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Article

Trichoderma-Induced Improvement in Growth, Photosynthetic Pigments, Proline, and Glutathione Levels in *Cucurbita pepo* Seedlings under Salt Stress

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Abstract: Salt stress is one of the major abiotic stress in plants. However, traditional approaches are not always efficient in conferring salt tolerance. Experiments were conducted to understand the role of *Trichoderma* spp. (*T. harzianum* and *T. viride*) in growth, chlorophyll (Chl) synthesis, and proline accumulation of *C. pepo* exposed to salinity stress. There were three salt stress (50, 100, and 150 mM NaCl) lavels and three different *Trichoderma* inoculation viz. *T. harzianum*, *T. viride*, and *T. harzianum* + *T. viride*. Salt stress significantly declined the growth in terms of the shoot and root lengths; however, it was improved by the inoculation of *Trichoderma* spp. *C. pepo* inoculated with *Trichoderma* exhibited increased synthesis of pigments like chl *a*, chl *b*, carotenoids, and anthocyanins under normal conditions. It was interesting to observe that such positive effects were maintained under salt-stressed conditions, as reflected by the amelioration of the salinity-mediated decline in growth, physiology and antioxidant defense. The inoculation of *Trichoderma* spp. enhanced the synthesis of proline, glutathione, proteins and increased the relative water content. In addition, *Trichoderma* inoculation increased membrane stability and reduced the generation of hydrogen peroxide. Therefore, *Trichoderma* spp. can be exploited either individually or in combination to enhance the growth and physiology of *C. pepo* under saline conditions.

Keywords: Vegetable crop; antioxidant; proline; NaCl; Cucurbita; plant-microbe interaction

1 Introduction

It has been accepted that sessile are often exposed to environmental extremes throughout their life cycles, leading to sizeable alterations in growth and metabolism. Among these critical environmental factors, salinity has been reported to induce harmful effects on the growth, biochemical process pattern, and development of plants [1–3]. Generally, increasing salinity is a grave concern worldwide, particularly in arid and semiarid regions. Salinity has rendered most of the agricultural land to barren wasteland. On the other hand, excessive use of saline water for irrigation incessantly adds salt-affected soils, therefore changing productive agricultural lands into the saline-affected waste. Globally, it has been calculated that 5–7% of the land is salt-affected [4]. This increased salt concentration directly affects the expansion of existing flora by imposing osmotic and ionic stress, resulting in obstructions to many physiological and biochemical processes like photosynthesis and ion homeostasis [5,6,7,8]. Salinity-induced alterations in plant functions, such as photosynthesis, which is related directly to carbon and nitrogen metabolism, decrease the yield productivity [8]. High salinity exposure triggers the generation of toxic reactive oxygen species (ROS), resulting in damage to membranes and different cellular structures [9,10]. Among toxic ROS are radicals like superoxide (O_2^-), the hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), which might negatively affect plant structural and purposeful stability [10,11]. As immediate effects, ROS mediate the oxidization of essential molecules like lipids and proteins and cause injury to nucleic acids [11–13].

Nevertheless, plants use mechanisms to avert the stress-induced toxic effects, and such mechanisms embody the up-regulation of the inhibitor system to hold off the scavenging of ROS. Also, the accumulation of compatible osmolytes like amino acids, glycine betaine, etc. is another crucial strategy to cope with stress [14,15]. Osmolytes maintain the water content of the cell, therefore preventing excessive harm from stresses. Selective uptake of ions like sodium and chloride from the growth media contributes to salt stress tolerance. It has been reported that the accumulation of solutes and efficient sequestration and compartmentalization of deleterious ions into the vacuoles are key determinant traits of salt tolerance in plants [7,16]. *T. harzianum* and *T. viride* are fungal species that are often used as fungicides or applied foliarly on seeds and soil to eliminate disease-causing fungal pathogens. Nowadays, the biotechnological products of *Trichoderma* spp., such as 3Tac, are available for commercial use for the control of *Botrytis*, *Fusarium*, etc. [17]. Being an endophytic plant symbiont, *Trichoderma* spp. are used as biofertilizers for plant growth promotion [18,19]. Also, the *Trichoderma* strains have been reported to help plants in withstanding biotic and abiotic stresses like salinity and drought. Inoculation with *Trichoderma* protects drought [20] and salt-stressed [21] plants by enhancing root growth and nutritional uptake and strengthening the stress-mitigating mechanisms.

