Alrashidi AA *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 1, Article number 12614
DOI:10.15835/nbha50112614
Research Article

Role of calcium and magnesium on dramatic physiological and anatomical responses in tomato plants

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Abstract

Minerals are the fundamental source of nutrients for plant functions such as photosynthesis, ATP currency, cellular respiration, metabolic activities, defense mechanisms, and tolerance to biotic and abiotic stressors. Minerals are the most significant component of plant nutrition and applying these minerals supplements can increase fruit output. The study's main aim was to make agricultural farming easier by foliar applying newly created nutrients like Lebosol-calcium and Magnesium. The four treatments: To (Control), T1 (Lebosol-Mg-Plus, 3 ml/L), T2 (Lebosol-Ca-Forte, 3 ml/L), and T3 (Lebosol-Mg-Plus and Lebosol-Ca-Forte, 3 ml/L) was applied as foliar spray to the seedlings of tomato. It was found that T3 substantially enhanced tomato's morphological features and yield. The treatment T3 significantly increased total soluble protein, chlorophyll content, and antioxidant enzyme activity. Furthermore, the foliar application of T3 considerably improved phenolic and ascorbic acid contents. The general anatomical features of the leaf, stem, and roots of tomato were qualitatively affected by the treatments. Application of Lebosol-Ca provided the highest total thickness of lamina, number of vessel elements, total phloem area, chlorenchyma layer, total area of vessel elements, xylem ratio, and increased palisade layer thickness, vessel diameter. Furthermore, T3 treatment showed a diverse impact on the internal structure of tomato organs, with palisade and spongy parenchyma growing to maximum values and vessel diameters expanding. T3 had also posed remarkable alterations in morpho-physiological, biochemical, and anatomical aspects in tested plants.

Keywords: calcium; magnesium; mineral nutrition; tomato; yield increment

Received: 31 Dec 2021. Received in revised form: 17 Feb 2022. Accepted: 24 Feb 2022. Published online: 09 Mar 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetables globally due to the formation of soups, juices, purees, and sauces (Giudice *et al.*, 2017). The fruit of the tomato is a rich source of vitamins A and C (Raiola *et al.*, 2014; Ayenan *et al.*, 2019). Recent research found that tomatoes are the primary source of nutritional lycopene, a powerful antioxidant unlike nutrients in most fresh fruits. Tomatoes also contain other protective mechanisms, such as antithrombotic and anti-inflammatory functions (Kumar *et al.*, 2020). Tomatoes are an important part of the Mediterranean and other traditional diets. However, global food security is a significant concern for humanity, as demographic predictions put the human population at 9.5 billion by 2050. Under the threat of climate change, securing and optimizing crop yield is vital for the agriculture industry (Lucini *et al.*, 2019). In light of this crop's significance, developing novel management methods to improve resistance to abiotic stressors might help boost world food production (Fahad *et al.*, 2015a; Fahad *et al.*, 2015b). The application of mineral fertilizers is a common strategy for agronomists to enhance the bioavailability of minerals in soil (Bindraban *et al.*, 2015). Usage of modified inorganic fertilizers as an agricultural strategy to upsurge mineral nutrition content in crops has been effectively employed {Formatting Citation}). A balanced fertilizer and extra modified minerals that don't lead to environmental pollution can achieve higher efficiency and produce more yield and high-value crops (Yunju *et al.*, 2012; Ali *et al.*, 2020).

Minerals are chemicals that may be applied to seeds, plants, and soil and are either natural or synthetic and alter critical and structural processes in plants to impact plants' growth yield and quality (de Vasconcelos and Chaves, 2019). In their formulations, fertilizers may contain a range of organic components, such as humic and fulvic acids, algae extracts, vitamins, and amino acids, ascorbic acid, and other metal ions that may depend entirely on the product's supplier and precursors ingredients (Sible *et al.*, 2021). In the fertilizer industry, there is enormous opportunity for new products to be developed, both economically and socially (Balawejder *et al.*, 2020). Researchers found that plants treated with either organic or inorganic chemicals, and also with natural bio-stimulants, tends to have more activities of antioxidant enzymes (Teixeira *et al.*, 2017; Wozniak *et al.*, 2020).

The use of Lebosol-Ca and Mg act as therapeutics in plants which offers a novel solution to the problem of improving agricultural system sustainability while reducing the usage of undesirable chemical fertilizers (Povero *et al.*, 2016; Di Stasio *et al.*, 2018). Lebosol-Ca and Mg can be used as therapeutic agents for different plants as these are more soluble and exchangeable with other nutrients and can promote growth in plants and can be used as fertilizer through modern Lebosol technology (Guerrera *et al.*, 2009). Lebosol-Mg-Plus is a mineral fertilizer consisting of a unique Mg and potassium oxide formulation, phosphate soluble in mineral acid, urea, and trace ions. Ca is an essential mineral that influences plant development and metabolism through various physiological and biochemical processes (Pathak *et al.*, 2020). Ca is required for plant development, cell wall thickness, and restoration, as well as plant tissue (Hepler and Winship, 2010; Kudla *et al.*, 2010). Ca is engaged in cell expansion and reproduction, maintains cell pH, and functions via its effects on cells and cell membranes as a regulating component in the source-sink translocation mechanism (Hirschi, 2004). Ca^{2+} nutrition positively impacted tomato growth, fruit production, and quality. Mg is also crucial for plant growth because it has a direct role in physiological and biochemical systems. It increases root development, enhances water and nutrient absorption, facilitates carbohydrate export, and reduces ROS generation and photo-oxidative damage to cells during stress conditions (Verma *et al.*, 2019). Mg is an essential nutrient involved in numerous metabolic processes during plant growth and development (Cakmak and Yazici, 2010; Gransee and Führs, 2013). In chlorophyll, Mg is the most abundant element that drives photosynthesis(Cakmak and Yazici, 2010). Mg deficiency may result in programmed cell death due to oxidative damage in chloroplasts (Foyer and Noctor, 2005). The morphology of the leaves revealed a disorder of the thylakoids due to Mg insufficiency (Hermans *et al.*, 2004). Both Ca and Mg play a therapeutic role in vascular bundles that assist the plants in different mechanisms like anatomical diseases of cell walls, secondary xylem, and epithelial tissues that have a powerful role in plants' developmental processes (Massironi *et al.*, 2013). So these elements have a significant part in the survival of plants subjected to various environmental stresses (Hao and Papadopoulos, 2004).

Application of Ca and Mg through the modern technology of Lebosol has great importance for plant anatomy, and their deficiency causes severe damage in the internal integrities of plants (Gransee and Führs, 2013). Mineral shortage or scarcity can impact plant anatomy (Fontes, 2006). In light of the above statements, the current study was designed to demonstrate (a) the impact of the Lebosol-Ca-Forte SC and Lebosol-Mg-Plus on growth performance through the foliar application (b) investigates how Lebosol Ca or Mg modulated the vegetative growth and physiological characteristics and (c) induced amelioration as well as anatomical changes of tomato plants.

This article is concerned with the use of Lebosol®-Calcium-Forte SC which (a special calcium-formulation with manganese, zinc, and Aminosol. Aminosol improves the absorption of calcium, manganese supports photosynthetic pigments, zinc is important for the defense metabolism), and Lebosol-Mg-Plus that (contains Magnesium and potassium in the form of phosphite, which increases plant resistance to biotic and abiotic stresses also, works to increase the vegetative growth and supports the immune responses). So, Lebosol®-Calcium-Forte SC and Lebosol-Mg-Plus consider therapeutic nutrients with various advantages such as safe, biocompatible, low cost, and enhancement of plant health and productivity.

Materials and Methods

The experiment was performed in the research farm of Al-SALAM International for Development & Agricultural Investment, Egypt. Lebosol®-Magnesium-Plus and Lebosol®-Calcium-Forte were obtained from Lebosol Dünger GmbH as a bio-stimulant (Wiesengasse 28, 67471) Elmstein, Germany.

Experimental treatments

Lebosol®-Magnesium-Plus and Lebosol®-Calcium-Forte treatments were performed by foliar spraying (FS) method until dropping. The Seedlings were planted in 4 groups as follow:

1) **Control** (T0): - plants without any treatments and irrigated by tap water only.

