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Proceeding Paper

Micronutrient Fertilization Amplified the Antioxidant Capacity in Tomato Plants with Improved Growth and Yield †

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Abstract: Micronutrients play a critical role in plant growth and development, and their deficiency can cause adverse effects on plant performance. Also, these elements can influence plant physiological processes as they incorporate in the molecular structure of enzymes as cofactors. In this study, the impacts of micronutrients solution containing manganese (125 ppm), iron (200 ppm), zinc (60 ppm), and copper (20 ppm) were investigated on growth parameters, yield, and antioxidant enzymes activity of tomato (*Solanum lycopersicum*) plants. Greenhouse tomatoes (cultivar Jet Star F1) were irrigated with the mentioned concentrations of elements in a completely randomized design with five independent biological replicates. The micronutrient treatment increased the specific activities of superoxide dismutase, ascorbate peroxidase, glutathione reductase, guaiacol peroxidase, catalase, phenylalanine ammonia-lyase, as well as phenols and salicylic acid contents in tomato leaves. However, malondialdehyde level and electrolyte leakage index were unaffected. Analysis of plant growth parameters revealed that micronutrient increased the stem diameter, root length, the number of leaves, stem height, and fruit's fresh weight in the treated plants. Overall, our results indicated that micronutrients positively affected the growth and development of tomato plants without adverse effects on the health indices. Moreover, the application of micronutrients can magnify the antioxidant capacity of tomato plants through increasing enzymes activity as well as phenols and salicylic acid levels. These changes would benefit the plants under abiotic/biotic stress conditions where elevated levels of antioxidant activities are crucial.

Keywords: antioxidant enzymes; growth; micronutrients; tomato plant; yield

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1. Introduction

Micronutrients have a critical role in plant growth and development and serve numerous functions in plants, such as cofactors of antioxidant enzymes [1] and structural components in osmolites under stress conditions [2]. In addition, it is well-established that losses of micronutrients can lead to a decrease in plant performance and yield and may have adverse effects on sustainable agriculture [3]. Microelements consisting of manganese, iron, zinc, and copper are required in small amounts and are essential for agricultural plants production [4]. Tomato (*Solanum lycopersicum*) is the most cost-effective vegetable for growers in which micronutrient fertilizers are used to improve the yield [5].

Due to the importance of tomato sowing in the world, this paper describes the effects induced by micronutrients application on the antioxidant capacity and performance of tomato plants. The output of this study will help farmers obtaining a maximum yield through nutritional programs in tomato greenhouses, especially under stressful conditions.

2. Materials and Methods

Greenhouse tomato seeds (cultivar Jet Star F1) were planted and grown in plastic pots of sterilized soil composed of 1:1:2 cocopeat: peat moss: perlite. Plant growth was conducted in a greenhouse under optimal conditions. Then, micronutrient solution containing manganese (125 ppm), iron (200 ppm), zinc (60 ppm), and copper (20 ppm) irrigated at different doses in different growth stages of tomato seedlings (Table A1). Simultaneously, control plants were irrigated with distilled water. Physiological and morphological parameters of the treated and control plants were investigated at the harvest stage.

Biochemical analysis of harvested leaves was performed after preparation in a suitable buffer. For superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) activities, the method of Homayoonzadeh et al. [6] was adopted. After homogenizing 1 g fresh weight in 1 mL phosphate buffer (50 mM, pH 7) and centrifugation at 16,000× *g* for 15 min at 4 °C, the supernatant was used as the enzyme source. SOD activity was assayed after mixing the enzyme source with EDTA, methionine, NBT, and riboflavin, and spectrophotometrically measured at 560 nm. APX activity was carried out by mixing the enzyme source with H_2O_2 as substrate and ascorbic acid as reductant, and then absorbance was measured at 290 nm. GR activity was spectrophotometrically evaluated at 412 nm using a reaction mixture of NADPH, DTNB, and GSSG.