C. pepo, is a popular winter vegetables in many countries belonging to the family Cucurbitaceae, is an essential domesticated plant grown throughout the world that is exclusively consumed as a vegetable. However, increasing salinity poses a considerable threat to its yield. Therefore, in the present study, we aimed to study the role of *Trichoderma* spp. in mitigating the salinity-induced changes in *C. pepo*.

2 Materials and Methods

2.1 Fungal Isolate

The species of *Trichoderma*, i.e., *T. harzianum* and *T. viride*, were isolated from the infected roots of maize (*Zea mays*) plants and subsequently cultured using potato dextrose broth medium (PDB, DIFCO) in sterilized flasks. The entire preparation was shaken continuously at 28°C for five days. After that, the fungal mycelium from the cultures was mixed with powder coating for the coating of seeds overnight. Seeds of *C. pepo* were coated with three different conditions, i.e., *T. harzianum* (treatment 1), *T. viride* (treatment 2), and *T. harzianum* + *T. viride* (treatment 3).

2.2 Pot Experiment

Pots (15 × 20 cm) were filled with reconstituted soil composed of soil and peat moss (1:1). The coated seeds were initially maintained to grow for three weeks under day/night temperatures of 28/15°C and were irrigated by distilled water. After seed germination, some inoculum was applied below the soil surface by syringe to ensure infection. After that, different concentrations of NaCl (0, 50, 100, and 150 mM) were applied to the pots in the form of modified Hoagland solution, and control pots were treated with standard Hoagland solution. One hundred milliliters of Hoagland (normal and modified) nutrient solution was applied every alternate day to each pot, and pots were arranged in a completely randomized design with six replicates for each concentration. The concentration of saline water was gradually increased from 50 mM to 100 mM to 150 mM in all treatments to avoid osmotic shock. As the first irrigation, the three treatment groups (50, 100, 150 mM) received 50 mM. In the second irrigation, the first group received a concentration of 50 mM, while the 100- and 150-mM treatment groups received 100 mM. In the third irrigation, each treatment group received its named concentration until the end of the experiment. Completely randomized block design was used for this experiment and there were three replications. After 45 days of treatment, plants were subjected to analysis.

For the determination of growth parameters like the lengths and fresh weights of shoots and roots, plants were analyzed immediately after uprooting. Harvested tissue was dried at 80°C for 48 h to record the dry weight.

2.3 Estimation of Photosynthetic Pigments, Anthocyanins, and Relative Water Content

The methods described by Lichtenthaler et al. [22] and Lange et al. [23] were followed for the estimation of photosynthetic pigments (chl *a* and *b* and carotenoids) and anthocyanins, respectively. Chl *a* and *b* and carotenoids were extracted from fresh leaves using 80% acetone, and the extract was centrifuged at 3000 rpm for 10 minutes. The optical density of the supernatant was recorded at 480, 645, and 663 nm. For anthocyanin determination, fresh leaves were homogenized in 1% HCl (v/v) in methanol, and the homogenate was centrifuged at 3000 rpm for 10 min at 5°C and absorbance was measured spectrophotometrically at 535 and 650 nm.

The estimation of relative water content (RWC) in fresh leaves followed the method of Smart et al. [24] using the following formula:

$$\mathrm{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100 \quad (1)$$

2.4 Estimation of Total Protein and Proline

Protein was estimated by the method described by Bradford [25] using bovine serum albumin as a reference. Fresh plant tissue was extracted in a phosphate buffer (0.1 M, pH 7.5). After centrifugation, the supernatant was reacted with Bradford reagent, and the optical density was measured at 595 nm.

For the estimation of proline in 0.5 g of the fresh plant, the sample was macerated in 3% aqueous sulphosalicylic acid. The extract was subjected to centrifugation for 10 minutes at 5000 rpm. Two milliliters of supernatant was mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin reagent, and the mixture were incubated for 1 hour. After that, the tubes were kept on an ice bath to stop the reaction, proline (Pro) was separated using toluene, and absorbance was measured at 520 nm [26]. A standard curve of Pro was used for calculation.

2.5 Measurements of Hydrogen Peroxide (H_2O_2) and Membrane Stability Index

The concentration of H_2O_2 was determined by following the method of Sergiev et al. [27]. For estimation of H_2O_2, fresh leaves were macerated in ice-cold 3% trichloroacetic acid, and the extract was

subsequently centrifuged for 15 min at 12,000 rpm. To 1 mL of supernatant was mixed 1 mL of potassium phosphate buffer (100 mM, pH 7.0) and 2 mL of potassium iodide (1 M). After that, the absorbance of the mixture was measured at 390 nm, and the H_2O_2 content was calculated from a standard curve.

