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Research Article

IMPROVED PHYSIOLOGICAL RESPONSES AND STRESS TOLERANCE IN COTTON AGAINST *FUSARIUM* WILT WITH *TRICHODERMA HARZIANUM* TREATMENT

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ABSTRACT

Cotton is a vital cash crop in the agricultural economy. Many abiotic stresses and pathogenic diseases reduce cotton yield, with cotton wilt being a major threat caused by *Fusarium oxysporum* f.sp. *vasinfectum* (FOV). In the present study, the antagonistic potential of *Trichoderma harzianum*, along with its extracted volatile and non-volatile compounds, was evaluated against FOV. In a dual culture assay, *T. harzianum* showed 71.4% inhibition of FOV. The volatile compounds extracted from *T. harzianum* showed 54.9% inhibition of FOV, while the non-volatile compounds reduced the growth of *F. oxysporum* f.sp. *vasinfectum* by 64.21%. The evaluation of vegetative traits of cotton plants indicated increased biomass when treated with *T. harzianum*. The activity of antioxidant defense enzymes Catalase activity (CAT), Superoxide dismutase activity (SOD), and Peroxidase activity (POX) was highest in cotton plants treated with both *T. harzianum* and *F. oxysporum* f.sp. *vasinfectum*, indicating an activation of the plant defense mechanisms in response to these treatments. Protein content, Malondialdehyde Content (MDA), proline content, electrolyte leakage, and H_2O_2 content, which are stress markers, were at their maximum under *F. oxysporum* f.sp. *vasinfectum* stress. Physiological features, including stomatal conductance, photosynthesis rate, Soil Plant Analysis Development (SPAD) chlorophyll value, and relative water content, were highest in plants treated with *T. harzianum*, as *T. harzianum* improves and boosts plant growth. Hence, *T. harzianum* is an effective biological control agent against FOV.

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INTRODUCTION

Cotton is a vital cash crop worldwide, including in Asia, Australia, Africa, and the Americas (Ozyigit et al., 2022). For thousands of years, it has been central to textile production (Tariq et al., 2018). Fiber quality decreases, and yield losses are caused by various biotic and abiotic factors (Karaş, 2022). Mostly, biotic agents such as pathogenic fungi, viruses, and bacteria cause diseases like wilting (Zeeshan et al., 2023; Yaseen et al., 2024). Cotton production can be significantly reduced by more than sixty different diseases (Majeed et al., 2021).

Cotton wilt, originally caused by *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) (Liu et al., 2017), has spread throughout the Americas and Asia (Dastur et al., 2015). Fusarium symptoms appear from early seedling growth through full maturity. Diseased seedlings exhibit darkened veins, yellowing leaves, and early leaf withering. Cotton seedlings may wilt and die if their cotyledons become necrotic and fall off (Asif et al., 2023).

The application of synthetic fertilizers to mitigate stress has a harmful impact on both plant health and soil quality. Beneficial microorganisms are recognized for utilizing comparable strategies to promote plant growth. Additionally, beneficial microorganisms have shown strong efficacy compared to rhizobacteria in mitigating the adverse effects of FOV stresses on plants. Their ability to penetrate plant tissue is primarily assisted through the roots and other natural openings in the plant. Upon entering, beneficial microorganisms can distribute themselves throughout the host plant's tissues. Several initiatives have been introduced to better control wilt and mitigate the issue of plant disease caused by pathogens. Using microbial species is one of the best strategies to combat diseases without having a major environmental impact (Patel et al., 2023).

Many biocontrol strategies have been explored for the management of different pathogens (Mukhtar et al., 2013; Mukhtar, 2018; Iqbal and Mukhtar, 2020; Azeem et al., 2021; Mukhtar et al., 2021). *Bacillus* strains (Mahmood et al., 2022, 2023) produce antibiotics and lipopeptides with antimicrobial properties (Maalik et al., 2023; Malik et al., 2024). *Trichoderma* species are also used as biocontrol agents, and deploying more potent strains of *Trichoderma* species is one of the most promising methods for reducing fungicide use and ensuring safe food production. It is frequently used as a fungal biocontrol agent against soil-borne diseases due to its efficacy in preventing fungal infections. Mycoparasitism, competition for nutrients, antibiosis, induced systemic resistance, biofilm formation, volatile and enzyme release, and hyphal contacts contribute to its effectiveness against plant pathogens (Fatima et al., 2023).

Fusarium species harm various aspects of plant morphology, physiology, and metabolism (Geng et al., 2014). The presence of *Fusarium* species can have multiple negative impacts on plants, such as reduced plant biomass, increased oxidative stress, decreased activity of the enzymes Catalase activity (CAT), Superoxide dismutase activity (SOD), and Peroxidase activity (POX), a lower photosynthesis rate, changes in stomatal conductance, alterations in plant communities, and higher levels of H_2O_2, electrolyte leakage, MDA (malondialdehyde), protein, and proline content, which are indicators of stress produced in response to *Fusarium* species (Dikilitas et al., 2016; Maqsood et al., 2020).

