Alleviation of the adverse effects of NaCl stress on tomato seedlings (Solanum lycopersicum L.) by Trichoderma viride through the antioxidative defense system

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Research Article

Keywords: Trichoderma viride, tomato, seedling growth, reactive oxygen species, salt stress, proline content, H2O2 content, Antioxidant capacity

Posted Date: February 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1241160/v1

Abstract

Background: Species of *Trichoderma* are widely recognized for their biocontrol abilities, but little information regarding their mechanisms in promoting plant growth and enhancing its tolerance to salt stress are available. Salt stress is one of the main abiotic stresses restricting crop growth and productivity. Hence, this study aimed to investigate the NaCl effects on *Trichoderma viride* growth as well as on the seedlings morphological, physiological, and biochemical parameters of tomato (*Solanum lycopersicum* L.). Besides, the role of *T. viride* in promoting tomato seedling stress tolerance was scrutinized.

Results: Results showed that 100 mM NaCl decreased the colony diameter of *T. viride* by 13.4 % compared to the control. Exposure of tomato seedlings to salt stress resulted in an overall decrease in growth, relative water content (RWC), and protein contents. At the same time, increases were found in proline, H_2O_2 content, malondialdehyde (MDA), as well as the activities of peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO), and ascorbate peroxidase (APX). Even though, with *T. viride* application, the salt negative effects were mitigated to a greater extent. Moreover, *T. viride* increased proline and total antioxidant capacity (TAC) in tomato seedlings at 100 mM NaCl by an average of 20.66 and 43.82 % compared to their comparable control. *T. viride* increased the activities of CAT, PPO, and APX enzymes by 74.6, 58.48, and 61.61% at 50 mM NaCl compared to non-saline control seedlings. As well, *T. viride* decreased MDA and H_2O_2 contents by an average of 14 and 24.8 % in tomato seedlings at 50 mM NaCl compared to their comparable control.

Conclusion: Hence, our study provides new insight into the mechanisms of *T. viride* that can activate both enzymatic and non-enzymatic antioxidant defense systems and enhance tomato seedling tolerance to salt stress at morphological and physiological levels.

Background

Being sessile in nature, plants are often exposed to different biotic and abiotic stresses that affect up to 50% of the crop productivity, these stresses are interconnected resulting in biochemical, morphological, and physiological changes causing plant death [1, 2]. Tomato (*Solanum lycopersicum* L.) is the second most important produced and consumed solanaceous crop in the world after potato [3], with a worldwide production of 170 million tons in 2014 [4]. It is nutrient-enriched containing water, proteins, fibers, carbohydrates, calories, vitamins, and 37 minerals [5]. It acts as an antioxidant, preventing risks of cancer and eye diseases, lowering blood pressure, and reducing risks of kidney stone production [6]. In Egypt, tomato is cultivated on a total area of 216,400 hectares accounting for 36% of the total volume of vegetable production [7]. This demonstrates the need to increase the production of this crop at the national level. However, despite the use of resistant varieties, the cultivation of tomatoes is still subject to resistance to abiotic stresses that sometimes cause severe damage [6].

One of these abiotic stresses that restrict plant growth is salinity [8, 9]. Universally and also in Egypt, large areas of land were out of cultivation due to salt accumulation [10]. Recently, according to the Russian classification of the East Nile Delta area, soil salinization levels were 71 percent non-saline, 10.5 percent mild saline, 9 percent moderate saline, 3.8 percent strong saline, and 5.7 percent very strong saline [11]. Plants undergo dehydration, nutritional disorders, oxidative stress, membrane instability, and decreases in metabolic and photosynthetic activity in saline soil [9, 12, 13]. As well, salinity results in a toxic concentration of Na^+ and Cl^- in the cytosol and cell organelle and also affects the water uptake [14]. Significant efforts have been made to create salt-tolerant plant genotypes through traditional breeding or genetic engineering to reduce the negative effects of salt stress. However, these attempts have shown limited success, as transgenic plants can easily lose the functional genes responsible for salt tolerance [15].

The use of microbes that encourages plant growth is an alternative strategy to boost plant tolerance to salt stress. One of these growth-promoting microbes is arbuscular mycorrhizal fungi that have been reported to boost the ability of plants such as cowpea and fenugreek to cope with salinity [12]. Besides, *Trichoderma* species, a genus of plant beneficial fungi, can provide opportunistic symbionts to induce plant tolerance to abiotic stresses [16, 17]. These fungal species are often found in the rhizosphere and can provide beneficial effects on plant growth and yields [13, 18]. The ameliorative effects of *T. harzianum* (Th-6) on maize and rice below a hydroponic saline environment were examined [19]. Moreover, bio-priming of wheat seeds with *T. harzianum* alleviated the negative effects of salinity stress [20]. The mechanism of *Trichoderma* spp stimulating plant growth under salt stress is not clear, nevertheless, several reports with different species showed that some metabolic processes and pathways may be convoluted (19, 21, 22]. For instance, *T. virens* as well as *T. atroviride* enriched *Arabidopsis* seedlings growth via enhanced root development, osmolyte production, and Na^+ rejection [21]. Similarly, *T. asperellum* lessened the destruction effect of salt stress in the cucumber plant by changing the phytohormone levels and phosphate solubilization capacity (22). Moreover, *T. longibrachiatum* T6 boosted the wheat tolerance to NaCl stress via increased anti-oxidative defense [peroxidase (POD), and catalase (CAT)] [23]. Additionally, in Indian mustard roots, *T. harzianum* augmented the level of antioxidant enzymes [24]. Regardless of these various studies, there is not sufficient information to expansively understand the reactions of plants to salt stress.

In a previous study, we isolated *T. viride* strain from soil rhizosphere of the El-Sharkia governorate [18]. Our previous research showed that *T. viride* had a higher potential to enhance the onion plant growth under controlled conditions. However, our previous study was unable to examine whether this fungal strain can enhance plant tolerance under salt stress, and little is known about whether such function of the *T. viride* can be retained under different levels of salt stress. Therefore, the present study was conducted to examine some morphological and physiological attributes of the tomato seedlings to salinity as well as to explore the possible mechanism of *T. viride* in improving and mitigating induced by salt stress, which used to find a remedy for salinity which is economical and effective.

Results And Discussion

NaCl stress consequence on the colony diameter and mycelia weight of *T. viride*:

Salinity decreases the growth of plants and the extent of this decrease may be linked to the interaction between the host, microbe, as well salt level [16, 25]. Thus, it is necessary to study the effect of NaCl on the growth of *T. viride.* Our results (Fig. 1 and Table 2) showed that, after 5 days of incubation, low salt concentrations had an enhanced influence on the mycelia weight of *T. viride* as compared to the control one. Also, with increasing NaCl concentrations occurs a decrease in the dry weight of mycelia significantly ($p < 0.05$) (Table 1). Concerning *T. viride* colony diameter results, low salt concentrations (50 and 100 mM NaCl) cause a slight decrease in *T. viride* growth as shown in Fig. 1, however, the decrease is not significant. Nevertheless, the differential inhibitory effects were detected with higher salt concentrations (150, 200, 250, and 300 mM). This is maybe a result of enhancing the water potential of the substrate that reduces the growth of fungal colonies at high salt concentrations [25]. Also, high salt may affect cytoplasmic metabolic activity, such as intracellular proteins that may provide the extra osmotic potential to prevent plasmolysis [26]. Our results are compatible with Zhang et al. [25] with *T. longibrachiatum* T6. Moreover, Contreras-Cornejo et al. [21] indicated that low NaCl concentrations increase the colony diameter and the growth of *T. atroviride,* although higher salt concentrations caused a significant reduction. This indicates that the effect of NaCl on the growth of *Trichoderma* spp is dose-dependent, with high salinity inhibiting the growth, and low salinity promoting its growth [27]. Moreover, a study by Guo et al. [28] reported that the growth of *T. asperellum* can be promoted by 2% NaCl. Similarly, Rawat et al. [29] stated that five of forty-five *T. harzianum* wild-type strains can grow and form spores in growth containing up to 240 mM NaCl. The optical microscopic examination revealed that the treatment of *T. viride* with high salt concentrations (150 and 200 mM NaCl) caused abnormal mycelial growth and considerable morphological changes, mainly manifesting as deformation, contraction, collapse, globular swellings occurred at the tips of hyphal strands and deformity of the conidium (Fig.2 D, E, F and G). In contrast, the mycelia of the control and that of low salt concentration (50 mM NaCl) were straight and well developed (Fig.2 A, B and C).