The method described by Sairam et al. [28] was followed for the determination of the membrane stability index (MSI). Fresh leaf samples (100 mg) were cut into small pieces in two test tube sets containing 10 mL of distilled water. One tube set was exposed to 40°C in a water bath for 30 minutes, and its electric conductivity was recorded (C_1). Another set was exposed to boiling temperature (100°C), and its electric conductivity (EC) was also measured (C_2) [28]. Calculation of MSI was done according to the following formula:

$$MSI = [1 - (C_1/C_2)] \times 100 \quad (2)$$

2.6 Estimation of Reduced Glutathione

The content of reduced glutathione (GSH) was measured spectrophotometrically by adopting the method of Griffith [29]. One gram of fresh plant material was homogenized in trichloroacetic acid (5%), and the homogenate was subjected to centrifugation at 10,000 rpm for 15 minutes. Subsequently, 2 mL of the extract was added to 8 mL of phosphate buffer solution and 1 mL of 5-5'-dithiobis 2-nitrobenzoic acid. After 10 minutes, the absorbance of the mixture was measured at 412 nm. A standard curve of GSH was used for calculation.

2.7 Statistical Analysis

The data shown are means (±SE) of four replicates. Statistical analysis was done using a completely randomized design by applying Duncan's Multiple Range Test and using One-way ANOVA and least significant differences (LSD). Significance was set at $p < 0.05$.

3 Results

Results regarding the influence of NaCl stress and *Trichoderma* spp. on growth parameters like shoot and root lengths are presented in Fig. 1. The exposure of *C. pepo* to salinity reduced the lengths of shoots and roots significantly, and inoculation of *Trichoderma* spp. ameliorated the negative impact considerably. Relative to the control, shoot and root lengths were maximally reduced by 53% and 58% at 150 mM NaCl, and maximum enhancement was observed when *Trichoderma* spp. were given in combination. Relative to the 150 mM stressed plants, plants treated with 150 + *T. harzianum* and *T. viride* showed 18% and 19% mitigation of the reductions in shoot and root lengths (Fig. 1).

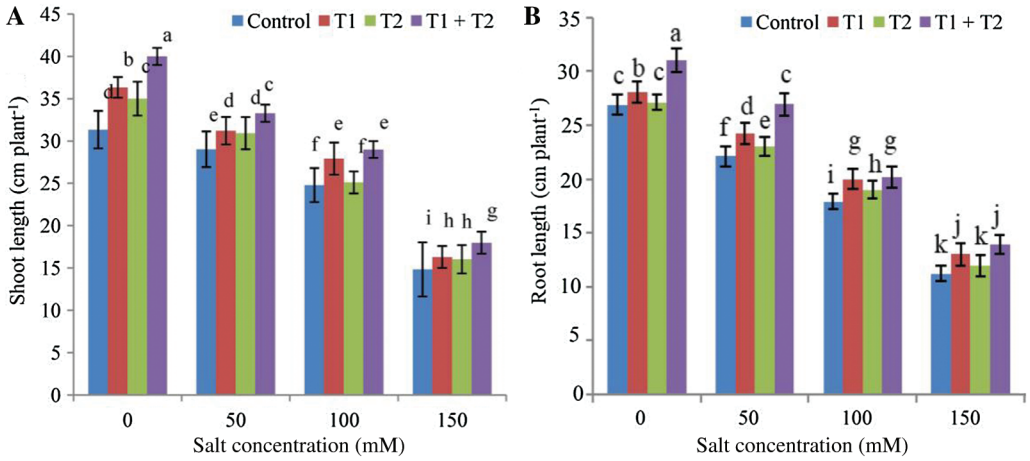

Figure 1: Effect of salinity stress (0, 50, 100, and 150 mM NaCl) on (A) shoot and (B) root lengths of *C. pepo* with and without *T. harzianum* (T1), *T. viride* (T2), and *T. harzianum* + *T. viride* (T1 + T2) treatment. Data presented are the means of four replicates and data followed by the same letters are not significantly different at $p < 0.05$

Salinity (150 mM NaCl) stressed *C. pepo* exhibited declines of 53%, 51%, 28%, and 55% in chl *a*, chl *b*, carotenoid, and anthocyanin contents, respectively, over the control (Fig. 2). *T. harzianum* and *T. viride* individually caused significant increases in all the pigments estimated and the maximal increase occurred when both species were given in combination. Treatment of *Trichoderma* spp. ameliorated the adverse effect significantly, and it was observed that when both species were inoculated in combination, maximal mitigation levels of 20%, 18%, 11%, and 20% were observed in chl *a*, chl *b*, carotenoid, and anthocyanin contents at 150 mM NaCl (Fig. 2).