Trichoderma isolates are well-suited for effective disease management due to their biocontrol features, such as enzyme production. Field testing is essential for improving promising bioagents against soil-borne wilt disease (Jeevidha et al., 2023). The objective of the present study was to identify and assess the efficacy of *Trichoderma* isolates and their volatiles and non-volatile metabolites against FOV isolates *in vitro*. The study also aimed to assess the efficacy of *Trichoderma* isolates as a biocontrol agent against FOV in cotton plants during field trials and to evaluate the impact of *Trichoderma* isolate inoculation on plant biomass accumulation.

MATERIALS AND METHODS

Fungal cultures

Samples were collected from cotton plant fields in Bahawalpur, Pakistan to isolate *Trichoderma* spp. The soil sample was diluted in 5 ml of double-distilled water, and 0.5 ml of the suspension was placed on a PDA plate. The plates were incubated at 28°C for five days. Colony pigments, green conidia, odor, and smell were observed (Kumar et al., 2012).

Five to six root samples were collected from wilted cotton plants at various sites across Bahawalpur, Punjab, Pakistan. Infected root cuttings, 1-2 cm in size, were placed on PDA plates. For the purification of FOV culture, the single spore technique was used (Leslie and Summerell, 2006).

Dual culture technique

A 3 mm block of *T. harzianum* was placed on a PDA plate

directly opposite a block of FOV of the same size (Morton and Stroube, 1955). The plates were incubated for five days at 25°C, and the radial growth of FOV was measured. One control plate of FOV was used for comparison, and each treatment was repeated thrice (Dubey et al., 2007).

Effect of volatile metabolites on FOV

To determine whether *T. harzianum* is capable of inhibiting pathogen growth via the production of volatile compounds, the evaluation methods described by Dennis and Webster (1971a) were used. The diameter of the colony was measured to assess the suppression of mycelial growth (Dubey et al., 2007).

Effect of non-volatile metabolites on FOV

The effect of non-volatile compounds produced by *T. harzianum* was evaluated using the techniques proposed by Dennis and Webster (1971b). Petri dishes were sealed with paraffin tape and incubated for five days at 25°C. The radial growth of FOV isolates was monitored to calculate the inhibition percentage (I %).

$$I\% = \frac{C - T}{C} \times 100$$

Where I = growth inhibition, C = colony diameter/radial growth of the pathogen in the control group, and T = colony diameter/radial growth of the pathogen in the treatment group (Dubey et al., 2007).

Greenhouse experiment

The research was conducted in the greenhouse at the Department of Plant Pathology, the Islamia University of Bahawalpur, Pakistan. The experiment consisted of pots with a 10 cm base diameter and a 14 cm height. There were four experimental units with three replicates, arranged in a completely randomized design. The treatments were as follows:

T1 = treated with *T. harzianum* (TH);

T2 = *F. oxysporum* f.sp. *vasinfectum* (FOV) stress;

T3 = *T. harzianum* + *F. oxysporum* f.sp. *vasinfectum* (TH+ FOV) and

T4 = healthy control (HC).

The experiment lasted for 60 days under the same conditions, and plant growth features were measured.

Physiological features

Before harvest, stomatal conductance (gs) and photosynthetic rate (Pn) were measured using an open infrared gas analyzer (IRGA) LI-COR system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) (Fatima et al., 2023).

Determination of defense enzymes

To extract enzymes, 3 g of crushed frozen leaf tissue was mixed with 6 ml of phosphate buffer solution (pH 7.6, 0.5 M) and 1% polyethylene pyrrole. The homogenate was centrifuged at 12,000 g for 10 minutes at 4°C. Catalase (CAT) activity was evaluated by measuring H_2O_2 reduction (Díaz-Vivancos et al., 2008). CAT activity was measured in units per milligram of fresh weight (UNIT mg^{-1} FW). Peroxidase (POX) activity was detected using guaiacol (Thomas et al., 1982). POX activity was also measured in units per milligram of fresh weight (UNIT mg^{-1} FW). Superoxide dismutase (SOD) activity was assessed by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopotitis and Ries, 1977). One unit of SOD activity is defined as the amount that reduces NBT reaction by 50%. SOD activity was measured in units per milligram of fresh weight (UNIT mg^{-1} FW).

MDA, proline, electrolyte leakage, and H_2O_2 content

MDA content measurement

The thiobarbituric acid method was used to detect MDA in leaves. First, 500 mg of leaf material was mixed thoroughly with 1.5 ml of 0.1% trichloroacetic acid. The mixture was centrifuged for 10 minutes at 12,000 g. Subsequently, 1 ml of the centrifuged material was combined with 4 ml of 20% trichloroacetic acid (TCA) and 0.025 ml of 0.5% thiobarbituric acid. The solution was heated for 30 minutes in a 90°C water bath. To stop the reaction, the tubes were cooled in water. The samples were then centrifuged again for 10 minutes at 12,000 g. Absorbance was measured at 532 nm (Guidi et al., 2000).