NaCl stress consequence on the appearance of cotyledonary leaves:

High salinity is one of the major environmental stresses that cause biochemical changes in plants and limits plant growth, according to earlier studies of Zhang et al. [23] and Mahmood et al. [30]. Our results showed that salinity reduces the number of tomato seedlings that show the first two cotyledonary leaves after 6 days of salt application by 59.3 and 37.5 % at 50 and 100 mM NaCl over their respective control ones. It means that salinity had negative effects on this parameter (Table 3). Furthermore, salinity increases the time of seed germination by lowering the water potential of the germination media, and

gradually reduced final germination as compared to regulation [31, 32]. Similarly, Tanveer et al. [6] found that salinity decreased the percentage of seeds that germinated since high salt levels result in low water and nutrient uptake, which affects seedling germination and development [33, 34].

Plant growth promotion in *T.viride*-treated tomato seedlings*:*

Seedling height, FW, and DW of tomato seedlings were measured 10 days after NaCl application to evaluate the growth-promoting effects of *T. viride* on tomato seedlings under salt stress (Fig. 3, 4 and 5). Our results showed that these growth parameters were significantly (p<0.05) inhibited with NaCl treatment; where, the seedling FW decreased by 33 and 26%, and DW decreased by 15 and 19% under 50 and 100 mM NaCl, respectively, over their respective control ones. The inhibitory effect of salt stress was in line with the results of Dief et al. [8], Metwally and Abdelhameed [12], Zhang et al. [23] in fenugreek and wheat. The explanations may be the non-availability of mineral nutrients and the outflow of energy to lessen the harmful effects of NaCl [35, 36]. Also, the consequence of osmotic stress, the toxicity of ions, and oxidative stress as a result of salt stress is known to delay growth [37]. However, the effect of salt was alleviated substantially with *T. viride* application, indicating that *T. viride* improves these measured variables significantly (Fig. 5). Our results are coherent with Metwally [17] and Metwally and Al-Amri [18] results about the improving capabilities of *T. viride* on onion growth and physiology. Likewise, Zhang et al. [23] observed that NaCl inhibited wheat seedling growth and that this inhibitory effect was alleviated by adding *T. longibrachiatum* T6. Compared to NaCl stressed seedlings, the height of tomato seedlings increased by 15 and 34% after being treated with *T. viride* at 50 and 100 mM NaCl; respectively (Fig. 5), where *T. viride* attained the maximum seedling height both in control and NaCl treatments. Fig. (4) shows the phenology of control or NaCl stressed tomato seedlings under *T. viride* fungal application.

According to related findings, *T. harzianum* enhanced seedling growth of cucumber after salt application [13]. Moreover, several previous studies have shown that *Trichoderma* sp form symbiotic relationships with a large variety of host plant roots and promote their growth and development [2]. Besides, *Trichoderma* sp. is also known to produce a range of antibiotics, including polyketides, trichodermin, peptaiboils, trichodermol, and steroids that promote plant development [38]. Also, the promotion of plant growth under saline condition largely relies on the increase of the activity of ACC-deaminase and the level of IAA production in *Trichoderma* as Zhang et al. [25] reported.

Seedling height stress index (SHSI)

The seedling height stress index was reduced under both salt concentrations as compared to the non-saline MS medium (Table 4). However, *T. viride* increased the seedling height stress index (SHSI) under both salt concentrations with 58.98 and 45.55, respectively. The lowest SHSI was detected in tomato seedlings subjected to 100 mM NaCl. Furthermore, AL-Mutawa [33] and Rawat et al. [34] stated that high

salt levels result in low water and nutrient uptake, which affects seedling germination and development. However, tomato seedlings treated with *T. viride* had a significantly higher percentage of SHSI under both salt concentrations. Besides, Zhang et al. [23] and Rawat et al. [34] indicated that the symbiotic colonization by *Trichoderma* enhances root growth and causes solubilization and sequestration of inorganic nutrients, which might be responsible for increased tolerance to osmotic stresses.

Seedling water status:

Regarding relative water content (RWC), our results showed that tomato seedlings have a maximum RWC with *T. viride* under non-saline MS medium and minimum for seedlings exposed to 100 mM NaCl (Table 4). RWC of tomato seedlings exposed to 50 and 100 mM NaCl decreased by 7.00 and 12.78% compared to control ones grown under non-saline MS medium. Even though, 50 and 100 mM NaCl reduce RWC and WC of tomato seedlings as compared to control; this decrease is not significant. This is in accordance with Metwally and Abdelhameed [12] and Chaudhuri and Choudhuri [39] that salt stress affects the water status of fenugreek and jute. Since plants grown under salt conditions are exposed to physiological drought as Na^+ and Cl^- ions bind water that is necessary for the plants growth [12, 40]. Of particular note, the inhibitory effect of salinity on tomato seedlings was mitigated to some extend by *T. viride* application. Furthermore, under salinity, WSD was substantially increased; however, these effects were diminished when *T. viride* was applied (Table 4); as *Trichoderma's* effects enable plants to more efficiently use water to maintain a lower CO_2 concentration within cells.

The consequence of NaCl and *T. viride* on H_2O_2 content and lipid peroxidation:

In consequence of superoxide radicals scavenging, H_2O_2 which is a toxic compound and injurious to plants is produced as a result of salt exposure. Higher concentrations of H_2O_2 in plants cause lipid peroxidation and membrane injury [23, 34]. Fig. (6 a and b) shows that under 50 and 100 mM NaCl, tomato seedlings treated or not with *T. viride* had a substantial increase in H_2O_2 content. Significantly higher levels were maintained in control seedlings under both salinity levels (5.16 and 5.53 mg/g FW). However, *T. viride* treatment reduces H_2O_2 accumulation (Fig. 6b), where under salt stress; H_2O_2 content of control seedlings was significantly higher than that of *T. viride* treated seedlings. The minimum level of H_2O_2 was observed in seedlings treated with *T. viride* (3.30 mg/g FW) followed by non-treated ones (3.42 mg/g FW) grown under non-saline MS medium or control condition. Our findings are coherent with Zhang et al. [13] and Rawat et al. [34] in cucumber and chickpea treated with *T. harzianum* under salt stress conditions. The decreasing levels of H_2O_2 in *T. viride* treated seedlings show that, at the cellular level, these seedlings are better fortified with an effective free radical quenching system that brings protection against oxidative stress.

Malondialdehyde (MDA), a product of lipid peroxidation, is mostly considered as an indicator of free radical damage to cell membranes caused by oxidative stress. Our result related to the effect of NaCl in the presence and absence of *T. viride* on MDA content is presented in Fig.6 a. MDA significantly increased in tomato seedlings subjected to 50 and 100 mM NaCl by 58.3 and 90% relative to the control ones. These results are consistent with Zhang et al. [13] in wheat seedlings; owing to salt exposure, the ROS formed in tomato cells causes peroxidation in membranous lipids and the formation of MDA [9, 41]. On the other hand, tomato seedlings with *T. viride* under salt stress were more effective than salt-stressed seedlings in lowering MDA contents, predicting membrane protection. Compared with their respective control, MDA content in tomato under control or 50 mM NaCl decreased by 11 and 14 percent with *T. viride* application. Our findings are consistent with Dief et al. [8] and Rawat et al. [20] that bio-priming wheat seeds with *P. chrysosporium* or *T. harzianum* decreased MDA accumulation.