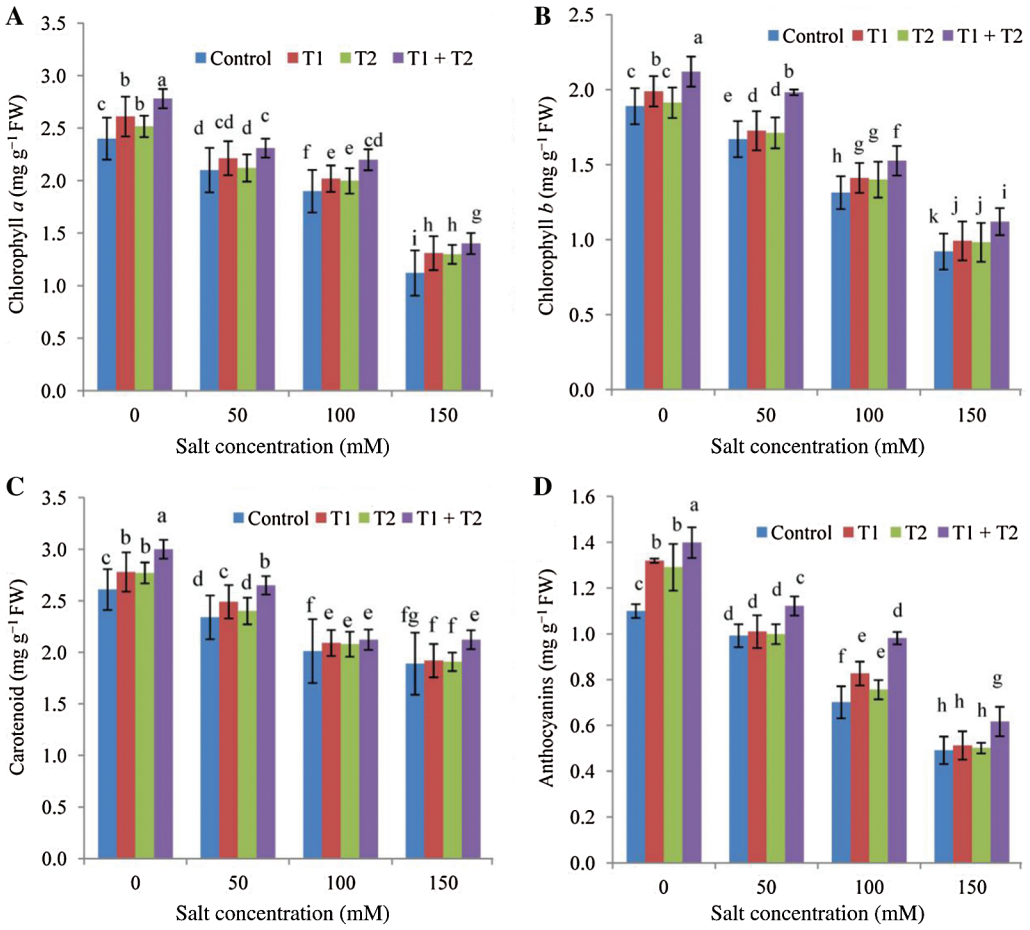

Figure 2: Effect of salinity stress (0, 50, 100, and 150 mM NaCl) on (A) chl *a*, (B) chl *b*, (C) carotenoids, and (D) anthocyanins in *C. pepo* with and without *T. harzianum* (T1), *T. viride* (T2), and *T. harzianum* + *T. viride* (T1 + T2). Data presented are the means of four replicates, and data followed by the same letters are not significantly different at $p < 0.05$

The exposure of *C. pepo* to NaCl increased the generation of H_2O_2, and this increase was maximally enhanced by 58% at 150 mM NaCl. Under normal conditions, H_2O_2 decreased by 10% with *T. harzianum*, by 8% with *T. viride*, and maximally by 22% when both *Trichoderma* spp. were given in combination. Salinity-induced enhancement in H_2O_2 caused an obvious decline in membrane stability. It was observed that salinity reduced the membrane stability by 12%, 27%, and 43% at 50, 100, and 150 mM NaCl, respectively. *Trichoderma* spp. improved the membrane stability by 1.16% (*T. harzianum*), 1% (*T. viride*), and 6% (*T. harzianum* + *T. viride*). However, relative to the control group, membrane stability was reduced by only 39%, 41%, and 35%, respectively, with *Trichoderma* spp. at 150 mM NaCl (Fig. 3).