Proline content measurement

The ninhydrin assay was used to examine free proline in the leaves. First, 0.5 g of leaf material was extracted with 10 ml of 3% (w/v) sulfosalicylic acid solution. A 2.0 ml aliquot of the filtered solution was added to 2.0 ml of acid ninhydrin, which consisted of 1.26 g of ninhydrin, 20 ml of 6 M ortho-phosphoric acid, and 30 ml of glacial acetic acid. An additional 2.0 ml of glacial acetic acid was then added. These samples were incubated at 80°C for 60 minutes and then placed in an ice bath to stop the reaction. After adding 4.0 ml of toluene and mixing for 30 seconds, the toluene chromophore was separated from the water phase. Absorbance was measured at 520 nm (Zulfiqar and Ashraf, 2023).

Electrolyte leakage (EL) measurement

Cotton leaf electrolyte leakage (EL) was assessed using the method of Dionisio-Sese and Tobita (1998). A 200 mg leaf sample was cut into 1 cm pieces and soaked in 20 ml of distilled water, then incubated at 35°C for 2

hours. The initial electrical conductivity (EC1) of the solution was measured using a conductivity meter. Each sample was then autoclaved at 121°C for 20 minutes, and after cooling to 25°C, the final electrical conductivity (EC2) was measured. EL was calculated using the equation (Velikova et al., 2000) which is given below

$$\text{EL}\ (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

Total soluble protein measurement

A 0.3 g leaf sample was homogenized with 8 ml of 0.05 M sodium phosphate buffer (pH 7.6). The mixture was centrifuged at 4°C for 15 minutes at 10,000 g. Then, 1 ml of the supernatant was mixed with 5 ml of 0.1% Coomassie Brilliant Blue G-250 solution and incubated for 10 minutes. Absorbance was measured at 595 nm to determine the soluble protein content (Bradford, 1976).

Relative water content (RWC)

To assess the relative water content (RWC), the fresh weight (FW), dry weight (DW), and turgid weight (TW) of the leaves were measured. Leaves from the sixth position from the top were selected for analysis. RWC was calculated using the formula (Kaya et al., 2003) which is given below

$$\text{RWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

SPAD chlorophyll value

The chlorophyll value of cotton leaves was measured 30 days post-treatment using a Konica Minolta SPAD-502 soil plant analysis development (SPAD) chlorophyll meter, focusing on the lateral margin of the central leaflet (Gratão et al., 2012).

Statistical analysis

All data were analyzed using a completely randomized design with the analysis of variance (ANOVA) approach. The statistical analysis was performed using SPSS software, and Tukey's HSD test was applied at $p \leq 0.05$ (Steel, 2001).

RESULTS

Fungal pathogens

The macroscopic and microscopic features of the cultures were observed by growing them on PDA plates and examining them under a microscope, respectively. The colony of *T. harzianum* (TH) was olive green with whitish margins. The pure colony of *F. oxysporum* f.sp. *vasinfectum* (FOV) exhibited light pink pigmentation on the underside of the plate. The conidia of TH were ellipsoidal to sub-globose in shape and displayed branching. The phialides had a flask-like shape and were arranged in 2-4 concentric circles. The spores of FOV exhibited various morphologies, including oval, ellipsoid, kidney-shaped, and tapered ovals. These spores were septate and comprised three cells. Conversely, chlamydospores were produced in a linear pattern. A pure colony of TH, a pure culture plate of FOV, and visualization of conidia produced by FOV at 100× magnification are shown in Figure 1.