2) **Lebosol®-Magnesium-Plus (T1): -** plants sprayed with 3 ml /L (foliar spray)

3) **Lebosol®-Calcium-Forte (T2): -** plants sprayed with 3 ml /L (foliar spray)

4) **Lebosol®-Magnesium-Plus & Lebosol®-Calcium-Forte Treatment (T3): -** plants sprayed with 3 ml /L of a solution containing both Mg and Ca (foliar spray).

The experimental site and setup

This study was carried out during the 2021 season on tomato plants (*Solanum lycopersicum* L. var. 023) in the experimental farm of Al-SALAM International for Development & Agricultural Investment. Four weeks old tomato seedlings (*Solanum lycopersicum* L. var. 023) were chosen due to its widespread use in Egypt's agricultural areas due to its excellent adaptability and productivity; (28 DAP) were received from the Agricultural Research Center, Giza, Egypt. The experiment was carried out as follows, four treatments, each treatment in a line of length 50 m. The plants were planted at a distance of 30 cm between each plant, meaning 165 plants in the line. The distance between the line was 1.5 m. The amount of water used depends on the need of the plant. The plants were not treated with any solutions for five days after transplantation, and regular irrigation continued. Afterward, the modified minerals (Lebosol®-Magnesium-Plus and Lebosol®-Calcium-Forte) were applied for four times (one time each week) (in the period before and after flowering). The different parameters were determined in samples that were taken at 60 DAP (60 days after planting). The sample from leaves, shoots, and roots (60 days vegetative), yield was taken (75 DAP) for each treatment, and the relevant parameters were estimated.

Vegetative growth and yield parameters

At 60 DAP, morphological characteristics of all plant samples were documented. Five plants with roots were taken from each treatment and rinsed under running tap water to remove dirt and adherent particles. Samples were sent to the laboratory for analysis of different growth characteristics, including shoot and root fresh weights. Dry weights of shoots and roots were determined after oven drying samples at 70 °C for 24 h or until they reached a constant weight. Further, the height of the plant, the root length, and the number of leaves per plant were recorded.

Photosynthetic measurements

Fresh 0.5 g leaf tissue was crushed in acetone (80%) using a pestle and mortar to estimate the pigment content. After centrifuging the filtrate for 5 min at 10,000×g, the absorbance of the filtrate was measured at 470, 652, and 665nm to estimate chlorophyll a, chlorophyll b and carotenoid content (Lichtenthaler and Buschmann, 2001).

Estimation of stress induced biomarkers

Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) method according to Heath and Packer (1968) and Attia *et al.*, 2021 with slight modification. The MDA content was determined according to its molar coefficient of absorbance of 155 mmol $\cdot L^{-1} \cdot cm^{-1}$ and expressed as nmolg^{-1} FW.

H_2O_2 content

Hydrogen peroxide levels were determined according to Velikova et al. (2000). The leaf was homogenized in 2mL 0.1% trichloroacetic acid (TCA) solution. After centrifugation at 12,000×g for 15 min, 0.5 mL of the supernatant was added to the reaction mixture containing 0.5 mL 10mM K phosphate buffer (pH 7.0) and 1ml of 1M KI. Absorbance was determined at 390 nm. The blank was prepared in the same manner except that 1 mL of 10 mM K phosphate buffer (pH 7.0) instead of the sample. The amount of H_2O_2 was calculated from calibrated samples using (1, 5, 10 mM H_2O_2) standard solutions, each standard solution was added to the reaction mixture containing 0.5mL 10mM K phosphate buffer (pH 7.0) and 1mL of 1M KI. Absorbance was determined at 390 nm.

Determination of the content of osmolytes

The soluble protein content was estimated following Lowry *et al.* (1951) using Folin phenol reagent, and absorbance was recorded at 700 nm using bovine serum albumin as standard.

The method of Bates *et al.* (1973) was used for the estimation of proline. Briefly, 0.5g dried leaves were extracted in 3% sulphosalicylic acid. After centrifugation at 10,000×g for 10min, the supernatant was mixed with ninhydrin reagent, and absorbance was taken at 520 nm. For measuring soluble sugar content, the anthrone method was used, and absorbance was measured at 625 nm (Irigoyen *et al.*, 1992).

Non-enzymatic antioxidant

Total phenolic content was estimated using the method Dai *et al.* (1994) described with slight modifications. A 100 µL volume of extract was added to 1.5 mL Folin-Ciocalteu reagent solution and incubated at room T for 1 min. Subsequently, 1.5 mL of a sodium carbonate solution was added and incubated for 90 min in the dark at room temperature. Absorbance was read at 765 nm.

The ascorbic acid (AsA) was determined according to Jagota and Dani (1982). Leaf samples (0.2 g) were ground with liquid N_2 and suspended in 2 ml of 5% TCA. The homogenate was centrifuged at 10,000×g for 15 min at 5 °C. AsA extraction solution was mixed with 10% TCA, vigorously shaken, and then placed in an ice bath for 5 min. 0.5 ml of the extract was diluted to 2.0 ml using double distilled water, and 0.2 ml of diluted

Folin-Ciocaiteu reagent was added to the previous mixture, and the absorbance of the blue color developed was measured after 10min at 760 nm. The AsA content was calculated using a standard curve of AsA.

Antioxidant enzymes assay

Fresh tomato (1.0 g) leaves were extracted in 100mM phosphate buffer (pH 7.8) containing PVP and EDTA where the homogenate was centrifuged at 15,000×g for 10 min and the supernatant was used for assaying enzyme activity.

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed following Bergmeyer (1974) and the ability of enzyme to auto-oxidize epinephrine was recorded at 480 nm.

Catalase activity (CAT; EC 1.11.1.6) was determined by Aebi (1974) and the disappearance of H_2O_2 was monitored at 240 nm for 3 min.

The method used in Bergmeyer (1974) was used to determine the activity of peroxidase (POD; EC 1.11.1.7) by monitoring the rate of guaiacol oxidation at 470 nm (ε= 26.6 $mM^{-1}cm^{-1}$). Polyphenol oxidase (PPO; EC.1.10.3.1) activity was detected by a Lavid *et al.* (2001) protocol. The purpurogallin production was monitored at 495 nm and the enzyme activity was expressed in U mg^{-1} $protein^{-1}$ min^{-1}.

Anatomical investigation

For anatomical slides production, 2-3 specimens of third internode mature blade and petiole were selected and preserved in Formalin Acetic Alcohol (FAA) to save internal structures for anatomical study. Then specimens were processed according to the paraffin wax method of (Shamso *et al.*, 2019) to prepare samples for microtome sectioning at 10-15 µm thickness. Sections were fixed on glass slides through Haupt's adhesive (1gm gelatin dissolved in 50 ml warm distilled water then 7.5 ml glycerol added + small phenol crystal then kept in the refrigerator for 24 h till solidification) and left to dry for 24h. Then sections were stained with Safranin- Fast green standard double stain and mounted in Canada balsam. Digital images of different organs were obtained using a photomicroscope (Optica, Italy) fit with a digital camera (Premer). Ten measurements were carried out by using image analysis software (Image J). For all measurements, at least ten replicates (measures) were used, and the averages were transformed into percentages in relation to the total thickness.

Statistical analyses

Inferential statistics for evaluating and comparing between two different groups (control, Lebosol Ca, Lebosol Mg, and Lebosol Ca + Mg) using one-way analysis of variance, followed by Duncan's Multiple Range test at significance levels of 0.05. The statistical analysis was carried out using SPSS (IBM-SPSS ver. 26.0 for Mac OS). Data were collected, checked, revised, and organized in tables and figures using Microsoft Excel 2016. Data was subjected to outliers' detections and normality testing.

Results

From Table 1, it is evident that morphological growth parameters (plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, number of leaves, and root length) showed a significant increase through exogenous application of Lebasol-Ca and Mg. All these growth parameters were found maximum at T3 as compared to T1, T2, and T0, respectively (Figure 1). The application of Lebosol-Ca and Mg caused a significant increase in yield attributes, as shown in (Table 2; Figure 2). Maximum yield was obtained from T3 as compared to T1 and T2, and T0.

