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EFFECT OF *TRICHODERMA HARZIANUM* ON ENHANCING THE DEFENSE ABILITY OF TOMATO PLANTS AGAINST *ALTERNARIA CEREALIS* MT808477

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Alternaria cerealis **is the first record as a phytopathogen in tomato plants (*Solanum lycopersicum* L). This fungus has been identified as *A. cerealis* MT808477 in Egypt. In the present work, *Trichoderma harzianum* was used to control the leaf spot disease caused by *A. cerealis* at different concentrations of inoculum (10^5 and 10^7 spores/mL) in infected or non-infected plants after 2, 24, and 48 h. The interaction of *T. harzianum* is to enhance the redox buffer capacity to suppress *A. cerealis* infection and to stimulate many chemical signals that cause a considerable increase in the activity of some chemical defense. Polyphenol oxidase activity was increased in infected tomato plant, whereas its activity was significantly decreased after *T. harzianum* treatments. *T. harzianum* played a vital role in the resistance of tomato plants against *A. cerealis* leaf spot disease by enhancing the redox buffer capacity. The results indicated that the improvement of plant tolerance, activation of plant defense systems, modification of oxidative damage, and antioxidant mechanism varied with the type and duration of stress. Also, *T. harzianum* mediated protection against *A. cerealis*. This behavior may be associated with the alleviation of the oxidative burst in tomato leaves.**

Keywords: *Solanum lycopersicum* L., *Alternaria cerealis*, *Trichoderma harzianum*, *Oxidative stress*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetables in the world. It occupies the second most valuable product after potatoes in

many countries. In Egypt, approximately 8 million tons of fresh tomatoes are produced annually **(Ramezani *et al.*, 2019)**. *Alternaria* causes at least 20% of the agricultural spoilage in Egypt, the most extreme loss can be as high as 80% of the yield (**Nowicki *et al.*, 2012; Moghadam *et al.*, 2019)**. Tomato plants are constantly threatened by pathogenic microorganisms in the environment. Therefore, a physiological defense mechanism has been developed and beneficial endophytes are used for protection **(Wang *et al.*, 2020).** Various species of *Alternaria* are pathogens and cause various destructive diseases to tomatoes **(Kumar *et al.*, 2008).** *Alternaria* is a prevalent fungal genus with distribution as saprophytic and pathogenic species. The formation of necrotic spots in the concentric rings with yellow halos is a common symptom of *Alternaria* species **(Chaerani *et al.*, 2007; Kokaeva *et al.*, 2018).** Leaf spot symptoms can cause damage in tomatoes at all growth stages (**Blancard, 2012**). Several effective biological control agents have been reported to suppress important plant diseases and can effectively induce plants defense against various pathogens, such as *Trichoderma* sp., *Bacillus subtilis, Pseudomonas fluorescens,* and *Actinomycetes* **(Ramamoorthy *et al.*, 2001; Goudjal *et al.*, 2014; Ma *et al.*, 2015).** *Trichoderma* has a strong antagonistic and fungal parasitic effect on plant pathogens that induce defense mechanisms in several plants and has previously been reported as an effective biological control agent against several soil-borne pathogens (**Kobori *et al.*, 2015**; **Daryaei *et al.*, 2016**). Species of the genus *Trichoderma* use various mechanisms to control the growth and reproduction of harmful pathogens which are known as plant pathogen control agents **(Mukhopadhyay and Kumar, 2020).** *Trichoderma* induces an increase of defense-related enzymatic and non-enzymatic mechanisms and increases the level of soluble protein in leaves (**Yedidia *et al.*, 1999; Ahmed *et al.*, 2000; Wang *et al.*, 2020)**. *Trichoderma harzianum* inhibits the mycelial growth of fungal pathogens such as *A. solani* and *Phytophthora infestans* in tomato plants that have the ability to induce hormones (auxin and gibberellin) in plants and increase the level of plant defense mechanisms **(Chowdappa *et al.*, 2013).**

Phenolic compounds are produced and accumulated in the subcutaneous layer of plant tissues exposed to biotic and abiotic stress **(Schmitz-Hoerner and Weissenböck 2003; Clé *et al.*, 2008).** They act as

phytoalexins and phytoanticipin against soil-borne pathogens **(Lattanzio *et al.*, 2006).** Thus, phenolic compounds have a useful alternative for the chemical control of crop pathogens**.** They have a high tendency to chelate metals and are secondary metabolites of plants **(Cervilla *et al.*, 2012).** Plant phenolic compounds can remove harmful ROS and chelate heavy metals through hydroxyl and carboxylic acid **(Chen *et al.*, 2019).** These compounds have considerable physiological and morphological importance and exhibit physiological properties such as antioxidant and antimicrobial activities in plants **(Balasundram *et al.*, 2006).** Polyphenol oxidase catalyzes the oxidation of monophenols to quinones and, at the same time, interacts with oxygen and proteins to form (ROS), leading to the formation of complexes protein and brown melanin pigments**.** Hence, PPOs can affect local levels of oxygen and ROS **(Boeckx *et al.*, 2015a)**. The presence of PPO activity may be involved in the protecting cell against low-level oxidative stress in plants **(Boeckx *et al.*, 2015b)**.

To date, there is no information on the effect of *Trichoderma* in inducing the defense of plants against *A. cerealis* and there is no research on the physiological changes under the treatment of *Trichoderma* and *A. cerealis* in tomato plants. So, the purpose of this work was to use the *Trichoderma harzianum* as a biological control to evaluate its ability to reduce leaf spot disease caused by *A. cerealis* MT808477. Also, phenols, proteins, and oxidative stress as responses to infection were assessed.