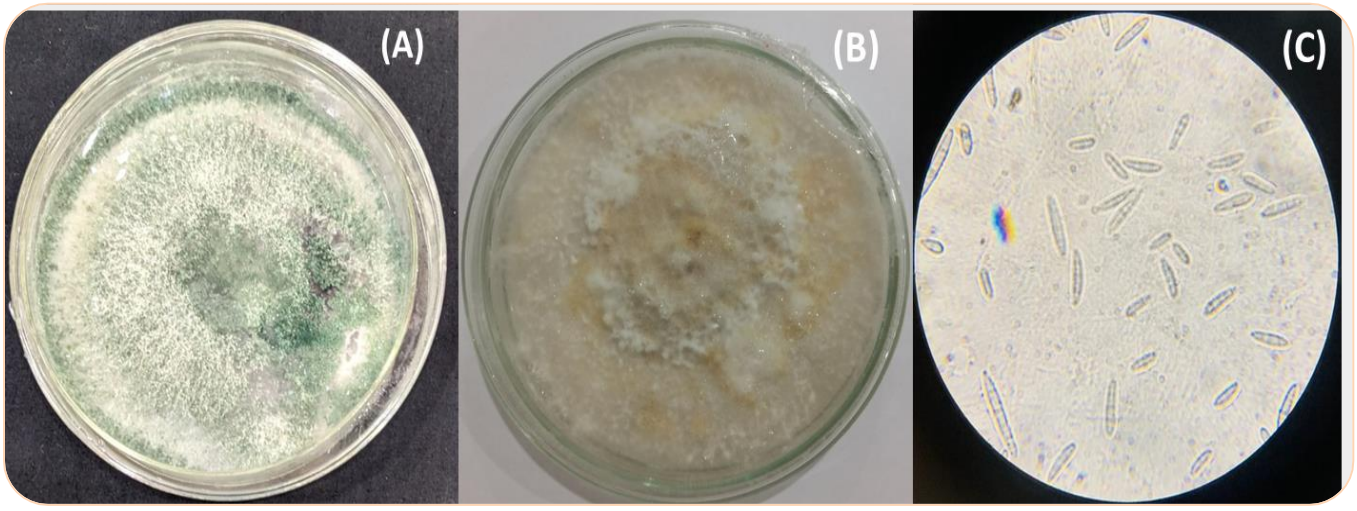

Figure 1. Pure colony of TH and FOV, Visualization of conidia produced by FOV at 100X, Pure colony of TH, Pure Culture plate FOV, Visualization of conidia produced by FOV at 100X. Pure colony of TH and FOV, Visualization of conidia produced by FOV at 100X. (A) Pure colony of TH (B) Pure Culture plate FOV (C) Visualization of conidia produced by FOV at 100X.

Effect of TH, volatile metabolites, and non-volatile metabolites produced by TH on FOV growth *in vitro*

The *in vitro* condition suppressed FOV by 71.4%. Volatile metabolites produced by TH suppressed FOV by 54.9%, and non-volatile metabolites produced by TH suppressed the growth of FOV by 64.21% compared to the control plate of FOV, which showed no inhibition zone, as shown in Figure 2.

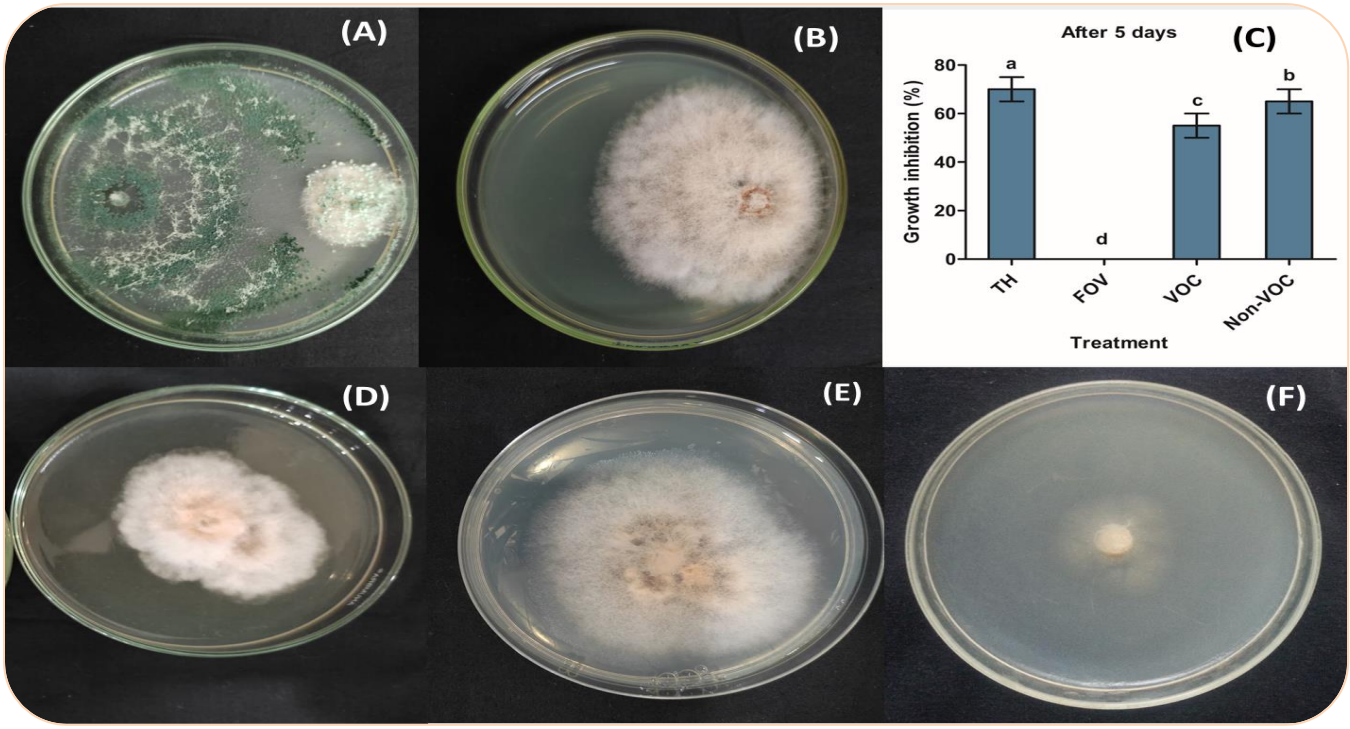

Figure 2. Effect of TH, Volatile metabolites suppress the FOV, and Non-Volatile metabolites produced by TH suppress the growth of FOV as compared to the control plate of FOV there is no inhibition zone, (A) Effect of TH on FOV in vitro condition (B) Control Plate of FOV (C) Graph represents the effects of TH on FOV (D) Volatile compounds produced by TH (E) control (FOV) for inverted plate assay for volatile and non-volatile (F) Non-Volatile compound produced by TH.