Assessment of guaiacol peroxidase (GPX), catalase (CAT), and phenylalanine ammonia-lyase (PAL) specific activities was done based on the method of Homayoonzadeh et al. [7]. For this, after homogenization of 1 g of fresh leaf tissue in Tris-HCl buffer (50 mM, pH 7.5) and centrifugation at 15,000× *g* for 10 min at 4 °C, the enzyme source was obtained by using the supernatant. In the GPX activity assay, the absorbance of the reaction mixture consisting of enzyme source, H_2O_2 as substrate, and guaiacol as electron donor was measured at 470 nm by spectrophotometer. The activity of CAT was recorded at 240 nm after mixing the enzyme source with H_2O_2 as substrate. PAL activity was estimated using phenylalanine as substrate and cinnamic acid production at 290 nm.

Contents of phenols and salicylic acid were measure using the method claimed by Homayoonzadeh et al**.** [8]. Phenols content was quantified spectrophotometrically at 760 nm using Folin-Ciocalteu as a reagent and gallic acid solutions as standards. The salicylic acid was extracted by homogenization in methanol and then analyzed with a HPLC apparatus equipped with a UV/VIS detector at 235 nm and a GLC-ODS C_{18} column (150 mm × 6 mm internal diameter). The mobile phase consisted of methanol/water (70/30) at 1 mL min^{-1}. The concentration of malondialdehyde as well as electrolyte leakage index were estimated according to the method described by Homayoonzadeh et al**.** [9]. The thiobarbituric acid was utilized for the malondialdehyde test, then absorbance was recorded at 600 nm. Assessment of ELI was done using a platinum electrode, and the percentages of initial to final conductivity were recorded.

The morphological parameters related to plant growth and yield comprising of stem diameter, root length, number of leaves, stem height, and fruit's fresh weight were also evaluated at the harvest stage in treated and control tomato plants.

Experiments consigned to a completely randomized design with five independent biological replicates. After the data passed Shapiro–Wilk's test for normality and Levene's test for equality of variances, an unpaired *t*-test was used for comparisons between the treatments. All analyses were carried out in GraphPad Prism version 8.2.0.

3. Results

Results showed that the antioxidant capacity of tomato plants amplified in response to the micronutrient solution without adverse effect on the plant's health indices. Specific activities of superoxide dismutase ($p = 0.0036$, $t = 2.164$, 1.33-fold), ascorbate peroxidase ($p = 0.0190$, $t = 3.256$, 1.25-fold), glutathione reductase ($p = 0.0091$, $t = 4.369$, 1.99-fold), guaiacol peroxidase ($p = 0.0028$, $t = 2.279$, 1.35-fold), catalase ($p = 0.0401$, $t = 3.387$, 1.14-fold), and phenylalanine ammonia-lyase ($p = 0.0299$, $t = 4.489$, 1.86-fold) were significantly higher in

the treated plants compared with control ones (Figure 1A–F). Moreover, analysis of phenols (p = 0.0213, t = 2.348) and salicylic acid (p = 0.0225, t = 3.856) contents revealed their significant increasing in treated plants compared to the controls by 1.22- and 1.41-folds, respectively (Figure 2A,B). In contrast, there were no significant changes in malondialdehyde content (p = 0.4420, t = 0.412) and electrolyte leakage index (p = 0.5200, t = 0.325) in response to micronutrient treatment (Figure 2C,D).

