%)
		2016	2017	Mean	
T_1	Seed dressing with *Trichoderma harzianum*- I	383.07±2.116^{cd}	378.84±2.116^{cd}	380.96±1.832^{e**}	8.76
T_2	Seed dressing with *Trichoderma harzianum*- II	376.72±5.599^{d}	368.25±3.665^{d}	372.49±1.058^{e}	6.34
T_3	Foliar spray of *T. harzianum*- I	400.00±12.698^{bc}	395.77±2.116^{b}	397.89±5.291^{b}	13.59
T_4	T_1+ foliar spray of *T. harzianum- I*	421.16±2.116^{a}	412.70±3.665^{a}	416.93±2.799^{a}	19.03

T_5	Foliar spray of *T. harzianum*- II	397.88±10.580 bcd	389.42±4.232^{bc}	393.65±6.608^{cd}	12.38
T_6	T_2 + foliar spray of *Trichoderma harzianum- II*	416.93±4.232^{ab}	397.88±5.599^{b}	407.41±4.612^{ab}	16.31
T_7	Foliar spray of Indofil M-45 @ 0.25%	385.19±4.233^{cd}	383.07±2.116^{c}	384.13±3.174^{de}	9.67
T_8	Control	344.97±2.116^{e}	355.56±3.665^{e}	350.27±2.116^{f}	0.00
	CD (0.05%)	2.839	1.830	1.849	

*The values are the mean ±Standard Error of experiment repeated in 2016 and 2017.
**The treatment means in a column with different lowercase alphabets show statistically significant difference among treatments at p<0.05.
The treatment means in a column with common lowercase alphabets are statistically at par with each other at p<0.05.

Table 4: Influence of *Trichoderma harzianum* isolates on peroxidase (POX) enzyme activity in maize leaves

Treatment		Peroxidase enzyme activity (U mg^{-1} protein)*					
		Time after inoculation (hours)					
		0	12	24	36	48	72
1	Indofil M-45	1.87±0.262 bc**	2.12±0.363^{b}	3.15±0.542^{bc}	2.62±0.451^{bc}	2.40±0.428^{ab}	2.04±0.526^{bc}
2	*T. harzianum* I	3.29±0.447^{a}	4.28±0.358^{a}	4.95±0.449^{a}	4.49±0.514^{a}	3.89±0.556^{a}	3.62±0.439^{c}
3	*T. harzianum* II	2.85±0.293^{a} b	3.23±0.336^{a}	4.32±0.274^{ab}	3.59±0.422^{ab}	2.70±0.435^{a}	2.64±0.596^{b}
4	Control	0.87±0.319^{c}	1.37±0.228^{b}	2.13±0.227^{c}	1.39±0.123^{c}	1.27±0.221^{b}	1.01±0.239^{c}
CD (0.05%)		0.480	0.563	0.618	0.748	0.701	0.619
F- VALUE		62.505	64.096	51.057	39.373	29.286	38.968
P-VALUE		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

*The values are the mean ±Standard Error.
**The treatment means in a column with different lowercase alphabets show statistically significant difference among treatments at p<0.05.
The treatment means in a column with common lowercase alphabets are statistically at par with each other at p<0.05.

Table 5: Influence of *Trichoderma harzianum* isolates on superoxide dismutase (SOD) enzyme activity in maize leaves

Treatment		Superoxide dismutase enzyme activity (U mg^{-1} protein)*					
		Time after inoculation (hours)					
		0	12	24	36	48	72
1	Indofil M-45	12.96±0.545^{c**}	20.07±0.582^{b}	30.41±0.391^{c}	24.48±0.245^{c}	28.11±0.294^{c}	19.25±0.258^{b}
2	*T.*	19.99±0.32	28.09±0.3	39.05±0.4	32.02±0.4	35.27±0.2	24.12±0.3

	harzianum I	9[a]	35[a]	35[a]	59[a]	73[a]	03[a]
3	*T. harzianum* II	15.31±0.545[b]	20.98±1.000[b]	31.99±0.571[b]	26.86±0.524[b]	29.03±0.220[b]	23.13±0.445[a]
4	Control	11.76±0.606[c]	14.64±0.334[c]	18.75±0.384[d]	17.39±0.308[d]	18.32±0.182[d]	14.48±0.283[c]
CD (0.05%)		1.359	2.291	1.489	1.223	0.390	1.036
F- VALUE		89.009	72.388	397.936	307.379	4016.624	222.549
P-VALUE		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

458 *The values are the mean ±Standard Error.
459 **The treatment means in a column with different lowercase alphabets show statistically
460 significant difference among treatments at p<0.05.
461 The treatment means in a column with common lowercase alphabets are statistically at par
462 with each other at p<0.05.
463

464 **Table 6: Influence of *Trichoderma harzianum* isolates on catalase (CAT)enzyme**
465 **activity in maize leaves**

Treatment		Catalase enzyme activity (U mg^{-1} protein)*					
		Time after inoculation (hours)					
		0	12	24	36	48	72
1	Indofil M-45	10.38±0.313[c**]	11.79±0.146[c]	21.14±0.329[c]	29.09±0.218[a]	24.14±0.255[b]	16.98±0.110[b]
2	*T. harzianum* I	12.94±0.158[a]	14.83±0.482[a]	27.61±0.308[a]	29.91±0.144[a]	26.02±0.401[a]	19.61±0.339[a]
3	*T. harzianum* II	11.40±0.307[b]	13.40±0.228[b]	24.34±0.357[b]	27.24±0.352[b]	22.74±0.257[c]	17.87±0.217[b]
4	Control	9.09±0.087[d]	10.07±0.536[d]	17.97±0.294[d]	22.16±0.277[c]	19.01±0.392[d]	14.98±0.427[c]
CD (0.05%)		0.620	0.992	0.489	0.398	1.121	0.502
F- VALUE		86.117	53.303	894.348	949.334	87.082	182.936
P-VALUE		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

466 *The values are the mean ±Standard Error.
467 **The treatment means in a column with different lowercase alphabets show statistically
468 significant difference among treatments at p<0.05.
469 The treatment means in a column with common lowercase alphabets are statistically at par
470 with each other at p<0.05.
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