Greenhouse experiment

TH enhanced the growth of cotton plants as observed in field trials, while FOV caused wilting in cotton plants and reduced plant length and weight. Overall, the growth parameters were boosted in the TH-treated plants. In the greenhouse experiment, TH acted as both a biofertilizer and a biopesticide, as shown in Figure 3.

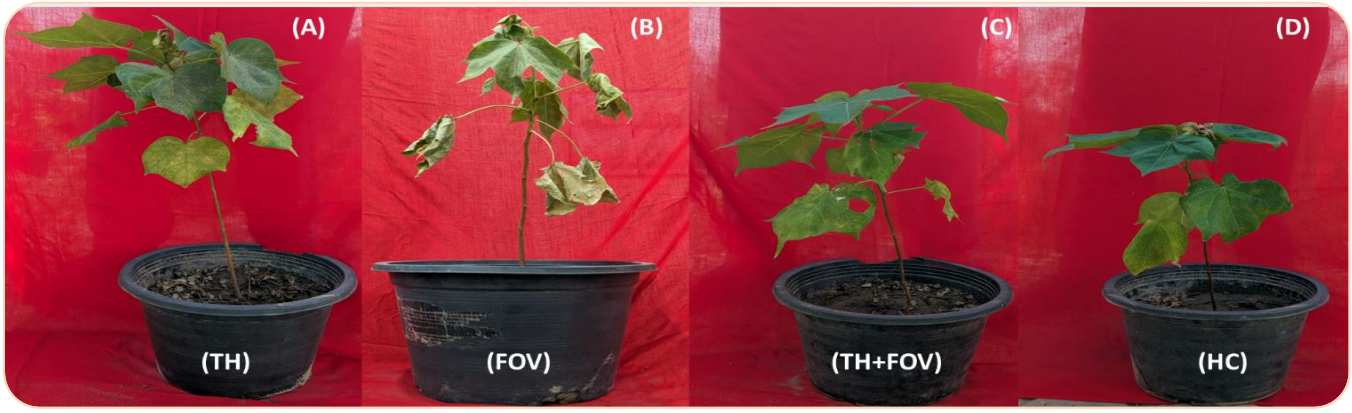

Figure 3. Experiment trail of cotton in the field, maximum growth was observed in TH treated cotton plants while minimum growth and wilting were recorded in FOV treated plants. (A) Effect of TH on cotton plant (B) Effects of FOV on cotton plant (C) Effects of TH and FOV on cotton plant (D) Healthy control.

Plant growth parameters

The highest growth parameters were observed in the TH-treated cotton plants, followed by the TH+FOV-treated plants. Healthy plants showed moderate growth, while the FOV-treated plants had the lowest growth due to wilting, as shown in Figure 4.