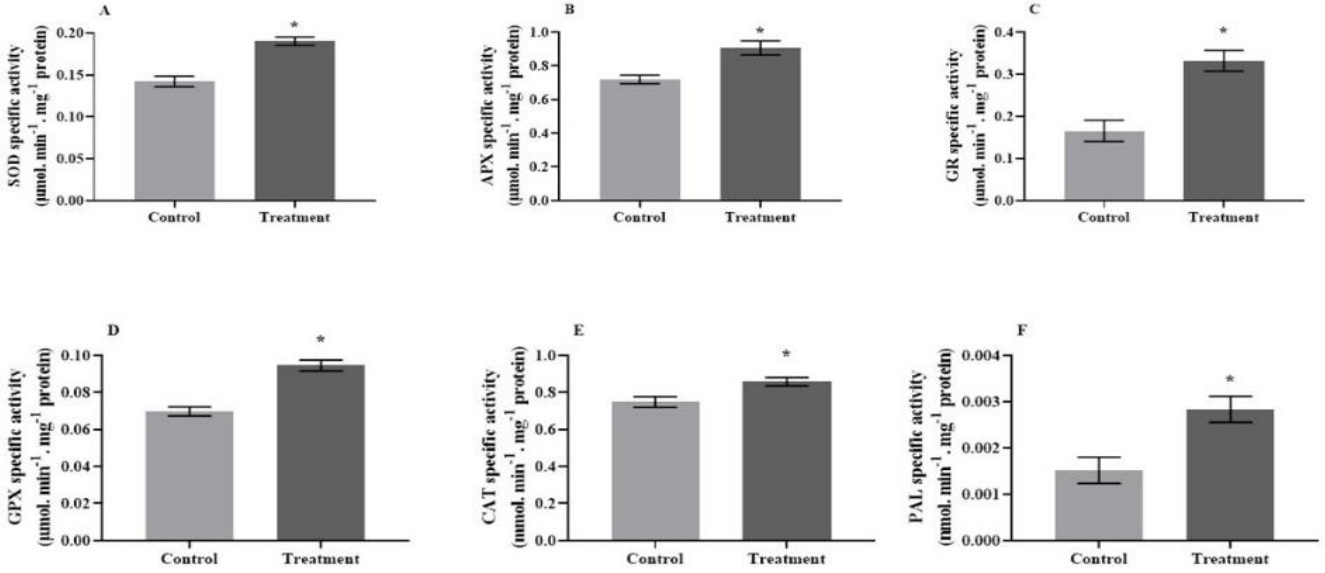

Figure 1. Mean (±SE) specific activities of (**A**) superoxide dismutase, (**B**) ascorbate peroxidase, (**C**) glutathione reductase, (**D**) guaiacol peroxidase, (**E**) catalase, and (**F**) phenylalanine ammonia-lyase in tomato leaves, when plants were treated with micronutrient solution (Treatment) or not (Conrol). The error bar shows standard errors. Asterisks were used to show statistically significant differences between treated and control plants.

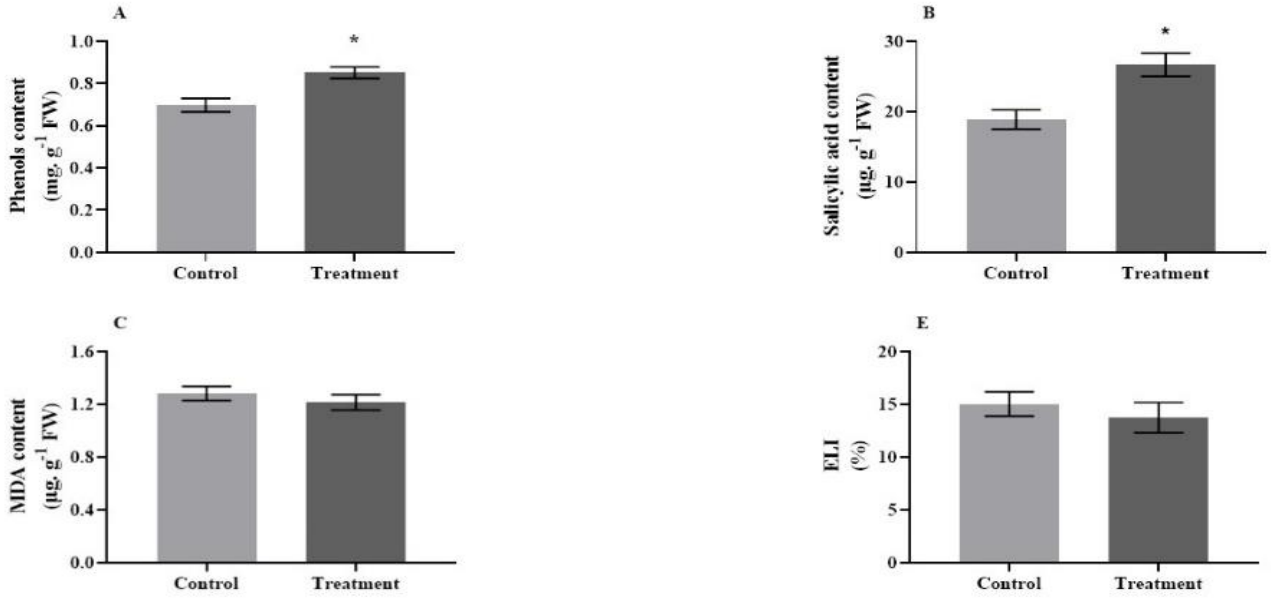

Figure 2. Mean (±SE) contents of (**A**) phenols, (**B**) salicylic acid, (**C**) malondialdehyde, and (**D**) electrolyte leakage index in tomato leaves, when plants were treated with micronutrient solution (Treatment) or not (Control). The error bar shows standard errors. Asterisks were used to show statistically significant differences between treated and control plants.