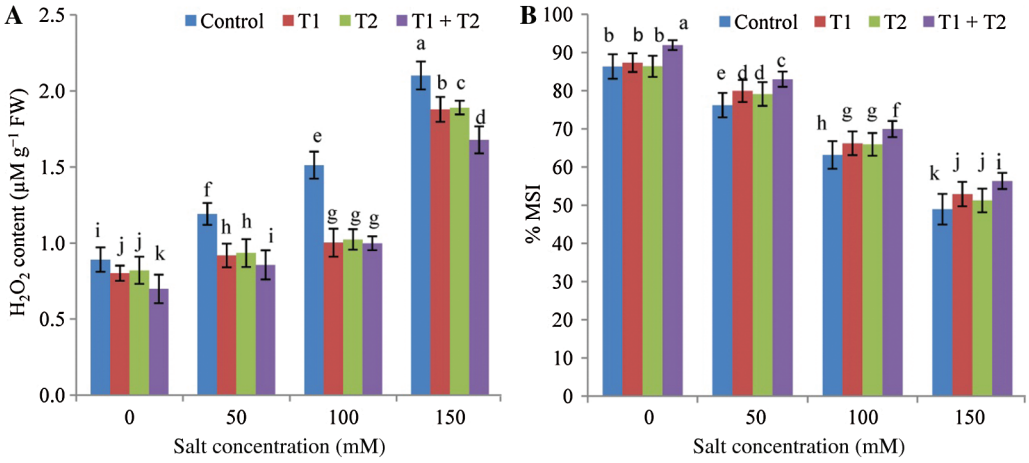

Figure 3: Effect of salinity stress (0, 50, 100, and 150 mM NaCl) on (A) hydrogen peroxide and (B) the membrane stability index in *C. pepo* with and without *T. harzianum* (T1), *T. viride* (T2), and *T. harzianum* + *T. viride* (T1 + T2). Data presented are the means of four replicates and data followed by the same letters are not significantly different at $p < 0.05$

Salinity stress increased the synthesis of proline, while it reduced the protein and relative water content. At 50, 100, and 150 mM NaCl, proline was observed to increase by 14%, 37%, and 45%, respectively, over the control; however, in plants treated with *T. harzianum*, *T. viride*, and *T. harzianum* + *T. viride*, increases of 20%, 17%, and 39%, respectively, were observed. *T.* spp. maintained this effect even under salinity-stressed conditions. Maximal (60%) proline accumulation was observed in plants treated with 150 mM NaCl + *T. harzianum* + *T. viride* relative to the control. Relative to the control, the protein content in salinity-stressed plants was reduced by 12% at 50 mM, 34% at 100 mM, and 52% at 150 mM, respectively. Under normal growth conditions, the protein content increased by 9% with *T. harzianum*, by 8% with *T. viride*, and by 19% with *T. harzianum* + *T. viride*. Relative to the control, RWC was decreased by 10%, 23%, and 38% at 50, 100, and 150 mM NaCl, respectively. Under normal conditions, *Trichoderma* spp. resulted in increases in RWC by 4% (*T. harzianum*), 2% (*T. viride*), and 7.15% (*T. harzianum* + *T. viride*). *Trichoderma* spp. maintained their beneficial effects, even when inoculated with salinity-stressed plants, and reductions of only 37% with 150 + *T. harzianum*, 38% with 150 + *T. viride*, and 35% with 150 + *T. harzianum* + *T. viride* occurred (Fig. 4).

The glutathione content increased with an increasing salinity concentration up to 100 mM and showed a steep decline at 150 mM. Both species of *Trichoderma* showed significant increases in GSH content of 10%, 6%, and 26% with *T. harzianum*, *T. viride*, and *T. harzianum* + *T. viride*, respectively. Relative to the control, GSH increased by 44% with 100 + *T. harzianum*, by 41% with 100 + *T. viride*, and by 49% with 100 + *T. harzianum* + *T. viride* (Fig. 5).