MATERIALS AND METHODS

Isolation and identification of tomato leaf spot disease

Alternaria cerealis AUMC 14484 was originally isolated from pathogenic leaves of tomato and maintained on Potato Dextrose Agar (PDA) at 28±2°C. It was detected and identified by morphological and molecular methods according to **White *et al.* (1990)** and **Simmons (2007)**. Morphologically and genetically, *Alternaria cerealis* AUMC 14484 was amplified using the polymerase chain reaction (PCR) technique in which two universal fungal primers ITS1 and ITS4 were used.

Pathogenicity test

Conidial suspension of *Alternaria cerealis* isolated from infected tomato was prepared from 7 days old culture grown on PDA. Conidia were adjusted to 10^5/mL using the hemocytometer count technique. Pathogenicity test of *A. cerealis* was done using four weeks of tomato seedlings growing in soil containing 56.7% clay, 30.9% silt, and 12.4% sand, pH 7.11 and EC 2.27, using 1×10^5 conidia/mL of *A. cerealis* inoculum as mentioned by **Blagojević *et al*,. (2020).**

Evaluation of *Trichoderma harzianum* as spore inoculums under greenhouse conditions

Pathogen inoculum preparation

Conidia of *A. cerealis* were collected in sterile potato dextrose broth containing a trace amount of Tween 80. The conidial suspension was sieved through a 45 μm sieve to remove mycelial clumps and adjusted to 1×10^5 conidia/mL (**Solino *et al*., 2017**). Conidial suspension of *A. cerealis* was inoculated into the tomato plant after making deep wounds in plant leaves, and then plants were collected at intervals 2, 24 and 48 hours.

Saprophytic spore inoculums

Trichoderma harzianum was prepared from cultures grown on PDA at 28 ± 2°C for 10 days on the static incubator. The fungal spores 10^5 and 10^7 spores/mL were scratched from the growth surface and suspended in 0.1% tween-80 in sterilized distilled water.

The greenhouse experiment

A greenhouse experiment was conducted to examine the effect of *T. harzianum* as a biological control agent on tomato seedlings after infection with *Alternaria cerealis*. *Solanum lycopersicum* L. (13-day-old) plants were cultured in a black polyethylene bag (30 cm) with 1K sterilized soil and grown under environmental conditions (relative humidity (RH) 42-55%, average maximum temperature (T) 35 ± 2^0C and average minimum temperature (T) 22 ± 2^0C) **(Dhouib *et al.*, 2019)**. Chemical soil

characterization was recorded in ppm as follows: sodium (Na^{+}) 186.3, potassium (K^{+}) 46.8, calcium (Ca^{++}) 574, chloride (Cl^{-}) 887.5, magnesium (Mg^{++}) 344.4 and carbonate (HCO^{-}) 1525. The physical properties of the soil texture were 56.7% clay, sand 12.4% and silt 30.9%; soil pH 7.11, electrical conductivity (EC) is 2.27 Mmhos/cm. The treatments were as following: (T1) tomato plants were treated with conidial suspension (10^5conidia/mL) of *A. cerealis* (T2) tomato samplings were only sprayed with spore suspension (10^5 and 10^7spores/mL); (T3) tomato saplings were treated with *A. cerealis* and *T. harzianum*; (T4) tomato plants without treatments were used as control. Three plots were used for each treatment. Plant leaves were examined after 2, 24 and 48h and the content of total phenol, soluble proteins, total antioxidant, MDA, H_2O_2 and polyphenol oxidase were analyzed **(de Oliveira *et al.*, 2021).**

Determination of total phenol

The Folin-Ciocalteu described by **Meda *et al.* (2005)** was used to determine the concentration of total phenol content. Fresh tomato leaves were dissolved in methanol (1 mL). The methanol extract (100 µL) was mixed with 750 µL of 1N Folin-Ciocolteu reagent (1:10). After standing at room temperature for 5 minutes, 60 µL Na_2CO_3 (7.5%) was added to the extract. The mixture was incubated for 30 minutes at room temperature. The absorbance of the reaction mixture was measured at 750 nm. The phenol concentration in the extract was determined from a standard curve prepared from gallic acid and expressed as µg g^{-1} Fresh Weight.

Soluble proteins determination

The content of soluble proteins was calculated according to the method of **Lowry *et al.* (1951).** The frozen leaf segments (1g) were ground in liquid nitrogen to a fine powder and then homogenised into 10 mL of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 Na_2-EDTA and 0.1 g polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 15000 rpm at 4^oC for 15 minutes and the supernatant was gathered and used to measure the content of soluble proteins content. The protein content was calculated as a standard using bovine serum albumin.

Determination of total antioxidants

Total antioxidant activity was calculated by combining an aliquot (50 µl) with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and 90 minutes of incubation in a water bath at 95 °C. The mixture of reactions was cooled at room temperature and measured at 695 nm. The result was expressed as µg g^{-1} protein **(Prieto *et al.*, 1999).**

Polyphenol oxidase

The enzyme activity of polyphenol oxidase was performed in 2 mL of 0.1 M phosphate buffer (pH 6), 1 mL of 0.1 M catechol and 200 µl of enzyme extract. The reaction mixture was incubated for 5 min at 25ºC. The absorbance of the purpurogallin formed was monitored at 495 nm. The PPO activity was expressed as DA_{495} mg $protein^{-1}$ min^{-1} **(Kumar and Khan, 1982)**.