T. viride and NaCl effects on ROS scavenging in tomato seedlings:

ROS act as signaling molecules at low concentration, though it's excessive accumulation damages plants under stress. These ROS can affect the integrity of cellular membranes and enzyme activities [2, 42]; resulting in oxidative stress which is one of the damaging causes in plants exposed to environmental stresses [9, 41]. For defense from oxidative stress damage, plants depend on non-enzymatic and/or enzymatic systems. Furthermore, inoculating plants with *Trichoderma* sp. resulted in a number of physiological improvements, including a rise in enzymatic and non-enzymatic antioxidants, which increased plant resistance to stress [23].

T. viride induces salt mitigation through a non-enzymatic mechanism (total soluble protein and proline content):

To assess whether *T. viride* induces salt tolerance in tomato seedlings due to a non-enzymatic mechanism, proline and soluble protein contents as substances capable of altering osmotic potentials (Fig. 6 c and d) were detected. Osmotic adjustment by lowering the osmotic potential plays a key role in cellular water retention and turgor maintenance, thereby minimizing the adverse effects of salt stress through balancing the solute potential [43], which then contributes to cell growth.

Proline stabilizes the membranes and prevents the degradation of proteins and enzymes under stress conditions [44]. We observed that *T. viride* application in MS medium with tomato seedlings resulted in a significant (p <0.05) increase in total soluble protein and proline contents, regardless of the severity of salt stress. Soluble protein content in tomato significantly decreased after NaCl treatment (% of decrease were 20.58 and 40 after 50 and 100 mM NaCl salt treatment compared to those under non-saline MS medium). This finding backs up the hypothesis that high Na^+ levels damage plants through disturbing protein synthesis [45]. Additionally, Rasool et al. [46] and Ahmad et al. [47] stated that plants cope with

overawed osmotic stress caused by salinity build-up osmolytes such as proline, soluble proteins, soluble sugars, and glycine betaine. Nevertheless, the protein content increased by 11.2, 25, and 23.04 %; respectively with *T. viride* application under control or 50 and 100 mM NaCl treatment, compared to their respective control seedlings. *T. viride* plays a key role in plant tolerance; where proteins serve as an energy reservoir or possibly an osmotic potential adjuster in plants that are exposed to salinity [2, 48]. These results are in line with Metwally [17] findings with onion plants inoculated with *T. viride*. Similarly, our results were supported by Zhang et al. [23] with *T. longibrachiatum* application in wheat seedlings. Moreover, Dief et al. [8] indicated that protein considerably decreased in the wheat seedlings after NaCl treatment; however, with *Phanerochaete chrysosporium* application its content increased greatly.

On the contrary, both NaCl concentrations cause a conspicuous increase in proline content of tomato seedlings, where its highest value was observed at 100 mM NaCl (Fig. 6 c). In harmony with this finding Ueda et al. [49] and Khomari et al. [50] stated that under salt stress, plants accumulate compatible osmolytes, such as proline, to facilitate osmotic adjustment leading to increased dehydration tolerance. Our findings are in line with those of Dief et al. [8] and Rawat et al. [20] that seed bio-priming with *P. chrysosporium* and *T. harzianum* increased proline content in wheat seedlings. Moreover, previous studies of Zhang et al. [23] and Khomari et al. [50] demonstrated that *Trichoderma* sp. and *T. longibrachiatum* had a highly significant effect on proline and protein contents in wheat and soybean under control and salt stress conditions. The augmentation in proline contents may be due to the enrichment in proline synthesizing enzymes activity and reduction in catabolizing ones or its circumscribed assimilation in protein synthesis [36]. As well, the further increase in their contents with *T. viride* indicates that *Trichoderma* could confer systemic resistance to the treated tomato seedlings by up-regulating the substances capable of causing major osmotic adjustments [46] as well as energy storage [23]. Moreover, increased proline content enhanced the ability of plants to detoxify the accumulated ROS and protect the seedlings from oxidative damage [24, 41].

Salt mitigation induced by T. viride is dependent on an enzymatic system:

In the direction of estimating whether the enzymatic system plays a role in *T. viride* prompted tolerance to salt by scavenging ROS, we examined the activities of some antioxidant enzymes in tomato seedlings such as POD, PPO, CAT, and APX as well as total antioxidant capacity (TAC) (Fig. 7 a-e). Results indicate that NaCl stress significantly induced an increase in all the assayed antioxidant enzymes besides TAC in tomato seedlings (Fig. 7). Furthermore, their activities were significantly increased after *T. viride* treatment under both saline and non-saline stress conditions, compared to their corresponding control. Under non-saline MS medium, *T. viride* increases POD, CAT, and APX by 15.03, 9.33, and 14.88%; respectively. Whereas, under 50 mM NaCl it increases these values by 60.2, 74.6, and 61.6% as compared to the control ones. Where, these antioxidant enzymes in tomato seedlings perform the main role in ROS scavenging and therefore preventing the oxidative stress prompted damaging effects on several sensitive molecules like nucleic acids, proteins as well as lipids [51, 52]. Our results corroborate with Zhang et al.

[13] and Zhang et al. [23] that *T. harzianum* and *T. longibrachiatum* T6 enhanced the tolerance of cucumber and wheat to salt stress through the increased antioxidative defense. Also, Dief et al. [8] reported a remarkable increase in CAT and APX enzymes activities in wheat leaves bio-primed with *P. chrysosporium* under salinity stress. These results indicate that *T. viride* could confer systemic resistance to tomato seedlings by improving the antioxidant enzyme activities, to result in higher FW, DW, and seedlings length (Fig. 5) in tomato seedlings.

Conclusions

From results of our study, it becomes clear that higher salt concentrations significantly affect the colony diameter and DW of *T. viride* as well as the tomato seedlings growth parameters. However, *T. viride* application mitigated these adverse effects of salinity up to a certain extent. Moreover, the improved salt tolerance of tomato seedlings may be due to multiple mechanisms of *T. viride*, such as the increase in ROS scavenging (CAT, POD, APX, and PPO), as well as maintained osmotic balance (protein and proline contents). Therefore, from these results, it is suggested that the application of *T. viride* is a good solution and can be recommended for usage by farmers as an effective besides an economical tool, against salinity.

Methods

Experiments were carried out at the Laboratory of Mycology, Faculty of Science, Zagazig University, Egypt. All treatments in the experiments described below had five replicates and each experiment was repeated twice over time.

1. Fungal, plant material, and salt treatment:

Fungal Material:

Trichoderma viride was previously isolated from soil rhizosphere of El-Sharkia governorate [18]. *T. viride* was cultured on potato dextrose agar (PDA) at 28 °C for 7 days until sporulation.

Plant material, salt treatment, and growth conditions:

Tomato (*Solanum lycopersicum* L.; Tomato HYBRID Seven F.1) was obtained from the local market of Minia Al-Qamh, El-Sharkia Governorate. Tomato seeds with a uniform shape and size were germinated in the laboratory at 25 ± 1°C in the dark in October 2020 on sterile filter paper for 4 days till the emergence of the radicle of 0.6 cm in length. Similar germinated seeds were surface sterilized with 7% sodium hypochlorite (NaOCl) for 8 min. After disinfection, all the germinated seeds were rinsed with sterile distilled water 5 times, left for dryness on sterilized filter paper, and then transferred under aseptic

conditions on agar plates containing MS medium (Murashige and Skoog basal salts mixture, Sigma-Aldrich). Six mm diameter of actively growing fungal agar discs of *T. viride* was inoculated in the opposite ends of 4-day-old germinated tomato seedlings (10 seedlings/ plate) on agar plates (MS medium with 50 and 100 mM NaCl or without salt as control). Table 1 showed the details of the study treatments. Plates were placed vertically at a 65° angle to allow root growth along the agar surface and unimpeded aerial growth of the hypocotyls and left for growth at 25°C in a chamber with a photoperiod of 16 h of light and 8 h of darkness. After 4 and 6 days of planting tomato seedlings on MS medium, the NaCl stress effects on the appearance of the first two cotyledonary leaves were recorded in Table 3. Moreover, 10 days after salt treatment, tomato seedlings were randomly collected and kept for further analysis.