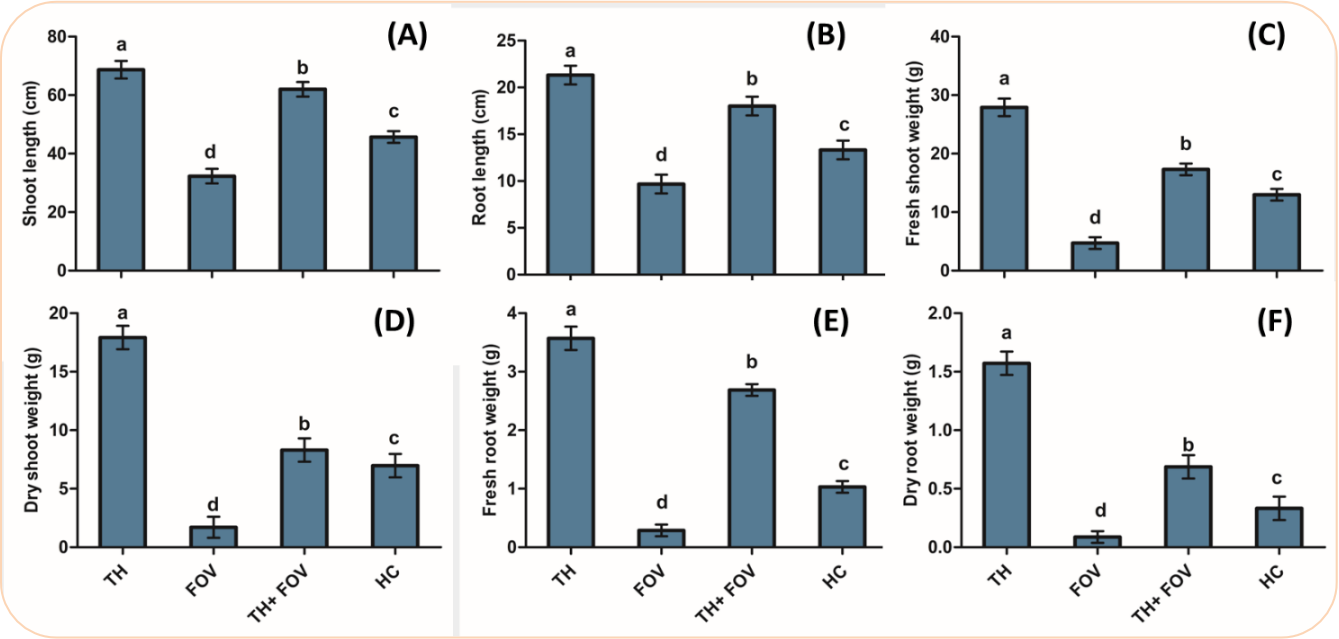

Figure 4. Shoot length (68.66 cm), root length (21.33 cm), fresh shoot weight (27.92 g), dry shoot weight (17.92 g), fresh root weight (3.57 g), dry root weight (1.57 g), (A) shoot length (cm) (B) root length (cm) (C) fresh shoot weight (g) (D) dry shoot weight (g) (R) fresh root weight (g) (F) dry root weight. Data were analyzed using the One-way ANOVA Tukey's HSD test (P<0.05). Different small letters have significant differences.

Antioxidant enzyme activity, protein content, MDA, proline, electrolyte leakage and H_2O_2 content, physiological features, SPAD chlorophyll value and RWC

Antioxidant defense enzymes, including CAT (UNIT mg^{-1} FW), SOD (UNIT mg^{-1} FW), and POX (UNIT mg^{-1} FW), showed the highest values in the TH+FOV treated cotton plants, while the lowest values were recorded in the healthy control. The protein content, proline content, MDA content, H_2O_2 content, and electrolyte leakage were highest in the FOV-stressed treatment in cotton plants. Physiological parameters, such as stomatal conductance, photosynthesis rate, SPAD chlorophyll value, and relative water content, were highest in the TH-treated cotton plants in field trials, with the minimum values recorded in the FOV-treated plants as shown in Figure 5.

DISCUSSION

In the present study, *T. harzianum* was isolated from a soil sample collected from a cotton field. The purified culture showed highly branching conidiophores with distinct continuous ring-like zones, as well as characteristic conidia, phialides, colony texture, and chlamydospores (Bhagat, 2008). FOV isolated from wilt-infected cotton plant roots exhibited features identical to those described by Leslie and Summerell (2006). FOV mycelium appeared white cottony to pinkish purple (Amini and Sidovich, 2010). When examined with a light microscope, three different spore types were observed for *F. oxysporum* f.sp. *vasinfectum*: microconidia, macroconidia, and chlamydospores (Asif et al., 2023).

The infection of FOV is a major factor in the worldwide decrease in cotton yield. In a laboratory experiment, *T. harzianum* effectively inhibited the growth of FOV through various mechanisms. These include competition for nutrients, production of antifungal chemicals, interaction with and destruction of fungal hyphae, and breakdown of the fungal cell wall. Previous studies have demonstrated that *T. harzianum* is the most effective antagonist in suppressing the growth of numerous plant diseases transmitted through soil and seeds (Dubey, 2003; Poddar et al., 2004).