Malondialdehyde (MDA) determination

The Malondialdehyde (MDA) content was determined using the thiobarbituric acid reaction as following; 0.5 g plant tissue were homogenized with 5 mL ethanol (80%), centrifuged for 10 minutes at 10,000 rpm. For every one mL aliquot of the supernatent, 3 mL of 20% trichloroacetic acid (TCA) containing 0.65% thiobarbituric acid (TBA) was added. The mixture was heated at 95 ºC for 30 minutes, then cooled quickly in an ice-bath. After that, the mixture was centrifuged at 10,000 rpm for 15 minutes and the absorbance of the supernatant was measured at 532 nm (**Hodges *et al.*, 1999).**

Hydrogen peroxide determination

Leaves (0.3 g) were homogenized in an ice bath with 5 mL of 0.1% Trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant (0.5 mL) was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. After 20 min, the absorbance of the supernatant was read at 390 nm. A calibration curve, using H_2O_2, was made and the concentration expressed as mg g^{-1} FW (**Velikova *et al.*, 2000)**.

Statistical analysis

A one-way analysis of variance (ANOVA) was used for statistical analysis of the data and the separation of the means was compared with the multiple range test performed by Duncan, all statistical analyses were performed by using SPSS ver 25. It was found that the difference was significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Pathogenicity of *A. cerealis* MT808477 on tomato plants

In the current experiment, tomato leaves were infected with *A. cerealis* AUMC 14484. The pathogen infected all the plants with 100% prevalence as confirmed as new tomato phytopathogen. The symptoms appeared as yellow lesions with small brown spots after two weeks of infection. The size of the spots was gradually increased, forming dark brown concentric circles. It's the first record of *A. cerealis* as tomato phytopathogen that causes dark spot lesions **(Figure 1),** which was identified molecularly by ITS sequencing, giving the Accession no. MT808477 **(Figure 2)**.

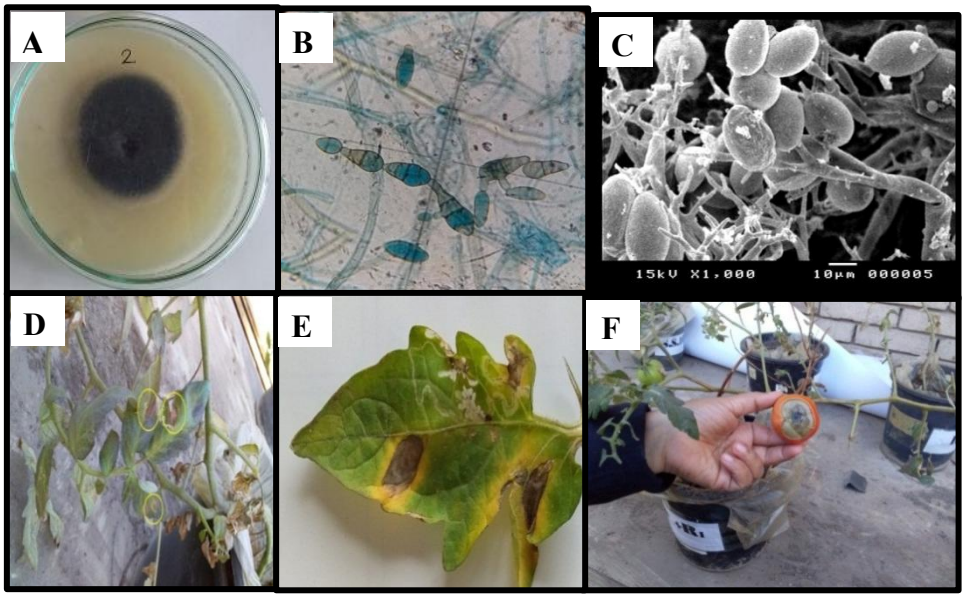

Figure 1: Symptoms of *Alternaria cerealis* MT808477 leaf spot disease in tomato (*Solanum lycopersicum* L.), (A) *Alternaria cerealis* on PDA medium, (B) Structure of conidia under the light microscope (40X), (C) Conidia under the Electronic microscope, (D & E) Disease symptoms on the leaves with brown spots, (F) The severity of disease by the pathogen in the fruit of tomato plant.