2. Measurements:

Effect of NaCl concentrations on the linear growth and mycelia dry weight of *T. viride*:

Sterilized PDA media containing (50, 100, 150, 200, 250, and 300 mM NaCl) salt concentrations were seeded respectively with *T. viride* mycelia discs (6 mm) of active culture and were incubated at 25°C with supplemental day/night lighting of 16/8 h. PDA media inoculated with *T. viride* mycelia disc without NaCl were considered as the control (0 mM NaCl). After 2 and 4 days of inoculation, the colony diameter was measured. The morphological deformations caused by NaCl concentrations on the mycelia of *T. viride* on PDA plates were directly examined. Hyphal strands at the end of the fungal colony were removed and examined under the light microscope observations (Leitz WETZLAR, Germany) for abnormalities and compared with the control.

Potato dextrose broth media with different NaCl concentrations (50, 100, 150, 200, 250, and 300 mM) distributed in 250 ml flasks each contain 100 ml were inoculated with 0.5 mL of spore suspension of *T. viride* 1×10^{8} (spores mL^{-1}). Media without NaCl solution were considered as the control (0 mM NaCl). Flasks were incubated at 25 °C for 5 days with shaking at 180 rpm min^{-1}. On day 5, the fermentation was filtered using sterilized filer three times, the mycelia were collected, oven-dried at 80 °C for 3 h, and weighed for mycelial dry weight.

Seedlings growth assessment:

Tomato seedlings were harvested 10 days after NaCl treatment. Seedlings height (cm) and fresh weight (FW) were recorded. For determination of dry weight (DW), all the samples of tomato seedlings were oven-dried at 60°C for 48 h to obtain a constant weight. Besides, seedling height stress index (SHSI) was calculated as follows:

$$\text{SHSI} = \frac{\text{Height of stressed seedlings}}{\text{Height of control seedlings}} \text{ X } 100$$

Seedling water status determination:

Water content (WC), relative water content (RWC), and water saturation deficit (WSD) of tomato seedlings were measured [53]. Tomato seedlings were cut into small segments and then weighed immediately to record FW. Seedling segments were floated in deionized water for 4 h at room temperature and light. After 4 h, surface water was removed and samples were weighted again to obtain a fully turgid weight (TW). Samples were dried in an oven at 60°C for 24 h and weighed again to obtain DW. WC, RWC, and WSD according to the following equations:

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

$$\text{WC (\%)} = \frac{(\text{FW} - \text{DW})}{\text{FW}} \text{ X } 100$$

$$\text{WSD (\%)} = 100 - \text{RWC}$$

* FW represents the fresh weight and DW represents the dry weight.

Lipid peroxidation and H_2O_2 assay in tomato seedling:

MDA accumulation in tomato seedlings treated or not with *T. viride* under 0, 50, and 100 mM NaCl was measured [54]. In brief, a known tomato seedlings FW was ground in 3 mL of 5% trichloroacetic acid (TCA) and the homogenate was centrifuged for 10 min at 6000 rpm. An aliquot of 2 mL of the supernatant was mixed with 2 mL of 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA) at 96°C for 25 min and then cooled quickly on an ice bath. The absorbance was recorded at 532 nm using a UV-visible spectrophotometer, RIGOL (Model Ultra-3660). The content of MDA was expressed as µmol g^{-1} FW.

As well, H_2O_2 content in a known FW of tomato seedlings was determined [55] by homogenizing with 3 mL of 0.1 % TCA. A known volume of the homogenate (0.5 mL) was added to 0.5 mL of potassium phosphate buffer (100 mM, pH 7) and 2 mL of 1M potassium iodide (KI). The reaction mixture was left for 1h in darkness at room temperature then centrifuged for 10 min. H_2O_2 content was calculated by measuring the absorption at 390 nm and expressed as µg g^{-1} of FW.

Proline contents in tomato seedling:

Proline content in a known tomato seedlings FW [56] was determined by homogenizing in 5 mL of 3% aqueous sulphosalicylic acid. After that, 2 mL of supernatant was mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin at 100 $^{\circ}$C for 1 h then the reaction was rapidly stopped in an ice bath. Afterward, 4 mL of toluene was added to the reaction mixture. The chromophore containing toluene was aspirated from the aqueous phase; the absorbance was read at 520 nm. The proline concentration was determined using standard curve and calculated as follow:

$$\text{Proline concentration } (\mu g/g\ FW) = \frac{(\mu g\ \text{proline}/\ mL\ \times\ mL\ \text{toluene})}{115.5\ x\ \ g\ FW\ \text{of sample}}$$

*115.5 is the molecular weight of proline

Protein and ROS scavenging enzymes extraction and determination:

Total soluble proteins, as well as antioxidant enzymes activities, were assayed using a known FW of tomato seedlings after 10 days of NaCl treatment by homogenizing with 5 ml of extraction buffer, which contained 1 mM ethylenediaminetetraacetic acid (EDTA) and 50 mM k-phosphate buffer (pH 7). Extracts were centrifuged at 10,000 rpm for 15 min and used for determining the activities of ROS scavenging enzymes. Peroxidase (POD) activity was assayed according to the method of Chance and Maehly [57] with pyrogallol as the substrate at 470 nm. Catalase activity was assayed according to the method of Aebi [58] and determined by calculating the decline decomposition of H_2O_2 in absorbance at 240 nm. Polyphenol oxidase (PPO) and ascorbate peroxidase (APX) activities were determined in the tomato seedlings extract according to Beyer and Fridovich [59] and Nakano and Asada [60] at 430 nm and 290 nm; respectively, and their activities were expressed as U g^{-1} FW. Also, the concentration of protein in the extracts was measured by Lowry et al. [61].

Assay of total antioxidant capacity (TAC) in tomato seedlings:

The TAC in an aliquot of 0.5 mL of the tomato seedlings extracts was evaluated according to Prieto et al. [62] by adding 4.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a boiling water bath at 95°C for 90 min then left to cool to room temperature; the absorbance was measured at 695 nm. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid and the TAC was reported as µg ascorbic acid equivalents/g FW.

3. Statistical Analysis:

The data was subject to one-way ANOVA using the SPSS package (SPSS V16.0, SPSS, Inc., Chicago, IL, USA). Treatment effects were determined using Duncan's multiple range test and the significances were expressed at $P < 0.05$.

Abbreviations

BSA: Bovine serum albumin; CAT: Catalase; DW: Dry weight; FW: Fresh weight; H_2O_2: hydrogen peroxide; MDA: Malondialdehyde; POD: Peroxidase; PPO: Polyphenol Oxidase; ROS: Reactive oxygen species; RWC: Relative water content; *T. viride*: *Trichoderma viride;* TBA: Thiobarbituric acid; TCA: Trichloroacetic acid; TW: Turgid weight; WC: Water content. WSD: Water saturation deficient.

Declarations

Ethics approval and consent to participate:

Not applicable

Experimental research and field studies on plants: "All relevant institutional, national and international guidelines and legislation were complied or adhered to in the production of this study"

Consent for publication:

Not applicable

Availability of data and material:

All data generated or analyzed during this study are included in this published article.

Competing interest:

The authors declare no competing interests.

Acknowledgements:

Not applicable.

Funding

This work was supported by funds from Zagazig University, Egypt.

Author contributions

Conceptualization, RAM and SAS; methodology, validation, formal analysis, investigation and data curation, RAM and SAS; writing-original draft preparation, review and editing, RAM and SAS. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1

Different treatments.

T_0	T_1	T_2	T_3	T_4	T_5
Control	*T. viride*	NaCl (50 mM)	NaCl (50 mM) + *T. viride*	NaCl (100mM)	NaCl (100mM) + *T. viride*

Table 2

Effect of NaCl concentrations on the linear growth and dry weight (g/100 mL) of *T. viride*.