4 Discussion

The current study evaluated the potential of two *Trichoderma* species to protect *Cucurbita pepo* from salinity stress. It was observed that salinity reduced the root and shoot growth, but growth was remarkably increased by inoculation with *Trichoderma* spp., either individually or in combination. Stress-induced reductions in morphological parameters have been ascribed to the inhibition of cell division and the restriction of cellular elongation [30,31]. Individual as well as co-inoculation of *Trichoderma* spp. enhanced the growth parameters under normal conditions and assuaged the adverse effects evoked by salinity to some appreciable extent. This ameliorating impact of *Trichoderma* spp. in improving the resilience of plants to stress is one of the vital components of proper growth maintenance under stressful

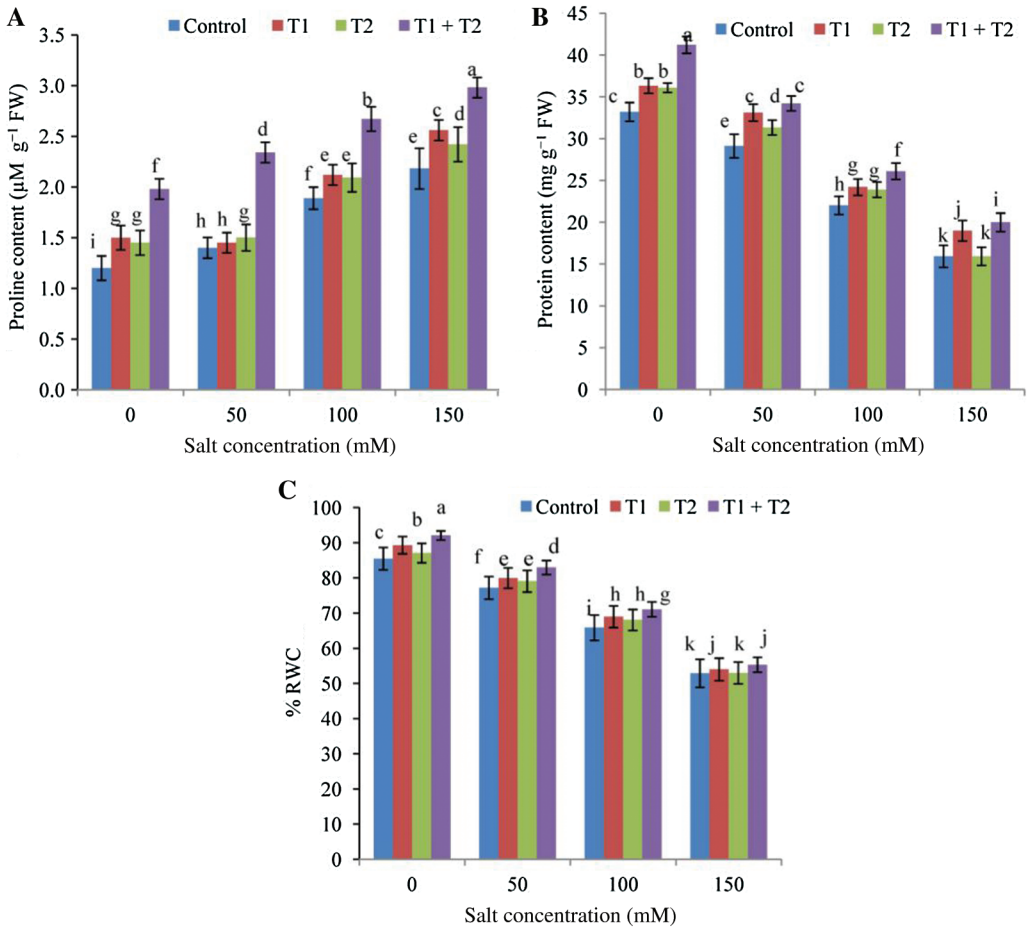

Figure 4: Effects of salinity stress (0, 50, 100, and 150 mM NaCl) on (A) proline (B) protein, and (C) the relative water content in *C. pepo* with and without *T. harzianum* (T1), *T. viride* (T2), and *T. harzianum* + *T. viride* (T1 + T2). Data presented are the means of four replicates and data followed by the same letters are not significantly different at $p < 0.05$

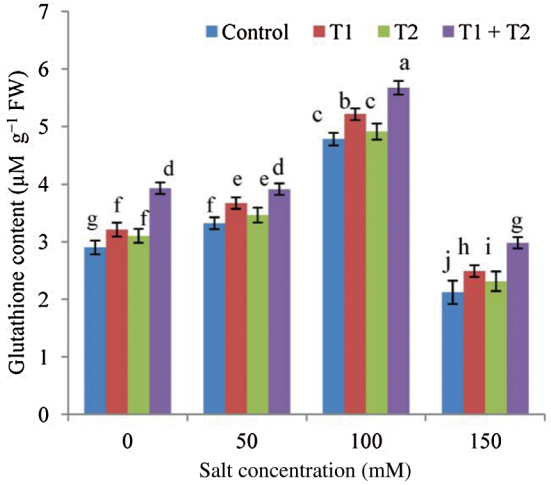

Figure 5: Effect of salinity stress (0, 50, 100, and 150 mM NaCl) on glutathione in *C. pepo* with and without *T. harzianum* (T1), *T. viride* (T2), and *T. harzianum* + *T. viride* (T1 + T2). Data presented are the means of four replicates, and data followed by the same letters are not significantly different at $p < 0.05$

conditions. Similar to our results, Yedidia et al. [32] and Ahmad et al. [21] demonstrated significant amelioration of salinity-induced damage through inoculation with *Trichoderma* spp., which might have a significant role in normal growth and development.