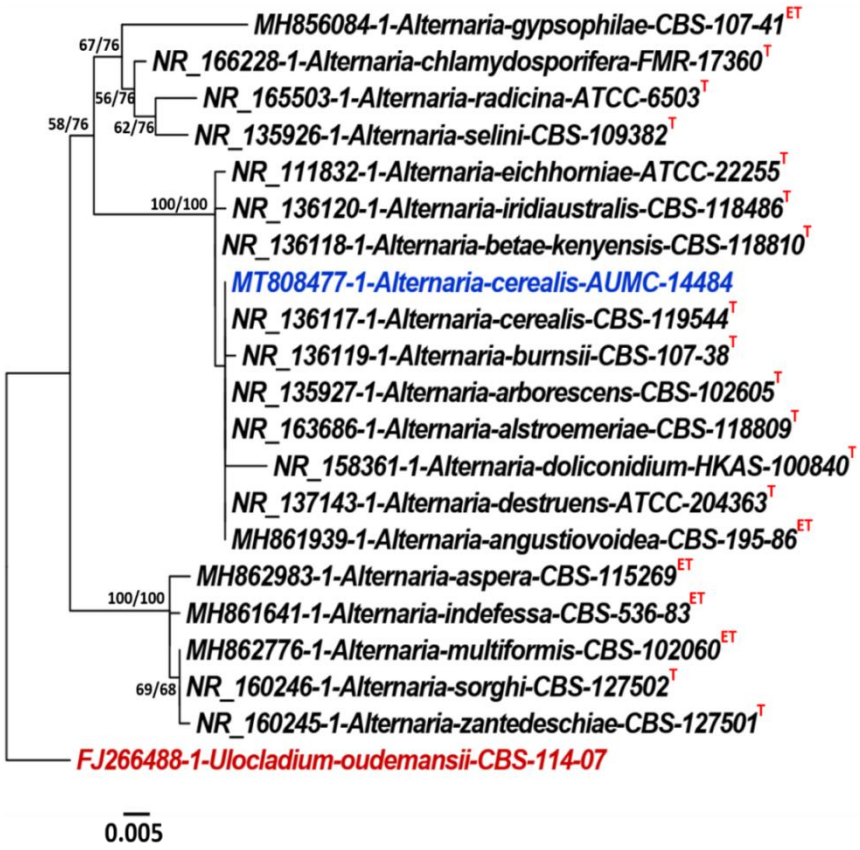

Figure 2: Phylogenetic tree based on DNA sequence data of ITS region of *A. cerealis* AUMC 14484 (MT808477) in blue color associated with other related genes in the ITS gene sequences belonging to *Alternaria*.

Tomato plants represent a rich source of minerals, vitamins and the antioxidant lycopene, contributing to a healthy and balanced diet (**Awan *et al.*, 2018**). Fungal pathogens can cause serious diseases in tomato plants, including; *Alternaria alternata*, *A. chlamydosporigena*, *Fusarium oxysporum*, *F. Solani*, *Rhizoctonia solani* and *Sclerotium rolfsii*, causing leaf spot, black mold and *Fusarium* wilt (**Shenashen *et al.*, 2017; Elshahawy *et al.*, 2018; Sadeghi and Mirzaei 2018; Hao *et al.*, 2020).** *Alternaria* is the common pathogen of leaf spot that causes diseases in tomato plants. It's the first record of *A. cerealis* as tomato phytopathogen that causes dark spot lesions. In this respect, **Akhtar *et al.*, (2004)** isolated 35 strains of *Alternata alternata* from rotten fruits in the fields and markets, and observed that only one isolate

was able to produce symptoms of leaf blight and was the first report of blight in Pakistan. Also, in Korea, leaf spots disease was first recorded in sesame plants (*Sesamum indicum* L.) caused by *A. simsimi* **(Choi *et al.,* 2014)**. The presence of *A. cerealis* MT808477 in tomato plants should be considered as the cause of food disqualification. In order to reduce the incapacity and loss in tomato plant production caused by this pathogen, a biological control agent was used to control the leaf spot disease caused by *A. cerealis. T. harzianum* is used in a wide range of crop plants as a biocontrol agent for the management of different pathogens **(Vinale *et al.,* 2008; Harman, 2011**).

Total phenolic content

The content of phenolic compounds in methanol extracts was determined after 2, 24 and 48 h in all treatments **(Figure 3).** The phenolic content was significantly increased in the plant infected with *A. cerealis* by 110.85±0.32 mg/g FW after 2 h, 104.79±0.51 mg/g FW after 24 h, and 101.23 ±1.77 mg/ g FW 48 h, compared to the control (66.225±0.76 mg/g FW). The current results indicated that phenolic content was significantly increased in the plant infected with *A. cerealis* spores compared to the control (66.225±0.76 mg/g FW), results in agreement with those obtained by **Aryal *et al.,* (2019)**. They observed that the methanol extract of *Alternanthera sessilis* produced a great phenolic content (292.65 ± 0.42 mg gallic acid equivalent (GAE)/g). The functions of phenolic compounds in plant physiology and their interactions with biotic and abiotic environments are difficult to overestimate. They play an important role in protective agents, inhibitors, and pesticides against fungal pathogens (**Bhattacharya *et al.,* 2010).** Also, phenolic compounds are good electron donors because their hydroxyl groups can directly promote antioxidant effects. Phenolic compounds exhibit free radical suppression and oxygen scavenging effects in biological systems, and prevent the oxidative stress **(Bendary *et al.,* 2013; Aryal *et al.,* 2019).** Also, phenolic compounds were enhanced in healthy plants treated with *T. harzianum* spores, but this improvement was less than that of infected plants. As shown in **Figure 3**, the total phenolic content in tomato saplings treated with the pathogen and *T. harzianum* was significantly increased in all treatment periods by 120.79 ± 1.66 mg/g FW, 143.29± 3.68 mg/g FW, 196.29± 2.09 mg/g FW, after 2, 24, and 48 h, respectively. The results also indicated that there were positive correlations between the total phenolic compounds and treatments with

saprophytic fungal spores in tomato plants by 0.340 and 0.146, respectively. **Ramamoorthy *et al.*, (2002)** mentioned that the induction of defensive enzymes involved in the accumulation of phenolic compounds limited the invasion of *Fusarium oxyporum* f.sp. *lycopersici* tomato root.