Table 1. Physico-chemical characteristics of the soil used in the experiment

Analysis of soil	Physicochemical analysis
Organic matter %	0.1
P %	0.44
N %	0.5
Saturation (%)	24
pH	7.8
ECe (dSm^{-1})	4.9
Cations (meq/L)	
K^+	3.2
Na^+	18
Mg^{++}	13
Ca^{++}	12.5
Anions(meq/L)	
$SO^=$	17
Cl^-	22.4
$HCO3^=$	0.0
$CO3^=$	6.9
Mechanical Analysis	
Soil texture	Sandy loam
Sand%	79.9
Silt%	12.2
Clay%	7.4

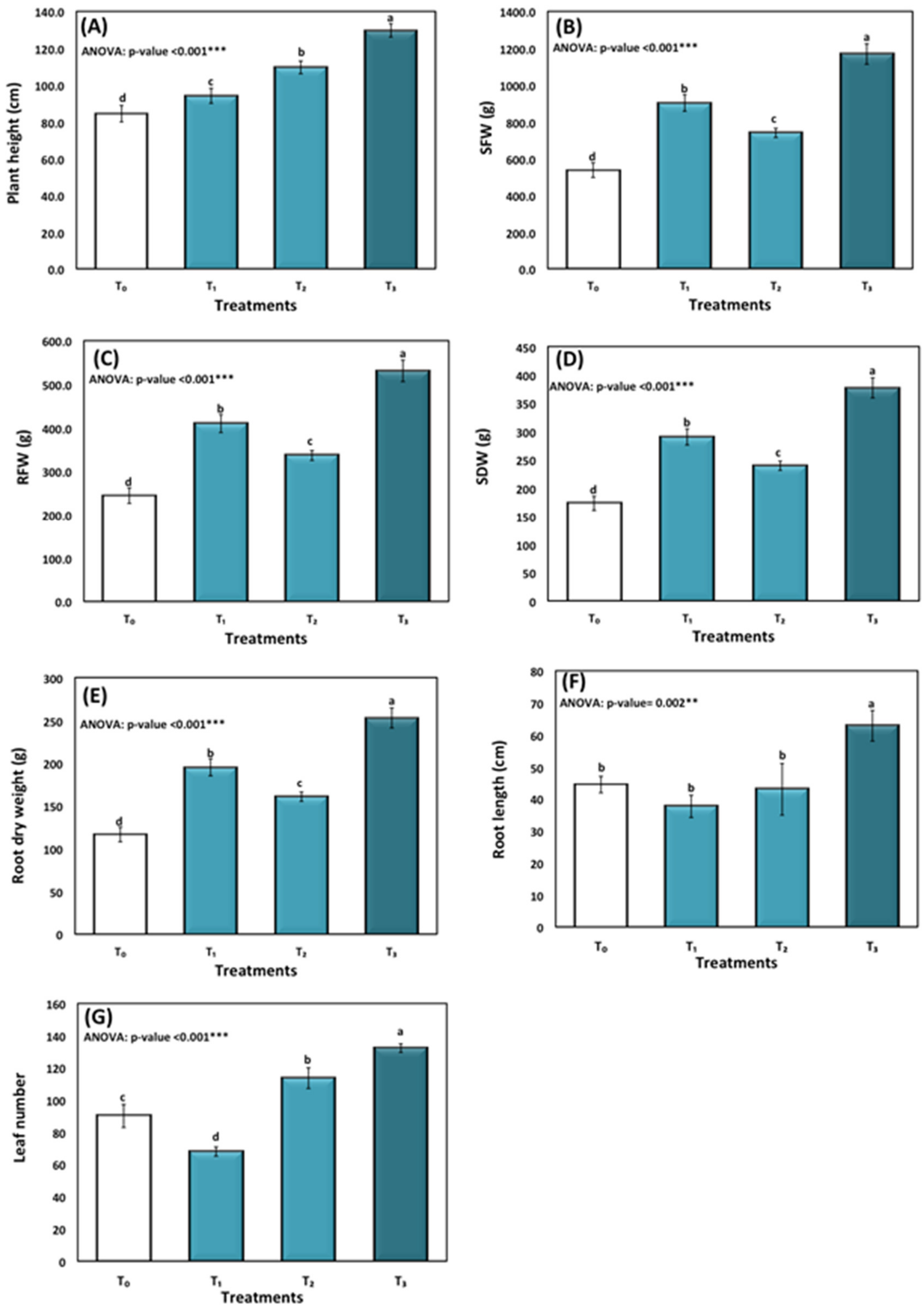

Figure 1. Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on the morphological growth parameters in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP

Data expressed as (A) plant height (cm), (B) shoot fresh weight, (C) root fresh weight, (D) shoot dry weight, (E) root dry weight, (F) Root length (cm) and (G) leaf number.

Values are mean (±SE) of three replicates and different letters represent significant difference at P<0.05. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg.

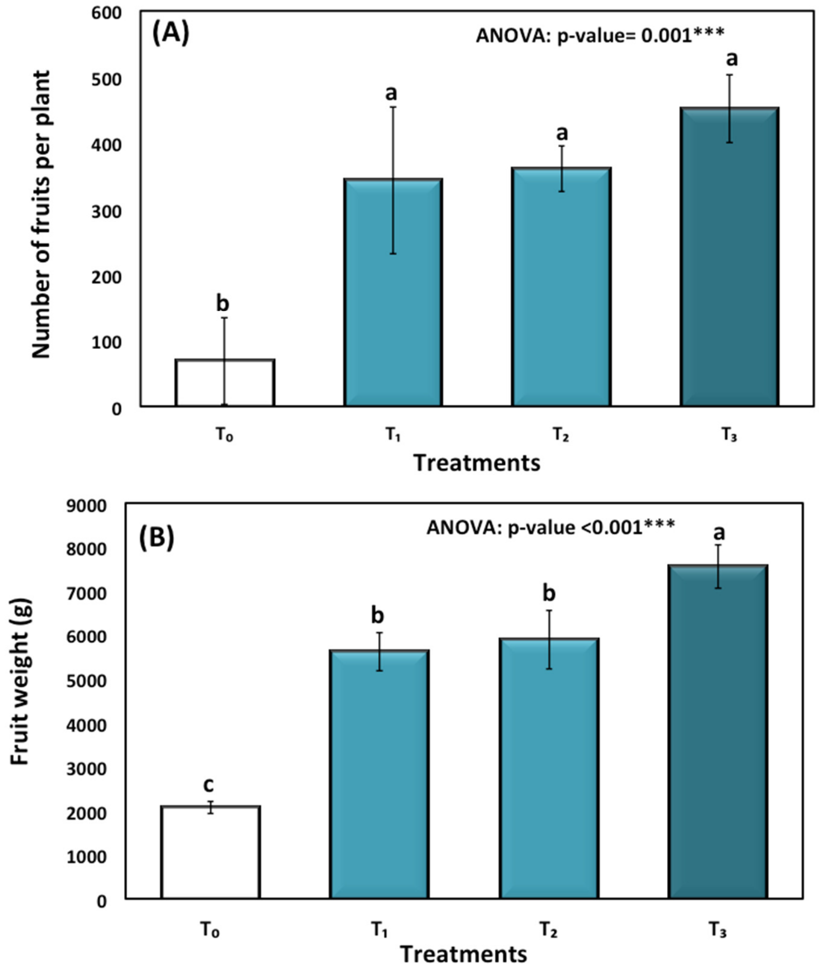

Figure 2. Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on the final yield of in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP
Data are expressed as (A) number of fruits per plant and (B) fruit weight (g). Values are mean (±SE) of three replicates and different letters represent significant difference at P<0.05. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg.

Chlorophyll a was observed maximum in T3 as compared to T1 and T2. Chlorophyll *b* was recorded in an optimum amount in T2, where Lebosol-Mg was used compared to T1 and T3. Carotenoid content showed a prominent increase through exogenous application of Lebosol-Ca, and it showed higher carotenoid content as compared to T2 and T3 (Table 2; Figure 3).