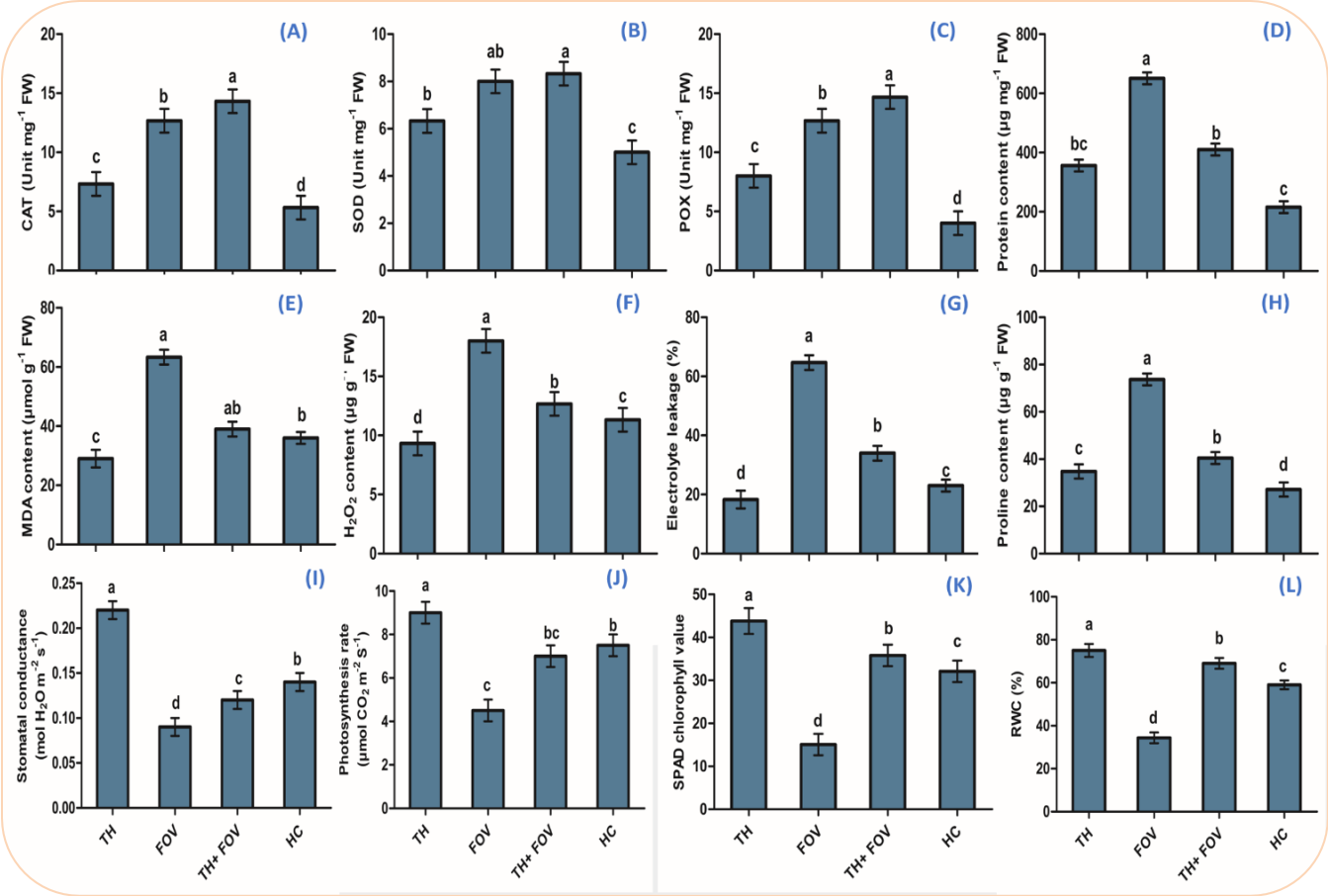

Figure 5. Antioxidant defense enzymes including CAT (14.33 UNIT mg^{-1} FW), SOD (8.33 UNIT mg^{-1} FW), and POX (14.66 UNIT mg^{-1} FW) observed high value in TH+FOV treated treatment in cotton plants, and minimum recorded in healthy control, protein content (650.87 µg mg^{-1} FW), MDA content (63.33 µmol g^{-1} FW), H_2O_2 content (18 µg g^{-1} FW), electrolyte leakage (64.66 %) proline content (73.73 µg g^{-1} FW), highest observed in FOV stress treated treatment in cotton plant, physiological parameters including stomatal conductance (0.22 mol H_2O m^{-2} s^{-1}), photosynthesis rate (9 µmol CO_2 m^{-2} s^{-1}), SPAD chlorophyll value 43.8, and relative water content (75 %) observed highest in TH treated in cotton plant in field trail while minimum value recorded in FOV treated. (A) Effect of TH on (A) CAT, (B) SOD, (C) POX (D) protein (E) MDA (F) H_2O_2 (G) electrolyte leakage (H) proline content (I) stomatal conductance (J) photosynthesis rate (K) SPAD chlorophyll value (L) RWC. Data were analyzed using the One-way ANOVA Tukey's HSD test ($P<0.05$). Different small letters have significant differences.

T. harzianum inhibits the growth of FOV by many mechanisms, such as direct antifungal activity, induction of stress response, interference with fungal signaling, modification of fungal morphology, and prevention of spore germination and mycelial growth. *T. harzianum* releases volatile metabolites that suppress the growth of *F. oxysporum* f.sp. *lycopersici* (Padmodaya and Reddy, 1996) and *Rhizoctonia solani* (Dubey and Patel, 2001).

T. harzianum synthesizes non-volatile metabolites, including antibiotics, cell wall-degrading enzymes, hydrolytic enzymes, and secondary metabolites, which effectively inhibit the growth of *F. oxysporum* f.sp. *vasinfectum*. In their study, Kumar and Dubey (2012) documented the inhibitory impact of a *T. harzianum* isolate on the growth of *F. solani* f.sp. *pisi*, the pathogenic fungus responsible for collar rot in pea plants. This inhibition is attributed to the production of non-volatile metabolites.

Recent research has demonstrated that the application of *T. harzianum* to cotton plants can improve multiple growth parameters. This treatment stimulates root development, enhances nutrient absorption, improves

soil health, and ultimately boosts plant growth. In contrast, when exposed to *F. oxysporum* f.sp. *vasinfectum*, these growth parameters diminish. The results indicate that *T. harzianum* has promise as a biocontrol agent and growth-enhancing inoculant, as demonstrated in a previous study (Sofy et al., 2022). *Trichoderma* species are plant symbionts that are highly versatile and have the ability to opportunistically invade plant roots (Brotman et al., 2013). Symbionts are recognized for their substantial interactions with host plants and their capacity to stimulate a wide range of resistance against plant diseases (Naseby et al., 2000; Yedidia et al., 2003; Harman et al., 2004). Previous research has shown that *Trichoderma* spp. can enhance plant growth.