NaCl concentration	Colony diameter (cm) Days of incubation		Dry weight (g/100 mL)
	2 days	4 days	
0 mM	5±0.265a	8.1±0.429a	0.5942±0.0314c
50 mM	4.53±0.239ab	7.11±0.376ab	0.8718±0.0461a
100 mM	4.33±0.229abc	7.38±0.391ab	0.7039±0.037b
150 mM	4.18±0.221bc	7.38±0.391ab	0.497±0.0263d
200 mM	3.83±0.203bc	6.46±0.342bc	0.342±0.0181e
250 mM	3.65±0.193cd	6.41±0.339bc	0.3236±0.0171e
300 mM	3.13±0.165d	5.78±0.306bc	0.2838±0.015e

* Data are mean of 5 replicates ± standard error; the dry weight was determined 5 days after inoculation. Different letters in the same column mean significant difference at the $p < 0.05$ level by Duncan's new multiple range test.

Table 3

Effect of NaCl stress on the appearance of the first two cotyledonary leaves of tomato seedlings.

Treatments	Number of seedlings (no)		Percentage % = Seedling no showing the first two cotyledonary leaves/ total seedling no*100	
	After 4 days	After 6 days	After 4 days	After 6 days
T_0	3±0.587a	8±0.577a	37.5	100
T_2	2±0.577a	4.33±0.88b	25	59.3
T_4	1.33±0.66a	3.33±0.667b	12.5	37.5

T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress. * Data are mean of 5 replicates ± standard error. Different letters in the same column mean significant difference at the $p < 0.05$ level by Duncan's new multiple range test.

Table 4

Influences of NaCl stress and *T. viride* on seedling height stress index (%) (SHSI) and the water status of tomato seedlings.

Treatments	Seedling height stress index (%) (SHSI)	Seedling water status		
		Water content (%) (WC)	Relative water content (%) (RWC)	Water saturation deficit (%) (WSD)
T_0	—	92.71±4.906a	91.56±4.845ab	8.44±0.446d
T_1	—	93.31±4.937a	96.54±5.108a	3.46±0.183e
T_2	36.69	89.49±4.735a	85.16±4.526ab	14.84±0.785b
T_3	58.98	91.53±4.843a	87.64±4.638ab	12.36±0.634c
T_4	24.94	88.61±4.688a	79.85±4.225b	20.15±1.066a
T_5	45.55	93.66±4.956a	84.58±4.476ab	15.42±0.816b

T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress, while T_1, T_3 and T_5 represent tomato seedlings grown on MS medium with *T. viride* under 0, 50 and 100 mM NaCl stress.*Data are mean of 5 replicates ± standard error. Different letters in the same column mean significant difference at the $p < 0.05$ level by Duncan's new multiple range test.

Figures

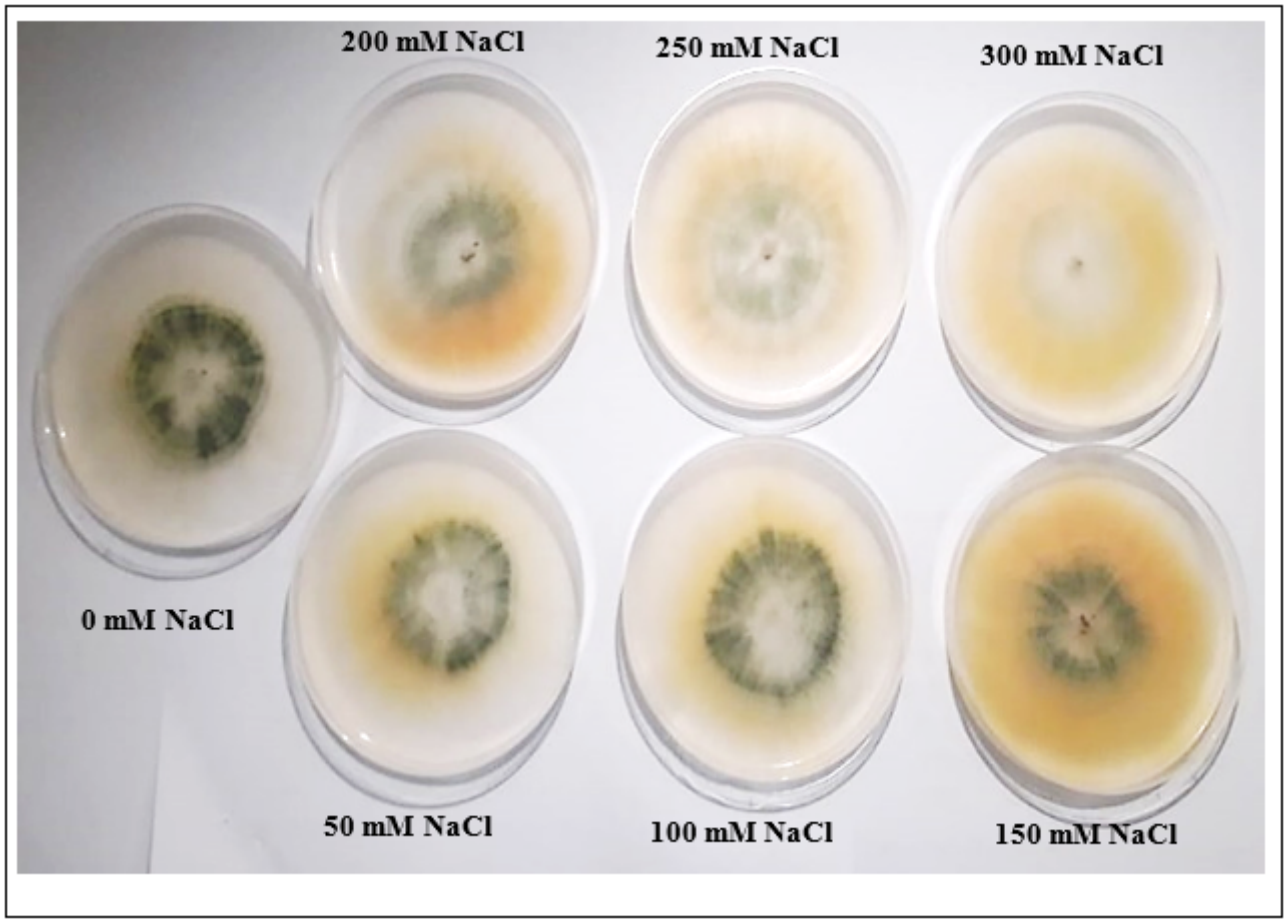

Figure 1

Illustrative photograph showing the effect of NaCl concentrations on *T. viride* growth colonies grown at 25°C and photographed after 2 days. To determine the effects of NaCl on *T. viride*, mycelia discs (6 mm) of active culture were used to inoculate PDA medium with or without NaCl.