The MDA content showed significant alteration due to exogenous application of Lebosol-Ca of Mg. It was highest at T1 and observed to be the minimum at T2 and T3. The H_2O_2 content showed the same results regarding all used treatments. T1, T2, and T3 led to relatively same results; hence minimum activity was observed in the control treatment (Table 2; Figure 4).

The total soluble sugars and proteins showed a significant increase under Lebosol-Ca and Mg treatments. At T3, maximum proteins and sugar content were observed with the least values in T1 and T2 treatments. Total proline content showed different results as compared to other biochemical attributes. The total proline content was found to be maximum in control treatment as compared to other treatments (Table 2; Figure 5)

Table 2. Showed mean square from analysis of variance for the data of growth, antioxidant, biochemical analysis, reactive oxygen species, enzymatic and non-enzymatic antioxidant and yield properties in tomato plants in response to Lebosol-calcium and magnesium

Dependent Variable		Source			
		Corrected Model	Lebosol-Ca	Lebosol-Mg	Lebosol-Ca * Mg
SFW	F	118.4	261.3	92.4	1.6
	Sig.	<0.001***	<0.001***	<0.001***	0.239
RFW	F	118.4	261.3	92.4	1.6
	Sig.	<0.001***	<0.001***	<0.001***	>0.05 ns
SDW	F	118.4	261.3	92.4	1.6
	Sig.	<0.001***	<0.001***	<0.001***	>0.05 ns
RDW	F	118.4	261.3	92.4	1.6
	Sig.	<0.001***	<0.001***	<0.001***	>0.05 ns
Leaf number	F	85.7	0.4	211.1	45.8
	Sig.	<0.001***	>0.05 ns	<0.001***	<0.001***
Root length	F	13.53	4.74	15.75	20.08
	Sig.	0.002**	>0.05 ns	0.004**	0.002**
Chl-a	F	41.5	97.03	24.05	3.63
	Sig.	<0.001***	<0.001***	0.001***	>0.05 ns
Chl-b	F	25.8	11.569	38.3	27.53
	Sig.	<0.001***	0.009**	<0.001***	0.001***
Caroteinoid	F	19.5	49.4	5.5	3.71
	Sig.	<0.001***	<0.001***	0.047*	>0.05 ns
MDA	F	2362.457	988.813	6092.482	6.076
	Sig.	<0.001***	<0.001***	<0.001***	0.039 *
H_2O_2	F	658.111	600.49	628.255	745.588
	Sig.	<0.001***	<0.001***	<0.001***	<0.001***
Totalphenols	F	504.386	824.02	533.824	155.314
	Sig.	<0.001***	<0.001***	<0.001***	<0.001***
Proline	F	53.248	16.49	141.667	1.588
	Sig.	<0.001***	0.004**	<0.001***	>0.05 ns
TSS	F	214.974	412.255	216.176	16.49
	Sig.	<0.001***	<0.001***	<0.001***	0.004**
Total_protein	F	848.438	839.813	1252.484	453.017
	Sig.	<0.001***	<0.001***	<0.001***	<0.001***
AsA	F	132.327	269.302	122.366	5.312
	Sig.	<0.001***	<0.001***	<0.001***	0.05*
SOD	F	211.375	364.102	10.92	259.102
	Sig.	<0.001***	<0.001***	0.011*	<0.001***
CAT	F	572.585	1012.902	22.886	681.968
	Sig.	<0.001***	<0.001***	0.001***	<0.001***
Peroxidase	F	386.497	666.747	14.098	478.645
	Sig.	<0.001***	<0.001***	0.006**	<0.001***
PPO	F	103.887	188.828	1.297	121.537
	Sig.	<0.001***	<0.001***	>0.05 ns	<0.001***
Number of fruits	F	15.921	19.394	23.424	4.945
	Sig.	0.001***	0.002**	0.001***	>0.05 ns
Fruit-weight	F	69.321	88.438	108.015	11.512
	Sig.	<0.001***	<0.001***	<0.001***	0.009**

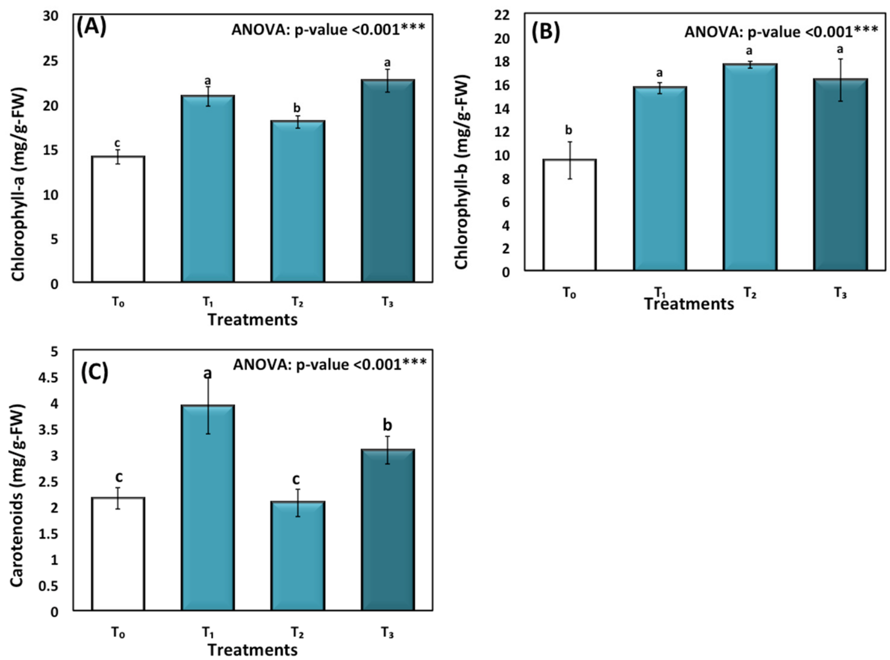

Figure 3. Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on pigment contents in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP

Data expressed as (A) chlorophyll a (Chl a), (B) chlorophyll b (Chl b), (C) carotenoid. Values are mean (±SE) of three replicates and different letters represent significant difference at P<0.05. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg

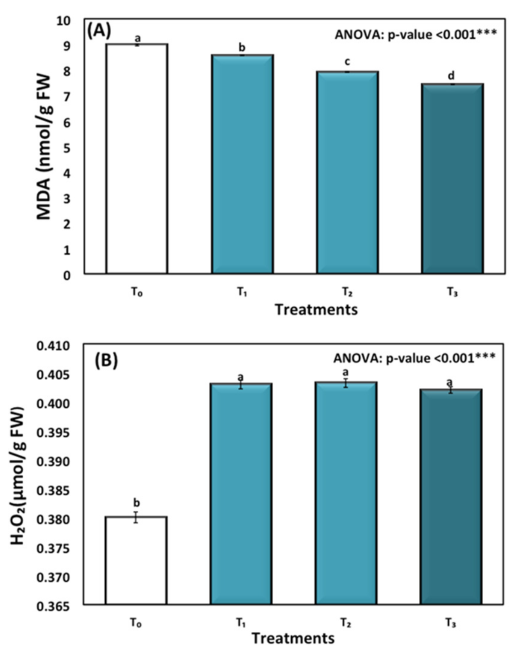

Figure 4. Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on changes in oxidative damage attributes in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP

Data expressed as (A) malondialdehyde content (MDA); and (B) Hydrogen peroxide (H_2O_2). Values are mean (±SE) of three replicates and different letters represent significant difference at P<0.05. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg.