In current studies, the antioxidant defense enzymes CAT, SOD, and POX showed the highest activity in treated cotton plants with *T. harzianum* and FOV, indicating the activation of plant defense mechanisms in response to these treatments. Minimum activity was recorded in healthy plants, similar to previous studies by Musheer et al. (2023). Protein content, MDA, proline content, electrolyte leakage, and H_2O_2 content, which are stress markers, were maximally assessed under *F. oxysporum* f.sp. *vasinfectum* stress, indicating oxidative stress and cellular damage in cotton plants, while minimum levels were detected in healthy and *T. harzianum*-treated cotton plants, as shown in previous investigations by Sutherland (2013).

Physiological features, including stomatal conductance, photosynthesis rate, SPAD chlorophyll value, and relative water content, recorded the highest readings in *T. harzianum*-treated plants. This is because *T. harzianum* acts as a biofertilizer and growth-promoting agent, boosting plant growth, which aligns with findings from previous research by Shukla et al. (2012).

The excessive synthesis of reactive oxygen species (ROS) is a biochemical response to FOV stress and is the primary cause of damage to macromolecules and cellular structures in plants due to FOV presence. Plants possess various enzymatic defense mechanisms to combat the detrimental effects of excessive ROS production (Rubio et al., 2012). The enzymatic antioxidant system is a vital plant defense mechanism, functioning through the synchronized activity of enzymes, including SOD, POX, and CAT.

A previous study by da Silva et al. (2008) found that chickpea seedlings exposed to FOV stress had higher levels of SOD, POX, and CAT activity compared to control samples. Regardless of the FOV concentration, the use of *T. harzianum* led to an increase in the activities of SOD, POX, and CAT in chickpea seedlings. This study demonstrated that *T. harzianum* significantly reduced the negative impact of FOV stress on Indian mustard (*Brassica juncea*) by enhancing the antioxidative defense system. The results of our study suggest that POX and CAT activity, along with SOD activity, is essential for safeguarding wheat seedlings against the harmful effects of ROS, such as O_2- and H_2O_2, when exposed to *T. harzianum* treatment.

Our study revealed that cotton plants, when exposed to FOV stress, demonstrated heightened production of antioxidants, including SOD, POX, and catalase CAT. Agricultural plants utilize increased antioxidant production as a defensive strategy against osmotic stress (Hayat et al., 2012). Previous studies (Irfan et al., 2014) have shown that these antioxidants accelerate the breakdown of ROS, superoxide, converting it into hydrogen peroxide (H_2O_2) and molecular oxygen. This phenomenon occurs in soil environments undergoing stress. H_2O_2, also known as hydrogen peroxide, is a reactive oxygen species that acts as a harmful intermediate in plants.

Furthermore, the *T. harzianum* strain exhibited superior scavenging capabilities for superoxide (O_2-) and H_2O_2 compared to the control (Kumar et al., 2020), thus enhancing its ability to protect plants against oxidative damage. Additionally, stomatal conductance, photosynthesis rate, SPAD chlorophyll value, and relative water content were found to be responsive to *R. solani* infection. According to Bahuguna et al. (2012), the SPAD value primarily reflects the nitrogen level of the plant and is highly responsive to environmental stress. It is also a simple and convenient method for assessing the health of plant populations under various stress conditions.

Recent research findings indicate that the application of *T. harzianum* decreased the levels of photosynthetic pigments in cotton plants under FOV stress. This reduction in photosynthetic pigments, specifically SPAD concentrations, is attributed to the combined effects of FOV stress and oxidative stress, contributing to water scarcity in cut stems. Interestingly, our results show a positive correlation between the presence of *T. harzianum* and higher SPAD chlorophyll values, indicating an enhancement in photosynthetic pigments.

CONCLUSION

In conclusion, our results showed that *T. harzianum* could be a remarkable biological control agent for preventing *Fusarium* wilt in cotton, offering a promising biocontrol strategy. This biocontrol agent has many beneficial effects, including reducing pathogen growth, inducing plant defense responses, and enhancing plant biomass. Therefore, *T. harzianum* and its volatile and non-volatile metabolites can be formulated as biofertilizers and biopesticides for the control of *Fusarium* wilt in cotton. They can be incorporated into the integrated disease management program for *Fusarium* wilt in cotton.

AUTHORS' CONTRIBUTIONS

TM and AM performed this research; MM, ARK, MR planned, designed, and conducted all the experiments; SQ, AQ, GMQ, and MSS helped to collect and analyze the experimental data; AM, MSB, ML and MA revised the whole manuscript with proofreading and scientific report writing.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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