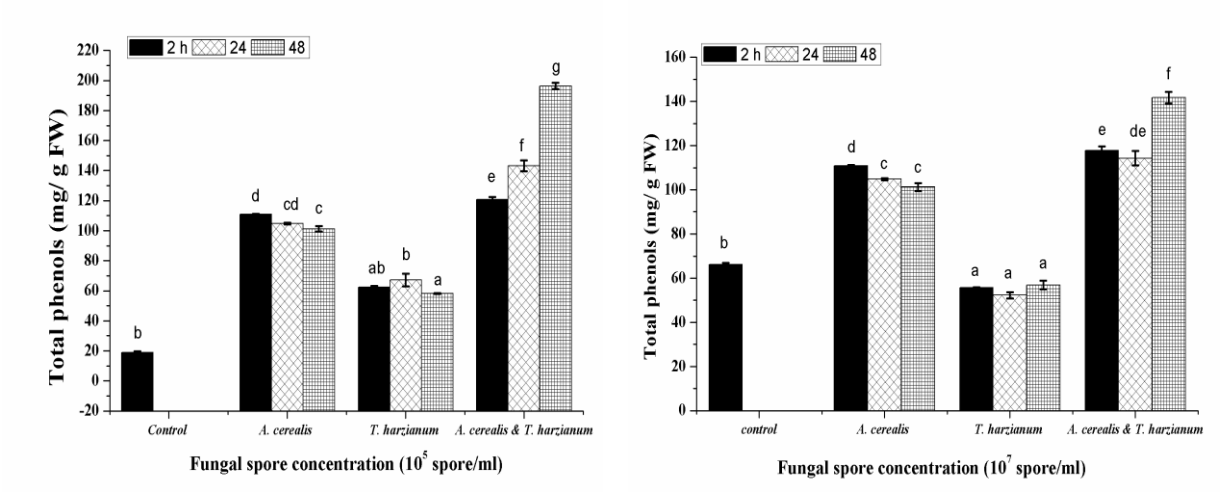

Figure 3: Total phenol of infected tomato leaves (*S. lycopersicum* L.) by *Alternaria cerealis* (MT808477). Data are means ± SD (n = 3), with statistically significant differences ($P \leq 0.05$).

Soluble proteins

Proteins are rich macromolecules that represent the main functions of living cells. Therefore, in this experiment, the result of assorted *A. cerealis* and *T. harzianum* spores inoculum (10^5 and 10^7 spores/mL) treatments on the content of soluble proteins in tomato plants was examined **(Figure 4).** The soluble proteins were significantly stimulated in healthy tomato plants treated with different concentrations of *T. harzianum* (10^5 and 10^7 spores/mL) in all incubation than the infected ones. Results additionally demonstrated that soluble proteins were correlated with the time of infection. The highest soluble protein was recorded after 48 h in tomato plants treated with *A. cerealis* MT808477 alone by 16.05± 0.35 mg/g FW. However, in the tomato plant treated with *T. harzianum* soluble proteins were recorded at 36.90 ± 0.09 mg/g FW in spore inoculum 10^7 spores/mL after 48 h. The highest concentration of soluble protein was found in the plant treated with *T. harzianum* and *A. cerealis* after 48 h by 39.75± 1.39 mg/g FW. It was worth mention that the correlation between soluble protein content and spores

inoculum concentration 10^5 or 10^7 spores/mL was strongly positive by 695** or 666**, respectively.

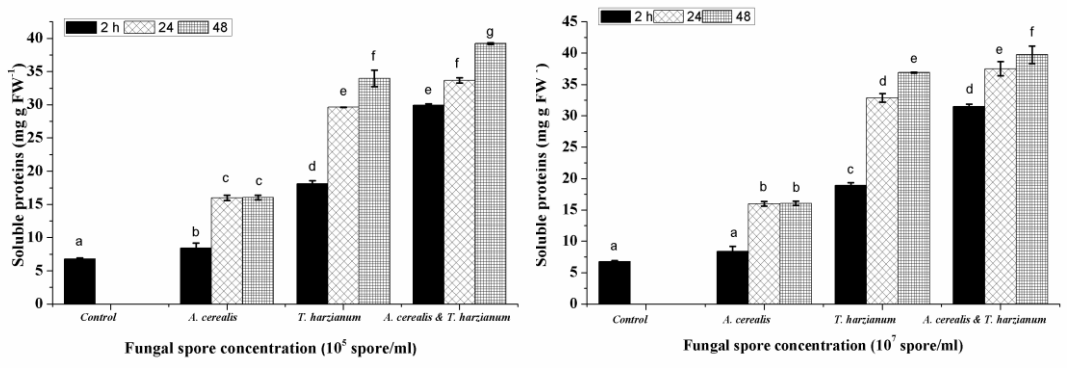

Figure 4: Soluble protein of infected tomato leaves (*S. lycopersicum* L.) by *Alternaria cerealis* (MT808477). Data are means ± SD (n = 3), with statistically significant differences ($P \leq 0.05$).

Total soluble proteins are studied as an index of metabolic changes under stress conditions; ROS cause serious damage by interaction with cellular components such as proteins, nucleic acids and lipids (**Sabatini *et al.*, 2009).** In tomato plants treated with *A. cerealis* MT808477 alone, the highest soluble protein (16.05± 0.35 mg/g FW) was recorded**. Antoniw *et al.*, (1980)** believed that protein is related to the defense of plants against pathogens. In the current study, the high concentration of soluble protein was found in plants treated with *T. harzianum* and *A. cerealis* after 48 h. This result was similar to the finding of **Wang *et al.*, (2020)** who showed that the soluble protein levels induced by the four *T. asperellum* strains may be indicated strong tolerance to stresses in the early stage of *Populus davidiana*.