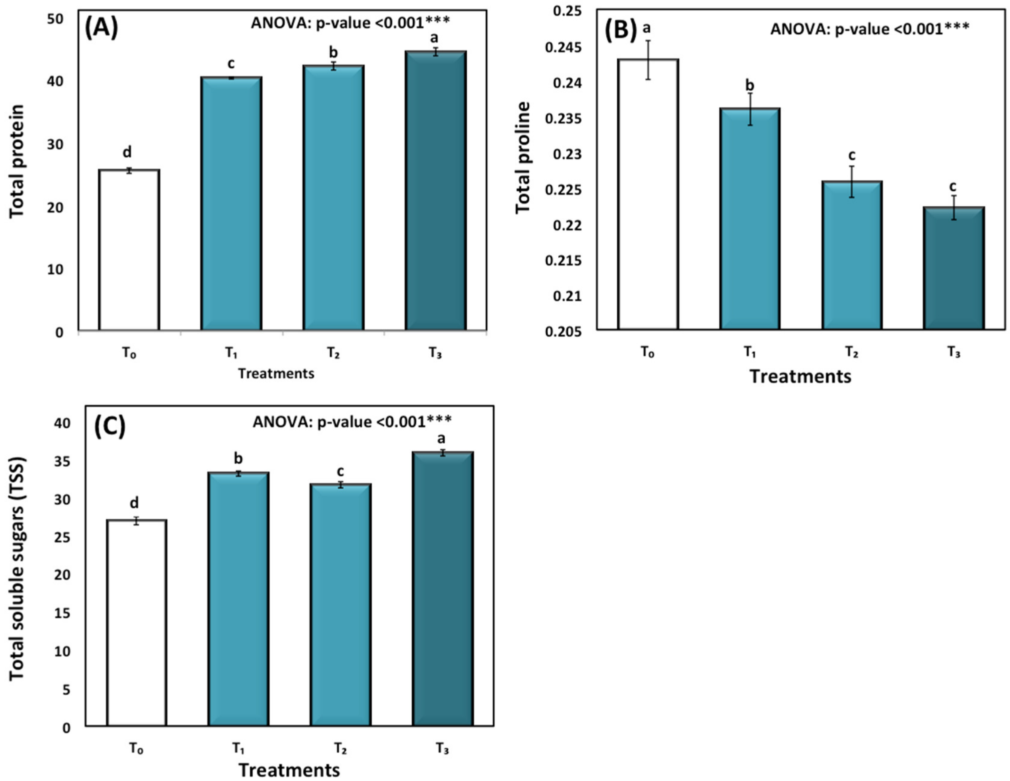

Figure 5. Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on changes in osmolyte concentrations in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP

Data expressed as (A) total soluble protein; (B) proline content, and (C) total soluble sugars (TSS). Values are mean (±SE) of three replicates and different letters represent significant difference at P<0.05. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg.

Non-enzymatic antioxidant (ascorbate and total phenolic) activity was observed maximum in response to Lebosol-calcium and Magnesium. Total phenolics and leaf ascorbic acid content were recorded maximum in T3 as compared to T1 and T2. Data related to non-enzymatic antioxidants is represented in Table 2 and Figure 6.

Enzymatic antioxidants showed maximum activity at T1 as compared to other treatments. Enzymatic antioxidants (SOD, POD, CAT, and PPO) posed a significant increase in response to Lebosol-calcium, and Lebosol-magnesium and Lebosol-calcium and Magnesium followed T1. In other words, T1 showed maximum enzymatic activity in tomatoes compared to T1 and T2. Data for enzymatic antioxidants are elaborated in Table 2 and Figure 7.

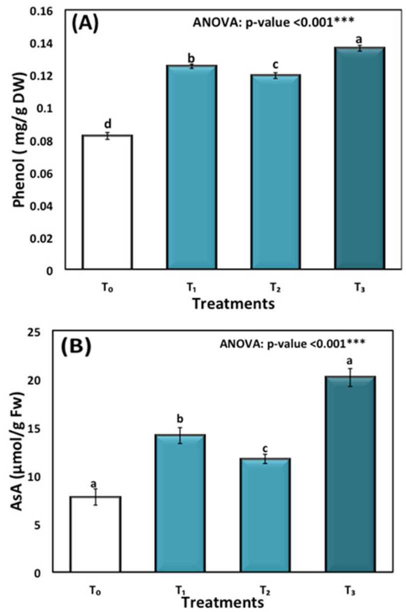

Figure 6: Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on changes in non-enzymatic antioxidants in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP

Data expressed as (A) phenol; (B) ascorbate content (AsA) in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP.Values are mean (±SE) of three replicates and different letters represent significant difference at $P<0.05$. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg.

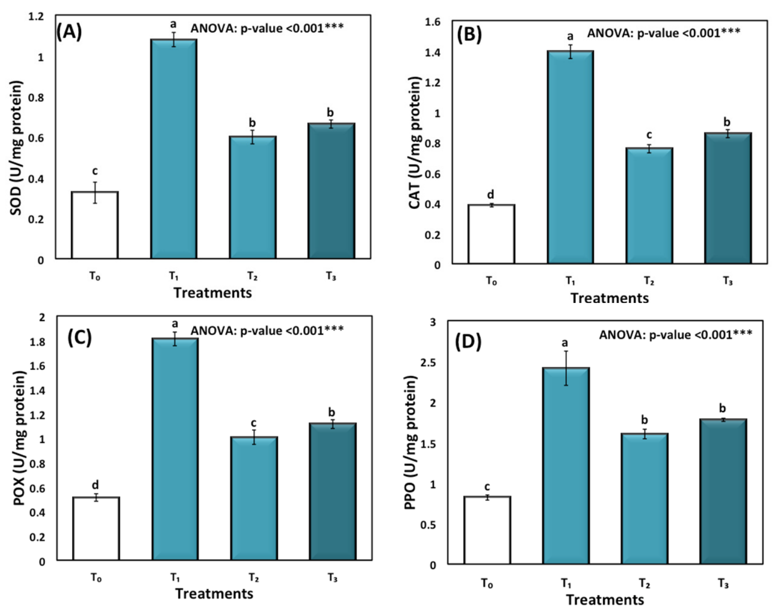

Figure 7. Effect of exogenous application of Lebosol-Calcium and Magnesium either individually or in combination on changes in enzymatic antioxidants in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP

Data expressed as (A) Superoxide dismutase (SOD, EC 1.15.1.1); (B) Catalase (CAT, EC 1.11.1.6); (C) Peroxidase (POX, EC 1.11.1.7); and (D) Polyphenol oxidase (PPO, EC 1.10.3.1) in tomato plants (*Solanum lycopersicum* L. *var.* 023) at 60 DAP.Values are mean (±SE) of three replicates and different letters represent significant difference at $P<0.05$. Treatments were organized as follow: T_0: control, T_1: Lebosol Ca, T_2: Lebosol Mg and T_3: Lebosol Ca + Mg.

Anatomical investigation

The lamina transverse section of control samples revealed the standard structure of the dicot leaf, epidermis uniseriate, covered by thin cuticle and multicellular, non-branched, non-glandular trichomes. The midrib was rounded, penetrated by seven bi-collateral vascular bundles with a large arc shape, surrounded by large ground tissue of thin parenchyma and little continuous palisade and spongy tissues. The mesophyll was dorsiventral with high content of sand crystals. The petiole was rounded with two small protuberances. The epidermis was uniseriate, followed by 1-2 layers of non-continuous chlorenchyma cells, 7-10 layers of angular collenchyma, and many thin parenchyma layers that extended to the pith. The vascular system consisted of many bicollateral vascular bundles arranged in an arc shape. (Plate 1).