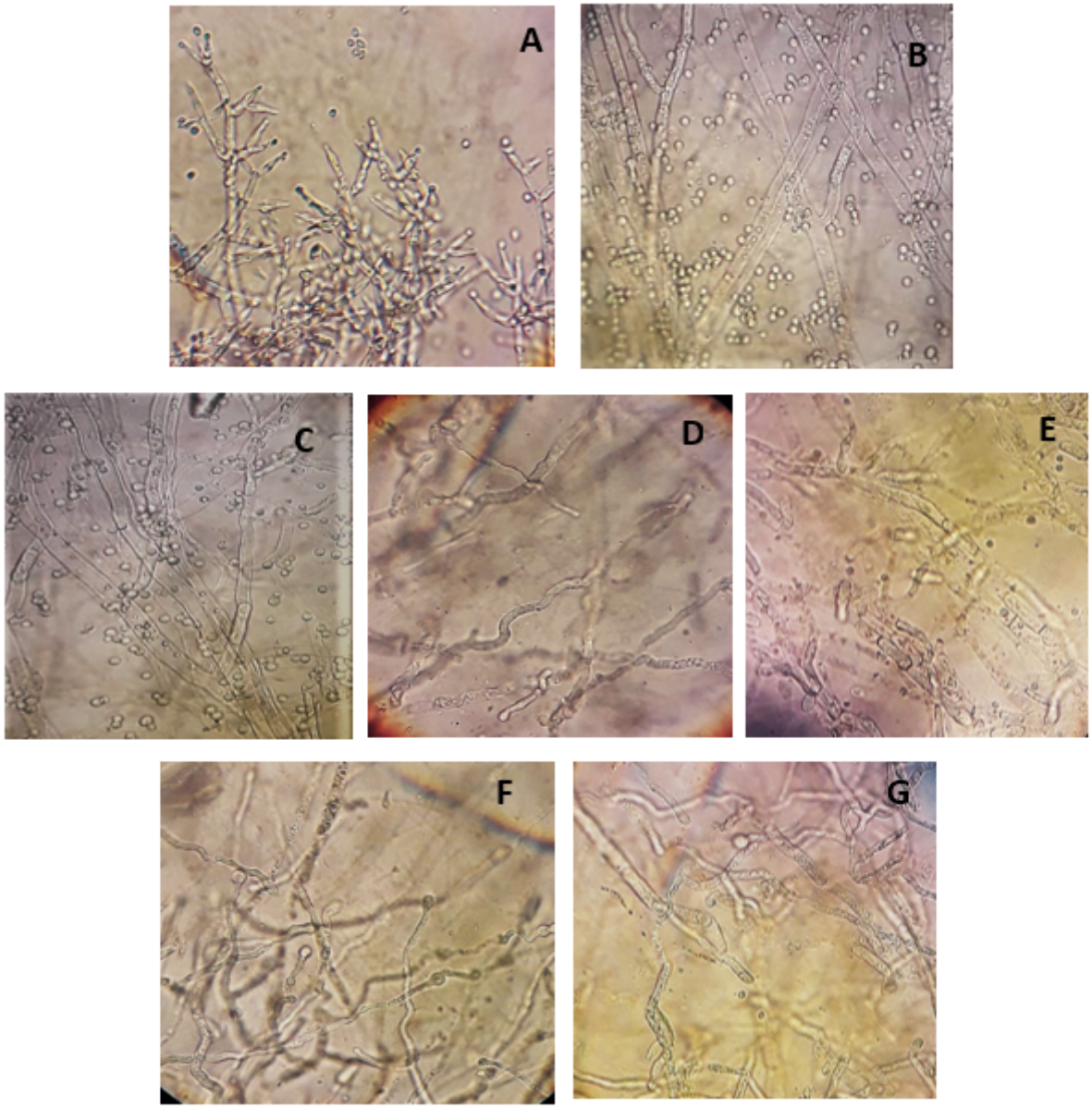

Figure 2

Effect of different salt concentrations (mM NaCl) on *Trichoderma viride* growth (**A**) and (**B**) Normal hypha (Control), (**C**) *Trichoderma viride* hypha under (50 mM NaCl) stress, (**D**) and (**E**) *Trichoderma viride* hypha under (150 mM NaCl) stress, (**F**) and (**G**) *Trichoderma viride* hypha under (300 mM NaCl) stress.

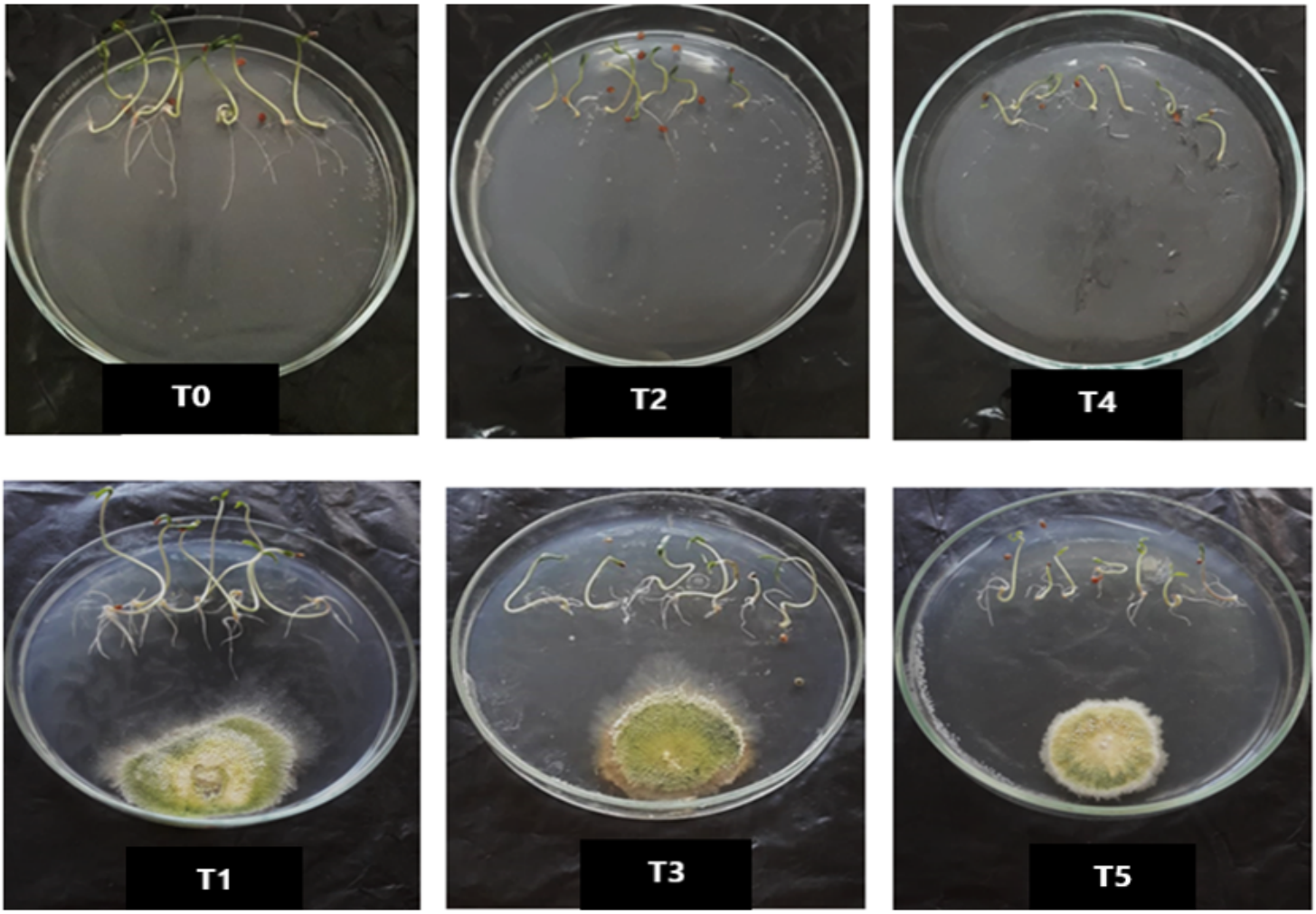

Figure 3

The phenology of control and NaCl stressed tomato seedlings under *T. viride* fungal application. T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress, while T_1, T_3 and T_5 represent tomato seedlings grown on MS medium with *T. viride* under 0, 50 and 100 mM NaCl stress.