In the present study, salinity stress resulted in a reduction in the chlorophyll (chl) content, and such results are confirmative of the findings of Ge et al. [33], and Ahanger et al. [11]. Several studies have concluded that stresses hamper the synthesis of chl pigments and tinker with the structure and functioning of the pigment–protein complex, resulting in a significant decline in the photosynthetic efficiency [34]. It has been earlier reported that reduced chlorophyll synthesis due to salinity declines photosynthesis and plant performance [35,36]. In the present study, *C. pepo* inoculated with *Trichoderma* spp. under salinity as well as normal growth conditions exhibited an enhancement in the chl content, which may be due to their involvement in allowing less Ca and Mg to leach from soil and increasing their uptake, with special consideration of Mg which forms a vital part of chl [36,37]. In agreement with our studies, enhanced chl synthesis under salinity stress due to inoculation of *Trichoderma* was also reported by Ahmad et al. [21], Mishra et al. [38], and Shukla et al. [36]. It should be noted that few reports are available depicting the beneficial role of *Trichoderma* spp. in stress mitigation. Plant growth promotion and development by *Trichoderma* spp. inoculation under salt stress condition has been reported in *Triticum aestivum* [39], *Brassica juncea* [40], *Ochradenus baccatus* [41], *Z. mays*, and *Oryza sativa* [42]. Hence, it is evident from the present study that *Trichoderma* spp. counterbalance the deleterious effects of salinity on photosynthetic pigment synthesis in *C. pepo*. This protective role of maintaining the pigment levels and associated components like anthocyanins in addition to other stress-alleviating mechanisms can be reflected in better growth performance of the plant [43]. Earlier, increased photosynthetic efficiency together with higher synthesis of photosynthetic pigments due to *T. harzianum* inoculation was reported in tomato plants [44].

C. pepo showed a considerable reduction in membrane functioning under moderate and severe salinity stress, and *Trichoderma* spp. inoculation significantly reversed this deleterious effect. The findings obtained by Ahmad [45], Rasool et al. [46], and Ahmad et al. [47] corroborate with our results. Ahmad et al. [47] recently showed that salinity exposure enhances the membrane peroxidation of *Solanum lycopersicum*, reducing the membrane strength. Earlier, it was confirmed that increased salinity intensifies the membrane leakage [48,49]. The stability of the membrane structure and functioning due to *Trichoderma* can be attributed to a substantial increase in tissue water maintenance, decreased accumulation of ROS, and upregulation of the antioxidant system, cumulatively contributing to the maintenance of optimal growth of plants under salinity conditions [40,44,50]. Moreover, Rawat et al. [39] and Hashem et al. [41] mentioned the significant role of T. *harzianum* in improving intercellular water relations through better water acquisition and nutrient uptake under salinity stress conditions. Several recent reports indicated that the increased peroxidation of polyunsaturated fatty acids in membranes could contribute to major loss of the membrane stability [7,46,47]. It has been reported that the inoculation of *Trichoderma* spp. in plants reduces the accumulation of H_2O_2 significantly by upregulating the antioxidant system and proline production, thereby leading to improved membrane stability [51,52].

During stress, the production and accumulation of toxic ROS increases manifold, leading to peroxidation of the unsaturated lipid components of membranes, thereby resulting in a loss of membrane integrity due to leakage and desiccation [53]. ROS can affect the integrity of cellular membranes, enzyme activities, and the plant photosynthetic apparatus. Among ROS, H_2O_2, has potential to diffuse through biological membranes and making the adverse effects evident in other parts of cells [53]. Our work supports the beneficial role of *Trichoderma* spp. under abiotic stresses. Excess ROS generated through stress, if not scavenged, results in oxidative stress [53]. It is suggested that *Trichoderma* spp. inoculation may enhance the salt tolerance of *C. pepo* by increasing the antioxidant potential, leading to quick neutralization of ROS for membrane and cellular protection [54]. In addition, the inoculation imparts