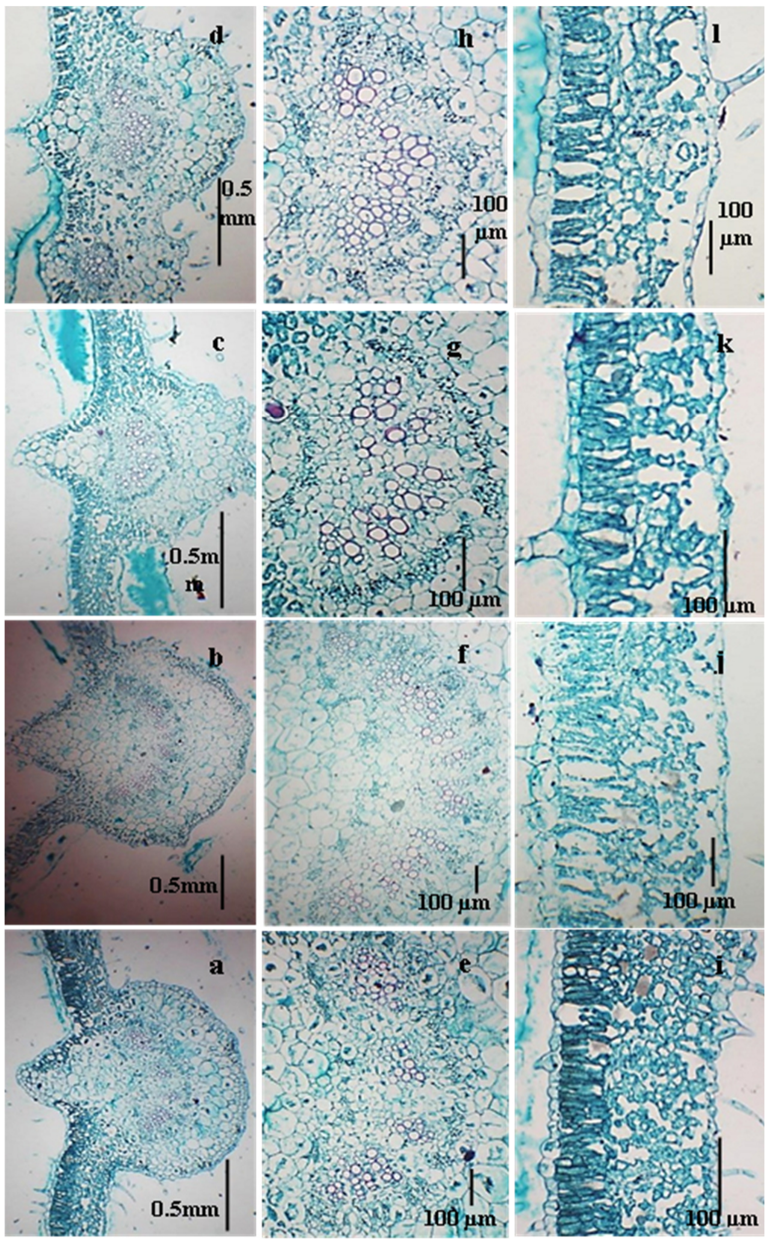

Plate 1. a, e, I - Control sample; b, f, j - Lebosol-Calcium; c, g, k - Lebosol-Magnesium; d, h, l - Ca & Mg mixture

The transverse section of the stem revealed that the epidermis was uniseriate, covered with thin cuticle and unicellular, non-branched, non-glandular trichomes. The cortex consisted of a thin chlorenchyma layer followed by angular collenchyma and thin parenchyma. The vascular cylinder consisted of a continuous bi-collateral vascular bundle and pith filed with large thin parenchyma (Plate 2). The root transverse section showed a wide periderm followed by a thin layer of phloem and large xylem (Plate 3).

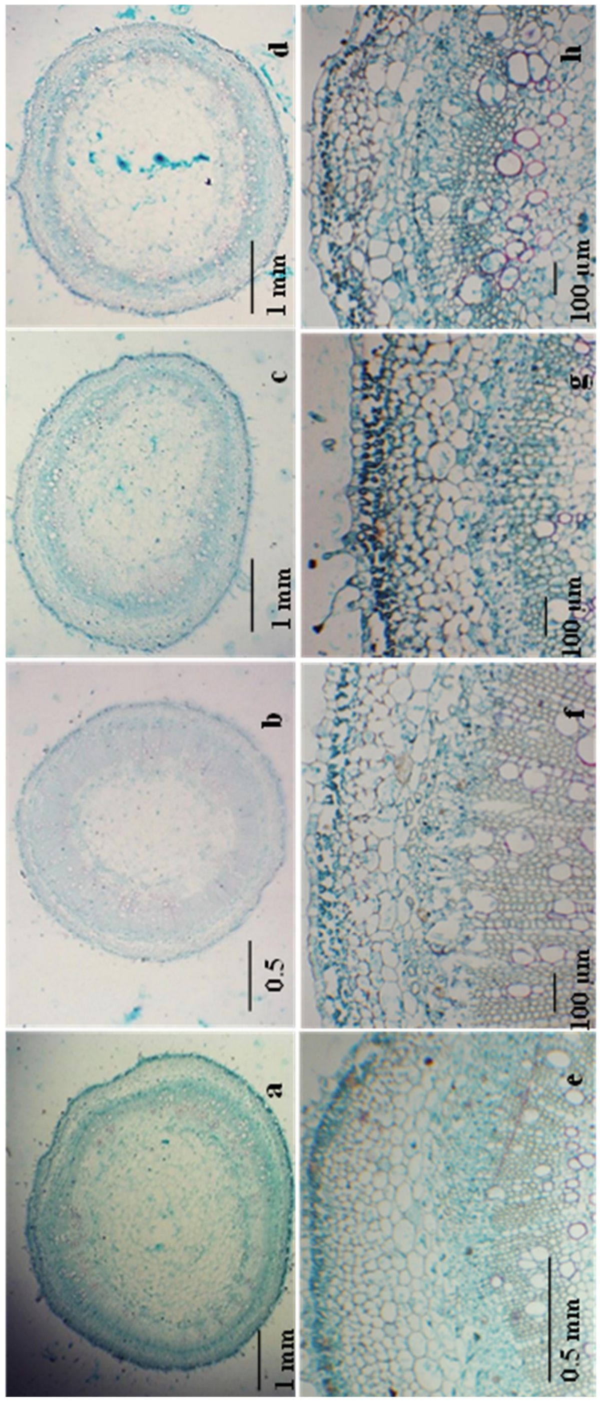

Plate 2. Stem anatomy of tomato: a and e - Control sample; b and f - Lebosol-Calcium; c and g - Lebosol-Magnesium; d and h - Ca and Mg mixture

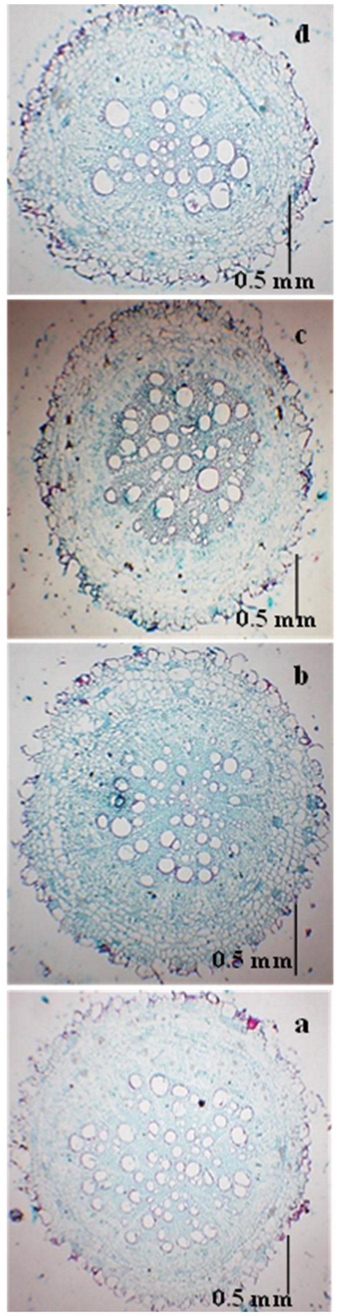

Plate 3. Root anatomy of tomato: (a) Control sample, (b) Lebosol-Calcium, (c) Lebosol-Magnesium and (d) Ca & Mg mixture

The general anatomical features of the leaf, stem, and roots of tomato, described above, were qualitatively affected by the treatments; however, the effect of tested treatment on the tomato plant showed quantitative differences. The highest thickness of lamina, number of vessel elements, total phloem area, chlorenchyma layer, the total area of vessel elements, xylem ratio, increased palisade layer thickness, and vessel diameter were observed under Lebosol-Ca on leaves, stems, and roots internal structure. The Lebosol-Mg showed increased palisade layer thickness, xylem ratio, vessel diameters, and total area of phloem elements. The combined application of Lebosol-Ca and Lebosol-Mg exhibited different effects on internal structures as palisade, and spongy parenchyma was increased to the highest value (Table 3).