Total antioxidants

The total antioxidant is a quick and easy method to measure the antioxidant pool. The total antioxidant was determined in tomato plants subjected to *A. cerealis* or *T. harzianum* to assess the quantity of scavenged ROS**.** As portrayed in **Figure 4,** there was an elevation in the total antioxidant content in tomato plants infected with *A. cerealis* after 2 h (51.05± 5.64 mg/g protein)**.** Then a decrease in total antioxidants was

observed after the remaining incubation periods (24 & 48 h). Results additionally explained that there was a correlation between total antioxidants and the concentration of spore inoculum (10^5 and 10^7 spores/mL) with increasing *T. harzianum* concentration in tomato plants treated with *T. harzianum* by 31.76±1.83 and 42.32± 1.07 mg/g protein in10^5 and 10^7spores/mL, respectively. On the other hand, the tomato plant infected with *A. cerealis* and treated with *T. harzianum* showed an increase in total antioxidant contents in all concentrations and incubation periods. Statistical analysis showed a strong negative correlation between the complex network of different inoculum concentrations of *T. harzianum* with *A. cerealis* and the total antioxidant in tomato leaves (- 0.705** and- 0.739 **, respectively).

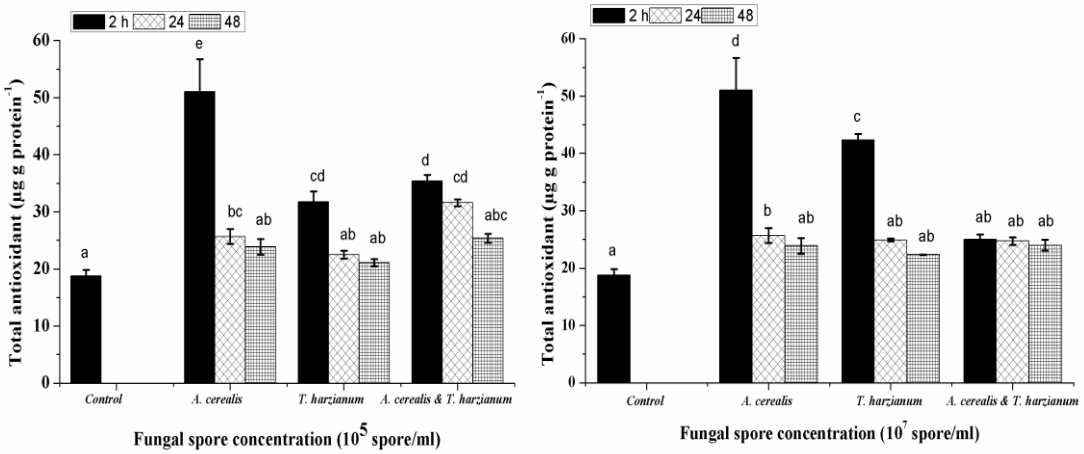

Figure 5: The total antioxidant content of infected tomato leaves (*S. lycopersicum* L.) by *Alternaria cerealis* (MT808477). Data are means ± SD (n = 3), with statistically significant differences ($P \leq 0.05$).

In the present study, there was an elevation in the total antioxidant content in tomato plants infected with *A. cerealis* after 2 h. Biochemical reactions that occur in the membrane are the main source of ROS produced by plant cells **(Farmer and Mueller, 2013).** Enhancement of antioxidant capacity might protect plants under stress conditions. Variations were observed in free radical scavenging response, the total antioxidant response was found directly linked with non-enzymatic antioxidative molecules, but

the direction of response depends on the plant species, tissue analyzed, the metal used for treatment, and also the intensity of metal stress. Our results explained that there was a correlation between total antioxidants and the concentration of spores (10^5 and 10^7 spores/mL) with increasing *T. harzianum* concentration in tomato plants treated with *T. harzianum*. In this instance, **Mastouri *et al*,. (2012)** reported that the ability of *T. harzianum* to improves tolerance in colonized plants by the enhanced redox buffer capacity of colonized plants. Plants exposed to biotic stress may produce high levels of (ROS), due to some signs of toxicity **(Mittler, 2002).** *Trichoderma* can enhance the enzymes in the glutathione-ascorbic acid cycle; recycle antioxidants more rapidly and reducing the impact of stress **(Harman, 2011)**. Also, *T. harzianum* treatment improves the antioxidant activity of grape plants **(Pascale *et al.*, 2017). Wang *et al.*, (2020)** reported that *Trichoderma aspereullm* induced the activity of ROS scavenging enzymes in plants and kept at a high level after inoculation, which may minimize the damage to the plants during the colonization of *T. aspereullm*.

Malondialdehyde (MDA)

The content of MDA, a product of polyunsaturated fatty acids peroxidation in the cells, was determined in tomato plants exposed to various treatments to assess the degree of membrane damage resulted from *A. cerealis* or *T. harzianum* stress. Results in **Figure 6** indicated that the MDA was significantly increased in infected plants or treated with *T. harzianum* or both, and it dramatically increased with increasing the incubation periods. The treatment with *A. cerealis* or *T. harzianum* displayed significant positive correlations between the increment in MDA content and incubation periods (R =0.831**) in the tomato plants.