significant changes in the plant's metabolic machinery, thereby benefiting the plant [55,56]. The overall positive effect of *Trichoderma* application in improving the antioxidant machinery performance was obviously reflected in higher levels of GSH content. GSH is an important antioxidant molecule and a redox component that contributes to the maintenance of the structural and functional integrity of cells [57,58,11]. In the present study, GSH accumulation was increased significantly by the inoculation of *Trichoderma* spp., either individually or in combination. Increased GSH following the inoculation of *Trichoderma* could have strengthened the antioxidant machinery to better counter the free radicals generated through salinity stress [59]. Plants maintaining a higher GSH concentration have been reported to withstand salinity better and prevent photosynthetic arrest more strongly through the maintenance of the cellular redox balance and the antioxidant system [60]. From the present study, it is evident that reduced generation of ROS together with increased antioxidant synthesis directly contributes to the maintenance of cellular functioning in *Trichoderma*-inoculated plants. Such results are in agreement with Zhang et al. [61], who reported that cucumber growth promotion was attributed to ROS hydrolysis by *Trichoderma* spp. under salinity conditions.

Greater accumulation of osmolytes provides protection to the cells [62] and is a well-observed tolerance strategy in plants for maintaining the cellular osmotic potential [63,64,11]. The results of increased proline accumulation due to salinity are in corroboration with Jaleel et al. [65], Azooz et al. [66], and [63]. Higher accumulation of proline results from the cumulative effect of stresses on the activities of proline synthesizing enzymes, which leads to upregulation and a significant decline in the catabolizing enzymes [67]. Greater proline accumulation leads to the maintenance of water balance in plants, allowing plants to withstand stress and insulating damage. Higher accumulation of proline and proteins in *Trichoderma*-inoculated *Cucurbita* plants could directly contribute to the maintenance of the relative water content in them. It has been reported that the maintenance of sufficient water content is essential for several plant functions including photosynthesis and membrane functioning [68]. Therefore, it could be inferred from the present study that a direct relation could occur between the accumulated proline concentration and membrane functioning, hence increasing the overall plant growth. Further, proline has a direct impact on the protein turnover and on the regulation of stress protective proteins [69]. *Trichoderma* spp. have proved beneficial for protecting *C. pepo* from the adverse effects of salinity stress.

The potential mechanism of *Trichoderma* in alleviating salt stress can be summarized as follows: First, under salinity stress conditions, the *Trichoderma* spp. restore the uptake of essential elements like Mg^{2+} and block the Na uptake that was negatively accumulated under saline conditions [70]. Second, many studies have reported that *Trichoderma* associated with plants subjected to salinity stress is triggered to synthesize higher amounts of plant growth regulators like α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA), cytokinin-like molecules that may aid in the mitigation of salinity stress [71]. Furthermore, *Trichoderma* induces phytohormones like salicylic acid and jasmonic acid [72]. The *Trichoderma* spp. have been found to lower the formation of abscisic acid during salinity stress and also facilitate the movement of cytokinins through the root to shoot system [73]. *Trichoderma* is responsible for the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, the primary precursor to ethylene breakdown and it triggers induced systemic tolerance in plants [74]. Third, reports confirm the role of *Trichoderma* in the activation of the antioxidant machinery to recover the oxidized ascorbate to enhance the tolerance mechanism under saline conditions [54]. *Trichoderma* treatment of salinity-stressed plants was shown to increase the GSH/GSSG ratio and act as a quencher for free radicals and cytotoxic compounds. Therefore, it aids in the cell's protection from oxidative damage [75]. Lastly, under salinity stress conditions, *Trichoderma* generates changes in host plants, and these changes are mainly associated with stress-related genes and proteins [76]. The inoculation of plant seedlings with *Trichoderma* significantly induced the expression of *MDAR, APX1*, and *GST* genes and triggered the antioxidant machinery to act against salinity-induced oxidative stress.

5 Conclusion

Salinity reduced the growth of *C. pepo* by affecting the key physiological and biochemical parameters studied. Membrane functioning was hampered due to excess generation of H_2O_2. However, *Trichoderma* spp. proved useful in protecting the *C. pepo* plants from salinity by improving the tolerance mechanisms including proline accumulation and GSH production. From the present study, it can be concluded that inoculation with *Trichoderma* spp., either individually or in combination, strengthens the salinity stress mitigating mechanisms in *C. pepo*.

Author Contributions: Mona Soliman and Amr Elkelish designed the research and conducted the experiments. All authors collected and analyzed the data, wrote the final text and approved the final version of manuscript.

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