Table 3. Quantitative anatomical characteristics of leaves, stems and roots of tomato plants grown under Lebosol-Calcium, Lebosol-Magnesium and their mixture

Characteristics	Treatments			
	Control	(+) Ca	(+) Mg	(+) Ca & Mg
Leaf lamina				
Total thickness (µm)	103.43	105.52*	58.52	83.56
% adaxial epidermis	7.68	10.28	7.86	11.43*
% palisade parenchyma	27.81	29.38	31.24	32.93*
Length of palisade parenchyma cells (µm)	17.97	26.15	14.35	33.54*
Width of palisade parenchyma cells (µm)	4.50	3.85	3.64	6.96*
% spongy parenchyma layer	59.09	54.19	51.27	60.18*
% abaxial epidermis	5.87	4.79	8.41	8.44*
% mesophyll	87.74*	83.22	83.11	79.15
Leaf midrib				
Number of vessel elements	68	140*	60	73
Diameter of vessel elements (µm)	4.6264	7.18*	6.0219	5.98
Total area of vessel elements (µm2)	2306.22	6075.82*	1651.40	2396.27
Total area of phloem elements (µm2)	2995.55	5465.62*	1536.58	1626.18
Stem				
Total thickness of sampled region (µm)	462.54*	313.09	347.52	341.59
% epidermis	4.36	4.85*	3.66	4.31
% chlorenchyma	6.36	7.97*	6.75	7.90
% collenchymas	8.68	14.78	21.14	24*
% cortical ground parenchyma	7.82	13.32*	5.97	6.85
% medullar ground parenchyma	57.24	55.46	69.11*	68.53
Total area of phloem elements (µm2)	23880	30851*	13456.51	4808.67
% phloem	7.47	17.46*	6.64	8.76
Total area of vessel elements (µm2)	39142.78	76358*	17534.49	13686
% xylem	14.43	41.85*	15.70	13.10
Diameter of vessel elements (µm)	13.51	23.93	21.96	26.38*
Root				
Total thickness of sampled region (µm)	566.95	588.98	529.2954	413.00
% periderm	33.64	43.81*	34.32	35.12
% phloem	7.88	9.44*	7.64	8.93
% xylem	46.9	55.99*	54.49	48.55
Diameter of vessel elements (µm)	18.61	19.82	21.43*	20.73
Total area of vessel elements (µm2)	57384.37	86051.42*	64480.75	33199.51
Total area of phloem elements (µm2)	3531.311	15325.58	6143.35*	2507.18

Discussion

Tomato is an essential and commercially used product that can be used for sauces, juices, salads, and other culinary purposes (Souri and Bakhtiarizade, 2019). It is well known that plant physiological immunity and productivity can be enhanced under various stress conditions and environmental challenges through biotic and abiotic inducers (Attia *et al.*, 2020; Attia *et al.*, 2021). Therapeutics in plants offer a novel solution to the problem of improving agricultural system sustainability while reducing the usage of undesirable chemical fertilizers (Povero *et al.*, 2016; Di Stasio *et al.*, 2018; Kopittke *et al.*, 2019). Plants face a variety of environmental stresses due to which growth, quality, and yield are reduced, which adversely affects the food chain and results in a shortage of products(Fahad *et al.*, 2019; Salehi *et al.*, 2019). Nevertheless, nutrient use efficiency plays a significant role in different growth phases of plants and agricultural productivity (Kopittke *et al.*, 2019). The main objective of using fertilizers in our cropping system is to achieve higher growth, yield, and quality (Langholtz *et al.*, 2021). The pH is an environmental factor that causes a reduction in growth and yield through fluctuations in ionic balance, which can be treated through newly commercialized products like Lebosol-Ca and Mg (Shi and Sheng, 2005). Lebosol is used as a secondary mineral in the form of Ca and Mg which maintains the electrolyte balance within plant's tissues and is generally recommended as amendments for growth, yield, quality and other physiological processes in plants (Yang *et al.*, 2007).

The judicious use of inorganic fertilizers in sustainable agriculture is a requirement and an essential part of Egypt's agricultural development. Because of recent advances in fertilizer technology and product innovation, farmers may now purchase high-quality products with controlled nutrient delivery for their crops. Agriculture farming can be boosted by modern technology and the use of significant minerals like Lebosol-Ca and Mg to bring sustainability to the cropping system (Lizarazo *et al.*, 2020). Lebosol-Ca and Mg have an important mineral composition that promotes growth, quality, and yield properties. Growth, yield, and quality of the fruits like a tomato can be encouraged through minerals like Lebosol-Ca and Mg. The Ca and Mg deficiency in plants caused alterations in growth, physiological, biochemical, and yield attributes, reducing fruit productivity (Petek *et al.*, 2019). Therefore, the current research was planned to demonstrate the efficacy of Lebosol-Ca and Mg in tomato plants by studying growth, physiological, biochemical, reactive oxygen species, yield, and quality traits.

The first standard to govern the occurrence of tolerance in tomato plants, foliar application with Lebosol solutions was the enhancement of growth parameters. In the current study, Lebosol-Ca and Mg posed a significant increase in the growth parameters of tomatoes. Growth parameters (shoot fresh and dry weight, root fresh and dry weight, root length, shoot length, number of leaves, and plant height) showed remarkable increment through exogenous application of Lebosol-Ca and Mg and these results matched with (Ilyas *et al.*, 2016; Nguyen *et al.*, 2017; She *et al.*, 2018) they reported that the plant height of tomato plants improved with the application of calcium and Magnesium.

Calcium is necessary for plant growth and development in both non-stressed and stressed conditions. As a result, it serves a dual purpose as an important factor in cell wall and membrane stability and as a second messenger in many developmental and physiological processes (Thor, 2019; White and Broadley, 2003). Moreover, Magnesium plays a vital role in the growth and improvement of new cells, and thus with the application of Magnesium, more change occurs (Li *et al.*, 2018; Ilyas *et al.*, 2021). These results are close to that of (Ilyas *et al.*, 2016), who stated that plant height increased with the foliar application of Magnesium.

Photosynthetic pigments were a vital positive sign due to the use of the Lebosol-Ca and Mg solutions and became a visible part of the indication of necessary treatments. Lebosol-Ca and Mg showed a significant increase in chlorophyll *a, b,* and carotenoid contents, and these results follow previous research (Nguyen *et al.*, 2017). Magnesium is an essential mineral involved in different functions like photosynthesis, generation and utilization of ATP, plant transpiration, and activation of several enzymes necessary for chlorophyll biosynthesis (Cakmak 2013; Fahad *et al.*, 2015b). Nonetheless, Ca promotes the activation of proteins channels through the root zone, due to which the availability of nutrients becomes sufficient and thus provides more surface area for root penetration (Fellet *et al.*, 2021). It was stated in (Kopittke *et al.*, 2020) that the application of calcium enhanced photosynthetic rates of tomato plants throughout enhancement photosynthetic pigments. This augmentation might be attributed to improved stomatal conductance, transpiration rate and/or cell size and number (Awan *et al.*, 2019).

Magnesium is an essential component of several biological processes in leaves, including CO_2 fixation in photosynthesis, photophosphorylation, protein and chlorophyll synthesis, phloem loading, and assimilate translocation (Cakmak and Yazici, 2010; Wang *et al.*, 2020). Photosynthetic assimilates from leaves are transported to sink organs (such as roots, shoot tips, and seeds) and stored as starch or converted to hexoses to increase crop yield under sufficient Mg status (Lemoine *et al.*, 2013). Invertase and sucrose synthase enzymes transport sucrose from source to sink tissues via the phloem (Welham *et al.*, 2009; Wamg *et al.*, 2020).

Oxidative stress caused by calcium and magnesium deficiency led to severe disruption to plant cells and increased the contents of MDA and H_2O_2 in the leaves of tomato plants. These findings are in harmony with (Li *et al.*, 2018; Sperdouli *et al.*, 2022). The MDA and H_2O_2 contents showed significant alteration in response to Lebosol-Ca and Mg as their activity was found to be increased. These results conform with earlier research (Sakhonwasee and Phingkasan, 2017). In conjunction with tending to stimulate development, Lebosol-Ca and Mg have many roles in plant life that are valuable to the biochemical characteristics of plants.