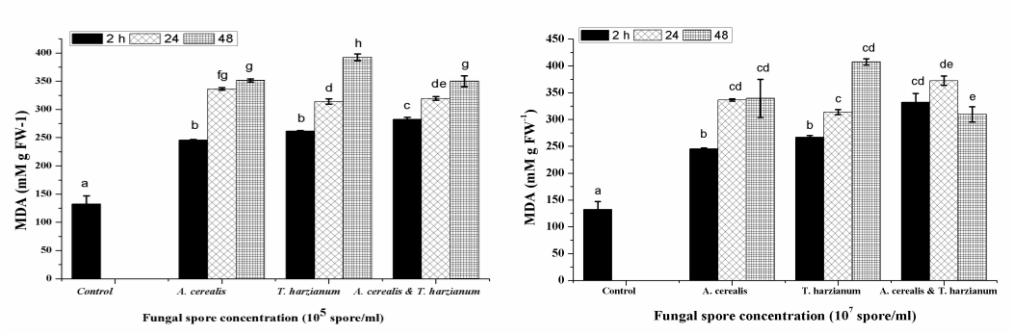

Figure 6: The MDA content of infected tomato leaves (*S. lycopersicum* L.) by *Alternaria cerealis* (MT808477). Data are means ± SD (n = 3), with statistically significant differences ($P \leq 0.05$).

Hydrogen peroxide

Hydrogen peroxide is generated in many biological systems and has been considered an important signaling molecule that mediates numerous physiological and biochemical processes in tomato plants. The data herein obtained clearly demonstrated that the H_2O_2 content in tomato plants was considerably increased in the infected plant or treated with *T. harzianum* and drastically increased in plants infected with the pathogen and treated with *T. harzianum* after 2 h specially at 10^5 spores (4.12± 0.03) (**Figure 7**). Moreover, the results indicated that H_2O_2 and MDA contents in the tomato plants were significantly positively correlated (R = 0.567**).

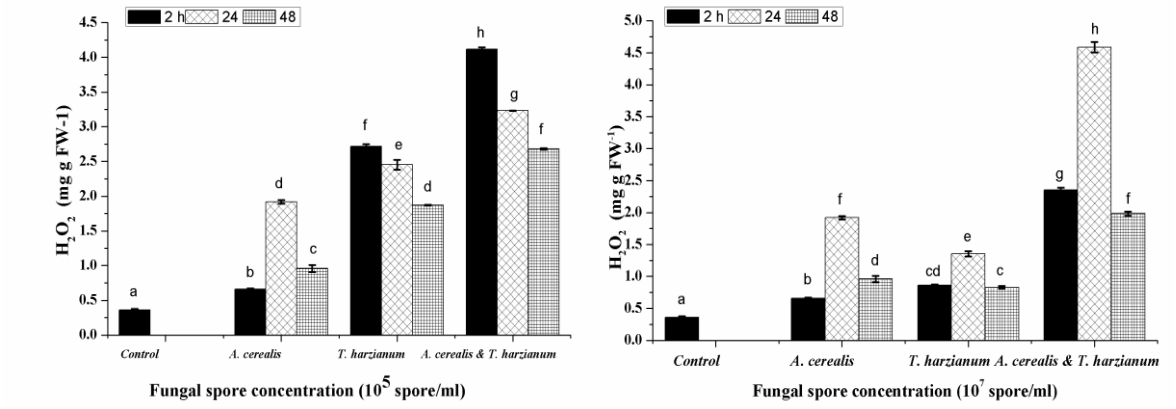

Figure 7: The H_2O_2 content of infected tomato leaves (*S. lycopersicum* L.) by *Alternaria cerealis* (MT808477). Data are means ± SD (n = 3), with statistically significant differences ($P \leq 0.05$).

Results indicated that MDA and H_2O_2 contents were significantly increased in infected plants or treated with *T. harzianum* or both and dramatically increased with increasing the incubation periods. MDA and H_2O_2 levels are commonly used as indicators of cell damage. Comparing this data with the data reported in previous studies, it was found that the MDA and H_2O_2 content in tomatoes was increased significantly after the stress, indicating the damage to the cell membrane in the later stage of the stress response. The high production of MDA in plant cells may be related to the decrease in salicylic acid production, which can protect the cell photosystem II **(Cervilla *et al.*, 2007).** Our results were greatly similar to the results obtained by **Zehra *et al.*, (2017)** who showed that the MDA and H_2O_2 content gradually increased in tomato plants that were infected with *Fusarium oxysporum*. Hydrogen peroxide represents a key metabolite in oxidative stress **(Sies *et al.*, 2017).** Our results showed that there was a higher H_2O_2 content in plants pretreated with a combination of *Trichoderma harzianum* and pathogen. **Zehra *et al.*, (2017)** showed that maximum H_2O_2 production after treatment with salicylic acid along with *Fusarium oxysporum* or in combination with *T. harzianum.*

Polyphenol oxidases (PPO)

Polyphenol oxidase catalyzes the oxidation of various phenols and some studies have reported a positive correlation between PPO expression and resistance/tolerance to biotic/abiotic stress. Data in **Figure 8** manifested that the PPO activity in tomato plants showed a significant increase in response to infection with *A. cerealis* especially after 2 h (2.88 ± 0.19). Whereas, the plants treated with the *T. harzianum* or both, the activity of PPO correlated drastically decreased till the end of the experiment. The PPO activity was negatively with all treatments (-0.637** and -0.586**) in tomato plants treated with the pathogen and *T. harzianum* spores inoculum (10^5 and 10^7 spores/mL), respectively.