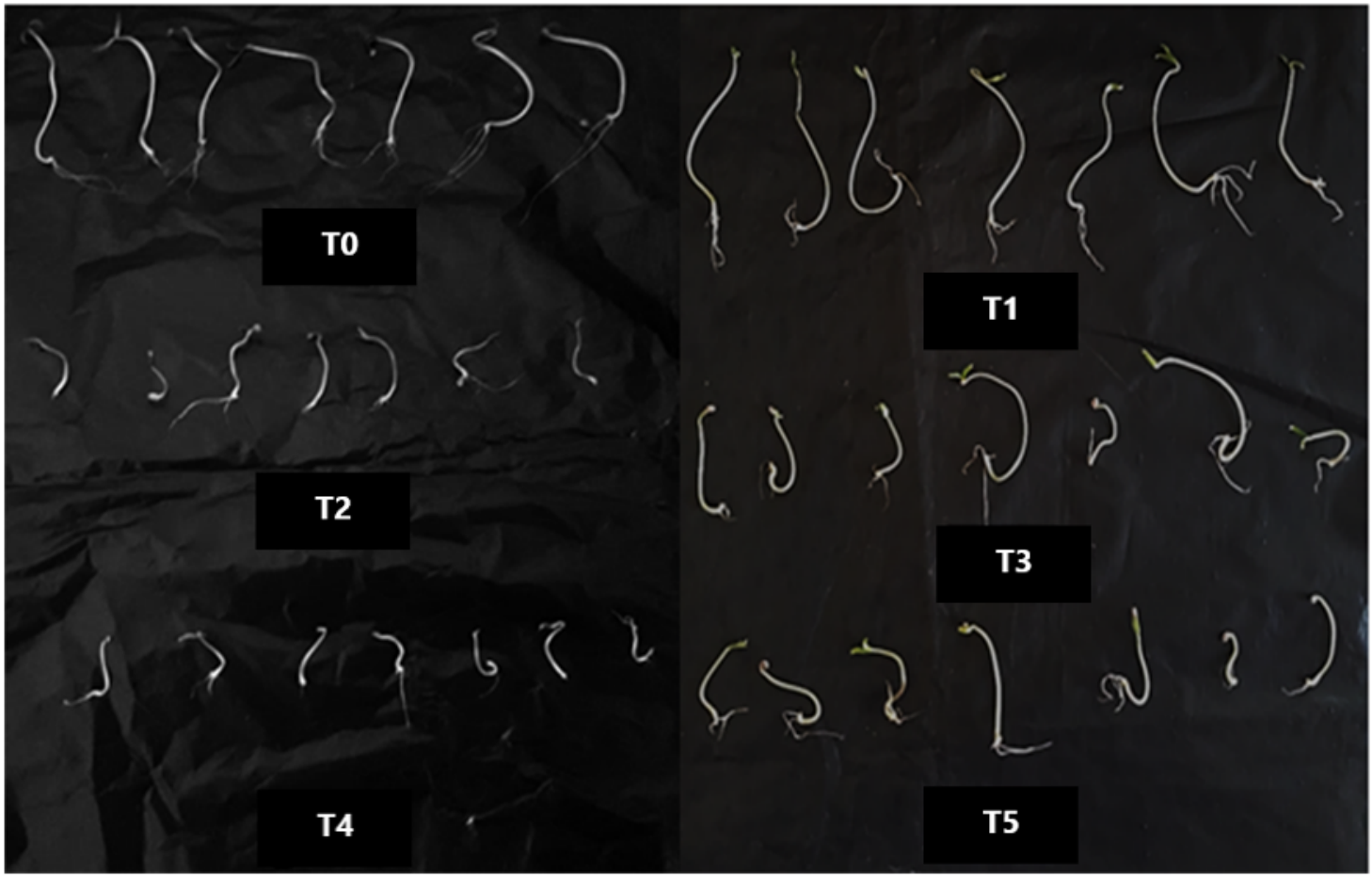

Figure 4

Photographs of tomato seedlings showing the differences between control and salt stressed seedlings under *T. viride* fungal application. T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress, while T_1, T_3 and T_5 represent tomato seedlings grown on MS medium with *T. viride* under 0, 50 and 100 mM NaCl stress.

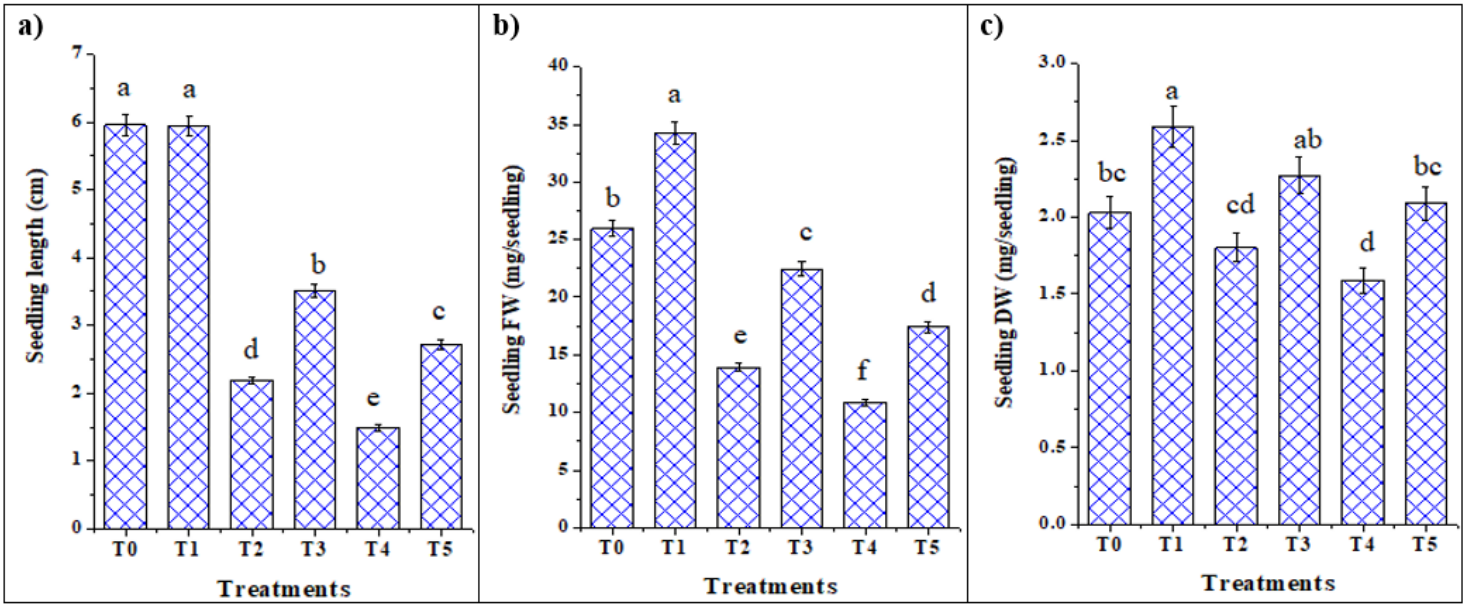

Figure 5

Effect of *T. viride* on seedling height, fresh (FW) and dry weights (DW) of tomato seedlings in response to salinity stress. T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress, while T_1, T_3 and T_5 represent tomato seedlings grown on MS medium with *T. viride* under 0, 50 and 100 mM NaCl stress. * Data are mean of 5 replicates ± standard error. Different letters above bars indicate a significant difference between treatments using ANOVA followed by Duncan's multiple range test ($p < 0.05$).