One of the most abundant groups of organic compounds in the plant kingdom is the carbohydrates (Zhao *et al.*, 2019). Biochemical aspects showed a significant trend during exogenous application of Lebosol-Ca and Mg and other biochemical contents (total soluble sugar, total proline and total soluble proteins) as these gets enhanced, which considerably promoted vital physiological processes in the plant. Calcium caused an enhancement in the contents of soluble sugars soluble proteins throughout its role in increasing the expression of enzymes involved in glycolysis (He *et al.*, 2012). The proline synthesis directly promotes the activation of proteins, due to which it is involved in various defense mechanisms during stress conditions, and these results had similarities with previous research (Fahad, 2015a; Akladious and Mohamed, 2018). Calcium has a significant role in the synthesis of the cell wall. It is an essential component of cell wall and plays a crucial role in synthesizing various chemical compounds necessary for plants during hazardous conditions, which in turn directly cause a remarkable increase in yield production (Lin *et al.*, 2016).

ROS scavenging in plants occurs in two ways, enzymatically and non-enzymatically, to prevent plant cells from oxidative damage. Non-enzymatic pathways include phenolic compounds and ascorbic acid, overcoming ROS production (Fahad *et al.*, 2019). The non-enzymatic antioxidants have several escaping mechanisms, and Lebosol-Ca and Mg can improve it. Lebosol-Ca and Mg had posed significant increments in leaf ascorbic acid and total phenolic. Leaf ascorbic acid and total phenolic activity are directly involved in plants' physiological activities, which results in an association for obtaining higher yield in plants (El Sabagh *et al.*, 2019). Our study is in accordance with the results on lettuce plants to check out the exogenous application of Ca and Mg (Galieni *et al.*, 2015). Lebosol-Ca and Mg have positive interaction with plants defense mechanisms due to which plants have adaptive features against various environmental stresses. Lebosol-Ca and Mg caused a significant trend in antioxidant properties in tomato plants under normal conditions. The increases as mentioned above in ascorbic acid and total phenol contents correlate with the reduction in MDA and H_2O_2. The accumulation of phenolic compounds and ascorbic acid is an adaptive strategy for biotic and abiotic stress (Arif *et al.*, 2019; Alharby *et al.*, 2020). The Ca and Mg directly activated the antioxidant mechanism in plants which involves ATP synthesis and defense mechanisms. The plants show different means to cope with salinity pressure as they increase the activity of certain antioxidant enzymes to keep ROS at the lower level in the cell (Ejaz *et al.*, 2020; Shah *et al.*, 2021). The antioxidants (SOD, POD, CAT, and PPO) significantly increased under Lebosol-Ca and Mg treatment. The enzymatic antioxidant activity increased due to Lebosol-Ca and Mg. These results for antioxidant activities were similar to previous research conducted on a tomato plant to determine Ca and Mg use efficiency through foliar applications (Attia *et al.*, 2021).

The yield and quality of the fruits are more critical for remarkable trading in the market. Lebosol-Ca and Mg induce a significant trend in the quality and yield data of the fruit. The output of the tomato was increased due to Lebosol-Ca and Mg treatment which resulted in improved fruit quality. The results are similar to previous studies, which were analyzed in the tomato plant to check the Ca and Mg influence, resulting in a significant increase in yield and quality of the fruit through foliar application of Ca and Mg (Hernández-Pérez *et al.*, 2020).

Calcium is not only a nutrient in and of itself; as a second messenger, it is also involved in signaling nutrient availability and changes. This has been reported for potassium, nitrate, iron, ammonium, and boron (Kudla *et al.*, 2018). Plant roots exhibit Calcium signals in response to minerals deficiency, especially K^+. The transport proteins responsible for the uptake of K^+ in *Arabidopsis* plants are both regulated via the same Ca^{2+}

decoding complex consisting of CBL1/9 and CIPK23 (Ragel *et al.*, 2015; Behera *et al.*, 2017). CIPK23 also controls the activity of the transceptor IRT1 (iron-regulated transporter 1), which transports not only iron but also other minerals as zinc, manganese, cobalt, and cadmium. Moreover, these minerals could enhance yield and quality of tomato fruits throughout activating enzymes that produce secondary metabolites (callose, glucosinolates, lignin, phenols, and phytoalexins) (Cabot *et al.*, 2019; Vadlamudi *et al.*, 2020).

The most evident anatomical responses to various treatments were seen in leaves, palisade, spongy, chlorenchyma, and vascular tissues. In the current study, Ca and Mg-availability produced leaves with positive changes in the proportion of chlorophyllous tissues where the percentage of palisade and spongy parenchyma were increased, which is responsible for the photosynthetic process. Ca –availability in leaf results in the highest total thickness of lamina, number of vessel elements, total phloem area, stem, chlorenchyma layer, a total area of vessel elements, and xylem ratio. In the stem, the chlorenchyma tissue increased by 2% in plants treated with lebosol-Ca, but what is more clearly evident is the increase of the total area of the vessel elements by 51.26%. The same results were explained by Algan (1992) and Martinez *et al.* (2020).

Nutrients have a vital role in plants' life, and their deficiency or scarcity cause a significant change in anatomical characteristics such as cell wall thickness, photosynthetic pigments, mesophyll cells, and epidermal tissue instead it can affect all aspects within anatomical features in plants (Chen *et al.*, 2018). Minerals are the most significant source of nutritional value for plants. These minerals like Ca and Mg, through the modern technology of Lebosol can be used as fertilizers for different functioning in plants which involves physiological, morphological, and anatomical aspects within plants. These minerals exert a role in pigmentation, cell formation, differentiation, and division in plants (Hao *et al.*, 2004). Mineral fertilization which incorporates Ca and Mg in plants poses therapeutic effects during hazardous environment stress and normal conditions (Cole *et al.*, 2016). Ca and Mg through Lebosol application were recommended fertilizer and therapeutic agent when used in optimum amount Martinez *et al.* (2020). Crop growth and yield can be improved through mineral fertilization and therapeutic agent for cell defaults as a treatment for pathogenic harms and different abuses during physiological processes in plants (Lu *et al.*, 2021).

Also, Mg-availability in leaf increases palisade layer thickness and stem xylem ratio, vessel diameters, and the total area of phloem elements. The chlorenchyma with larger and vacuolated cells and smaller intercellular spaces had more chloroplasts per unit area. As noticed in our study, plants treated with Lebosol-Ca showed the highest total thickness in the lamina, the number of vessel elements, total phloem area, chlorenchyma layer, total area of vessel elements, xylem ratio, also increasing palisade layer thickness and vessel diameter. The consistency in a lamina, number of vessel elements, total phloem area, chlorenchyma layer are the areas with high concentrations of Ca, which has essential structural functions; therefore, any change in the supply of this element can affect the formation of these structures (Hao *et al.*, 2004). The main evident sign of Ca or Mg deficiency in tomato stems is the noticeable reduction in the cell wall thickness of support cells which is located outside the phloem (Martinez *et al.*, 2020).

Conclusions

In conclusion, Lebosol-Ca and Mg caused a significant increase in all aspects of a tomato plant. Lebosol-Ca and Mg resulted in better growth, chlorophyll contents, phenolic compounds, antioxidant activity, yield, and quality of the tomato plant. Lebosol-Ca and Mg application through exogenous mode was an adaptive mechanism for the yield and quality of fruit. Our product can get maximum values in the market for trading. The exogenous application of Lebosol-Ca and Mg was the best method for obtaining higher yields and better fruit quality.

Authors' Contributions

Conceptualization, M.S.A., M.H.S. and A.M.S.; Methodology, M.S.A, M.H.S. and A.M.S.; Software, M.A.F, M.S.A. and M.M.A; Resources, A.A.A, M.S.A. and S.M.E; Formal analysis and investigation M.S.A., M.H.S, H.A.S.A and A.M.S; Writing - original draft preparation M.S.A., M.H.S. and A.M.S; Writing - review and editing M.S.A., M.H.S. and A.M.S.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors are grateful to Eng. Mahmud M. Elsayed, for his help during the study. The authors are also grateful to Al-SALAM International for Development & Agriculture Investment, Egypt for the financial and technical support offered during this work. The authors appreciate the scientific efforts of Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia, and motivates researchers to prove their claims. Also, the authors like to give special thanks to Dr. Mahumed Samy Osman (Faculty of Science (Boy), Al-Azhar University, Egypt), for his great help in revising the discussion part.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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