Further analysis on plant growth and yield clearly showed that the performance of treated tomato plants with micronutrient solution was improved. Morphological parameters including stem diameter (1.32-fold), root length (1.39-fold), number of leaves per plant (1.36-fold), stem height (1.14-fold), and fruit's fresh weight (1.17-fold) were significantly higher in the treated tomato plants compared with the controls (Table 1).

Table 1. T Mean (±SE) tomato plant growth and yield following treatment with micronutrient solution (Treatment) or not (Control). Asterisks were used to show statistically significant differences between treated and control plants.

Parameters	Control	Treatment	*p*-Value	*t*-Value
Stem diameter (mm)	8.44 ± 0.29	11.16 ± 0.36 *	0.031	2.559
Root length (m)	4.81 ± 0.45	6.69 ± 0.69 *	0.028	3.664
Number of leaves/plant	41.1 ± 2.31	56.3 ± 3.12 *	0.019	4.719
Stem height (m)	3.41 ± 0.19	3.89 ± 0.22 *	0.042	2.873
Fruit fresh weight (g)	82.1 ± 4.21	96.49 ± 3.81 *	0.036	3.964

4. Discussion

This paper proposes the framework of micronutrient application in tomato greenhouses that can have positive effects on the plants' antioxidant system as well as their performance. Some microelements are important cofactors of antioxidative enzymes involved in plant defenses. Manganese is a cofactor in the activation of SOD, CAT, and PAL [10]. Iron plays an activator role for APX, GPX, and CAT [11]. Zinc is a cofactor of transcriptional factors commonly involved in the expression of genes encoding antioxidative defense enzymes such as SOD, APX, and GR, which results in higher enzyme activity [12]. Copper is a cofactor of SOD, APX, and GST, which increases the catalysis of reactions [13]. According to results that demonstrated increases in antioxidant activities, it is plausible that treatment with micronutrients has positive and profound effects on the tomato plant defense systems, which may protect it against biotic and abiotic stresses.

Phenolics as reactive oxygen species quencher are produced by PAL activity because PAL is the key enzyme in the plant secondary metabolism, which catalyzes the first step in the phenylpropanoid pathway leading to the synthesis of the phenolic compounds [14]. Also, salicylic acid as a small phenolic compound makes a substantial contribution in the multiple physiological processes and activation of the plant defense system against biotic and abiotic stresses, which in turn could result in systemic resistance [15]. By contrast, malondialdehyde level and electrolyte leakage index, which did not significantly change in tomato plants in response to micronutrient treatment, may be related to inhibition of lipid peroxidation and cell injury by elevated levels of phenols [14] and salicylic acid [15] since they act as non-enzymatic antioxidants and cause decreasing membrane permeability and increasing cell viability.

Micronutrients such as manganese, iron, zinc, and copper have crucial roles in plants performance [16], and plants use these essential micronutrients to grow and complete their life cycle [17]. It is well-recognized that micronutrients promote plant growth and development by biosynthesis of free amino acid, carbohydrates, protein, and also plant yield through improving photosynthetic pigments function [18]. Thus, it can be concluded that the micronutrient regime utilized in this study has substantial benefits for tomato plant farming by amplifying the antioxidant capacity and improving growth and yield.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available in a publicly accessible repository.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Nutrition regime at used doses on different phenological stages of tomato plants.

Growth Stage	Days from Planting	Stage Duration (days)	Crop Age (days)	Dose (%)	Watering Volume (mL plant^{-1})	Watering Duration
Vegetative	1–14	14	14	0.5	300 mL	Every 7 Days
Budding	15–28	14	28	1.0	300 mL	Every 7 Days
Flowering	29–35	7	35	1.5	300 mL	Every 7 Days

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