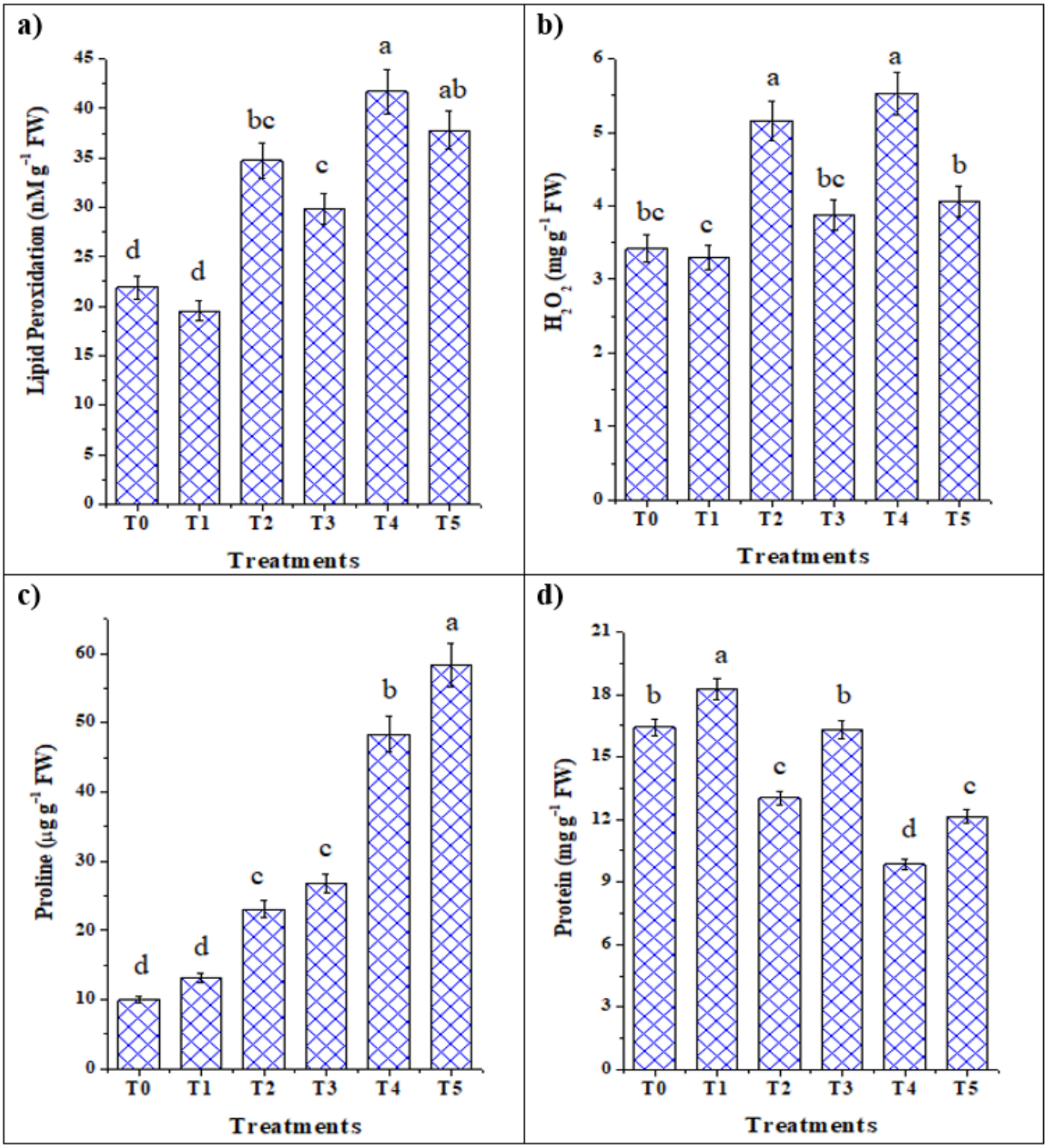

Figure 6

Effect of *T. viride* application on lipid peroxidation (nM/g FW), hydrogen peroxide (H_2O_2) content (mg/g FW), proline (µg/g FW) and protein (mg/g FW) contents of tomato seedlings in response to salinity stress. T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress, while T_1, T_3 and T_5 represent tomato seedlings grown on MS medium with *T. viride* under 0, 50 and 100 mM NaCl stress.*Data are the mean of five replicates ± standard error (n = 5). Different letters

above bars indicate a significant difference between treatments using ANOVA followed by Duncan's multiple range test ($p < 0.05$).

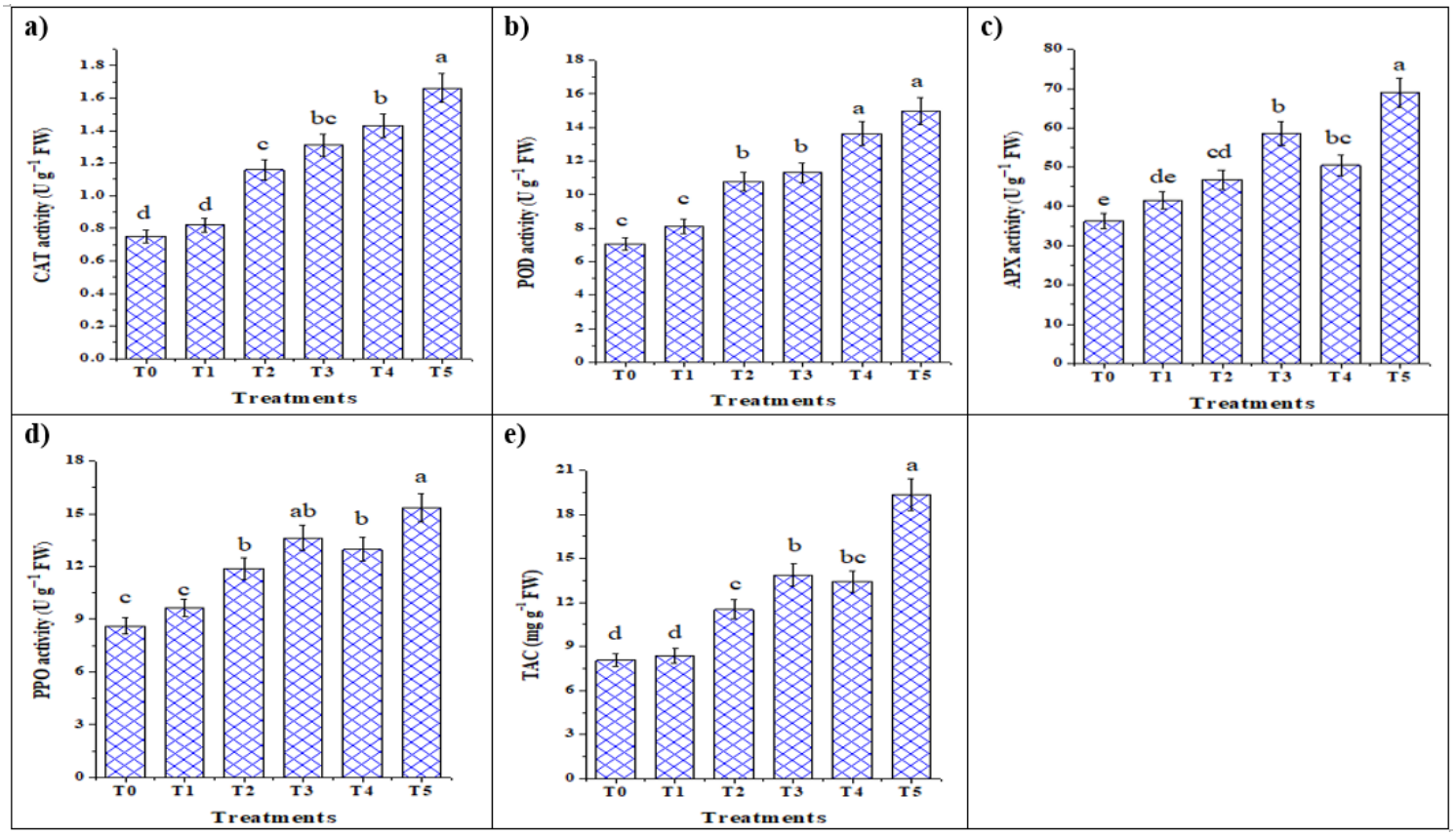

Figure 7

Effect of *T. viride* application on antioxidant enzymes system in tomato seedlings in response to salinity stress. T_0, T_2 and T_4 represent tomato seedlings grown on MS medium under 0, 50 and 100 mM NaCl stress, while T_1, T_3 and T_5 represent tomato seedlings grown on MS medium with *T. viride* under 0, 50 and 100 mM NaCl stress.*Data are the mean of five replicates ± standard error (n = 5). Different letters above bars indicate a significant difference between treatments using ANOVA followed by Duncan's multiple range test ($p < 0.05$)