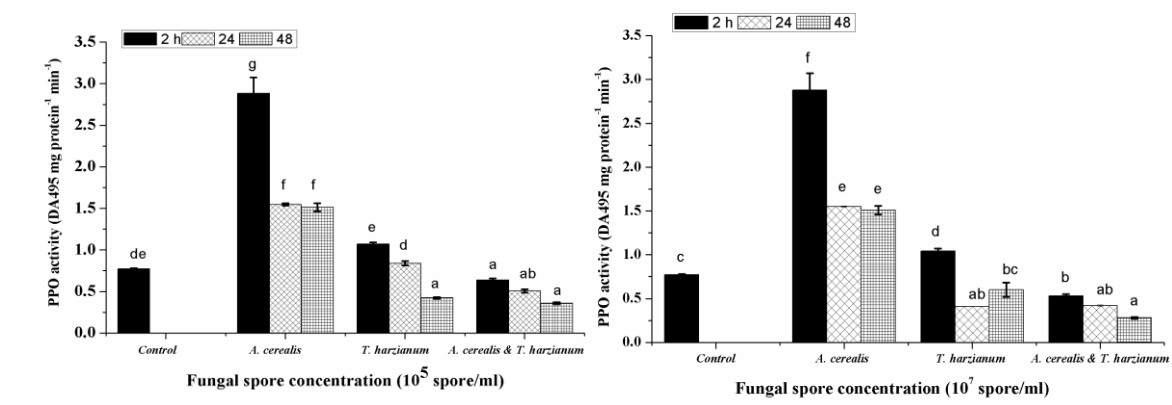

Figure 8: Polyphenol oxidase of infected tomato leaves (*S. lycopersicum* L.) by *Alternaria cerealis* (MT808477). Data are means ± SD (n = 3), with statistically significant differences ($P \leq 0.05$).

The activity of PPO in tomato plants showed a significant increase in response to infection with *A. cerealis* especially after 2 h. **Patil *et al.*, (2011)** found an increase in the concentration of polyphenol oxidase activities at the time of induction of systemic resistance (ISR) by non-pathogenic strains of *F. solani* and *F. moniliforme* against *Fusarium oxysporum* f.sp. *lycopersici*. *T. harzianum* improved the tolerance to various stresses, including excessive light water deficit, salinity, suboptimal temperature, and decreased seed quality **(Mastouri *et al.*, 2010; Shoresh *et al.*, 2010).** In tomato plants that

were treated with the pathogen and *T. harzianum*, the activity of PPO was drastically decreased till the end of the experiment. This behavior is related to the bioagents-treated plants recovered from the stress status of the pathogens. Our results were greatly similar to the results obtained by **Al-Tuwaijri (2009)** who demonstrated that treatment of cucumber plants with *T. viride* and *Bacillus subtilis* resulted in a significant reduction in the activity of all oxidative enzymes in the tissues of infected plants as compared to infected untreated plants.

CONCLUSION

The obtained results showed that the application of *T. harzianum* plays a vital role in the resistance of tomato plants to *A. cerealis* leaf spot disease. *T. harzianum* stimulated many chemical signals, thereby increasing the total phenol, soluble protein, total antioxidant, MDA and H_2O_2 after incubation periods in all treatments and the concentration of spore inoculum in tomato plants. Whereas polyphenol oxidase was increased in infected tomato plants but decreased significantly in combination with *T. harzianum*. These results confirm that *T. harzianum* has a stronger endurance and stress ability and mitigated the effect of oxidative burst by enhancing the redox buffer capacity, improving plant tolerance and activating plant defense systems.

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تأثير ترايكودرما هارزانيم في تعزيز القدرة الدفاعية لنباتات الطماطم ضد الالترناريا سريالس MT808477

تم تسجيل الالترناريا كفطر ممرض لنبات الطماطم لأول مره في مصر وتم تعريفه بإسم الالترناريا سريالس MT808477. تم استخدام ترايكودرما هارزانيم للسيطرة على مرض تبقع الأوراق الناتج عن الالترناريا سريالس بتركيزات مختلفة من اللقاح (١٠^{٥} و ١٠^{٧} جراثيم / مل) في النباتات المصابه أو غير المصابه بعد ٢ ، ٢٤ ، ٤٨ ساعة. اظهرت النتائج ان ترايكودرما هارزانيم مع الكائن الممرض يعزز القدرة التأكسدية لقمع عدوى الالترناريا سريالس وتحفيز العديد من الإشارات الكيميائية التي تسبب زيادة كبيرة في نشاط بعض الدفاعات الكيميائية. منها زيادة نشاط بوليفينول أوكسيديز في نبات الطماطم المصاب بينما انخفض بشكل ملحوظ في معاملات الترايكودرما هارزانيم بمفردها. لعبت ترايكودرما هارزانيم دورًا حيويًا في مقاومة نباتات الطماطم ضد الالترناريا سريالس المسببه لمرض تبقع الاوراق من خلال تعزيز القدرة التأكسدية. اوضحت النتائج إلى أن تحسين تحمل النبات، وتفعيل أنظمة الدفاع النباتي ، وتعديل الضرر التأكسدي ، وآلية مضادات الأكسدة تختلف باختلاف نوع ومدة الإجهاد ايضا أدت الترايكودرما هارزانيم الى الحماية ضد الالترناريا سريالس. وقد يرتبط هذا السلوك بتخفيف الانفجار التأكسدي في أوراق الطماطم.