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Article

# Melatonin Enhanced Drought Stress Tolerance and Productivity of *Pelargonium graveolens* L. (Herit) by Regulating Physiological and Biochemical Responses

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**Abstract:** As abiotic stress, drought limits plant growth and minimizes productivity. The increased request for valuable essential oil extracted from geranium (*Pelargonium graveolens* L.) is mainly associated with plant growth, which is adversely affected by drought. Melatonin (MT) has been used to enhance plant growth under abiotic stress, however, its impact to overcome drought stress of aromatic plants including geranium is poorly investigated. In the current investigation, MT application at 100  $\mu$ M was applied under 100 % (well-watered) or 50 % (drought stress) of FC to verify this role. Drought stress markedly reduced growth parameters, herb yield, and total chlorophyll; however, MT alleviated these effects. In contrast, drought enhanced the essential oil percentage in geranium leaves. Despite the reduction in oil yield caused by drought, MT application mitigated this reduction and improved both oil yield and oil components. Besides, MT treatment enhanced the accumulation of total phenols, glutathione, and proline and improved the activity of ascorbate peroxidase, catalase, and glutathione reductase with possible alleviation of drought-induced oxidative damage. Therefore, it reduced both H<sub>2</sub>O<sub>2</sub> and malondialdehyde accumulation, and finally maintained membrane integrity. Overall, this is the first report that reveals that MT application can improve geranium resistance to drought by enhancing the antioxidant potential and protecting the cell membrane from oxidative damage.

**Keywords:** essential oil; enzymatic antioxidants; drought; glutathione; lipid peroxidation; proline

## 1. Introduction

The limited water resources are caused by global warming that eventually affected the agriculture sector via drought [1]. Drought, as abiotic stress, adversely affects the growth and development of various species and therefore declines their productivity [2,3]. Drought stress greatly impacts the morphological, physiological, and biochemical processes of horticultural plants. Reduced root and shoot growth, impaired membrane integrity, imbalanced mineral accumulation, and damaged photosynthetic apparatus are observed as the most deleterious effects that face crop plants under drought stress [4,5]. Drought stress also triggers oxidative stress due to the overproduction of reactive oxygen species (ROS) which attack cellular membrane lipids, proteins, and nucleic acids [6,7]. Plants evolve various mechanisms to cope with the adverse effects of drought stress. Among these mechanisms, the accumulation of compatible solutes reduces the cellular osmotic potential to retain continuous water absorption and keep the cell turgidity [8]. In addition, plants combat drought stress-induced oxidative damage by enhancing the enzymatic and non-enzymatic antioxidants, which scavenge ROS and attenuate their hazardous effects [1,9,10].

Plants' responses to drought stress to combat its adversities seem to be not sufficient to completely mitigate them. Therefore, different approaches are necessary to alleviate these harsh effects, and exogenous applications of various natural and unnatural compounds supplied in

different protocols are adopted to improve drought stress tolerance [7]. In medicinal and aromatic plants, different treatments have been applied to enhance drought stress tolerance such as ketoconazole [11], calcium chloride [12], amino acids [13], mycorrhizal inoculation [14], silicon [3], polyamines [7] and chitosan [4]. Interestingly, the synthesis of secondary metabolites by aromatic plants is considered as functional stabilization of such plants to cope with abiotic stress conditions via a signaling pathway [15]. In support, the accumulation of secondary metabolites has been markedly increased in several aromatic plants including black cumin [16], *Salvia* species [17], Lamiaceae [18], and *Citrus* [19] in response to stress conditions. However, it is essential to use effective eco-friendly applications for the sustainable cultivation of medicinal and aromatic plants to enhance tolerance to drought stress [2]. Among them, exogenous treatment of bio-stimulators or phytohormones is considered a useful and powerful method to improve crop adaptability and protection against stressful conditions [4,20,21]. One of the bio-stimulators that has been shown to play crucial roles in plant growth, development, and response to abiotic stresses is Melatonin (MT); it is a multifunctional hormone [22].

MT, a tryptophan derivative, is an important indoleamine plant hormone that is commonly produced in several species [23]. MT is a potential bio-stimulator phytohormone that helps to regulate several functions in horticultural crops, especially under abiotic stresses mainly via improvements in osmolyte production and membrane stability [24]. MT plays an important role in numerous biological processes including germination, growth regulation, flowering, photosynthesis, senescence, postharvest quality, and abiotic stress tolerance [25–27]. MT is also an antioxidant and a powerful free radical scavenger, boosting the plant's performance against oxidative injury [28]. Therefore, MT ameliorates the abiotic stress by enhancing the antioxidant machinery (enzymatic and non-enzymatic) that directly eliminates ROS [29,30]. As such, MT has been demonstrated to ameliorate the adverse effects of abiotic stress in several horticultural species such as cucumber [31], apple [32], Moldavian balm [33], rapeseed [34], tomato [35], and *Citrus* [19]. Noticeably, the available information concerning MT functions in enhancing drought stress resistance is mainly from field crops or other horticultural plants, but its role in promoting drought stress tolerance in aromatic plants is rarely investigated. For example, MT treatment ameliorated drought's adverse impact on lemon verbena by protecting photosynthetic pigments, accumulating proline, and soluble sugars, increasing total phenolic compounds, and preventing oxidative damage, resulting in enhanced growth and essential oil content [36].

Geranium (*Pelargonium graveolens* L. Herit), belonging to the Geraniaceae family, is an imperative perennial aromatic plant that is usually cultivated for essential oil production [37]. The valuable geranium essential oil is mainly used in cosmetics, perfumery, and flavor industries [38]. Due to various oxygenated monoterpenes that are detected in geranium essential oil such as linalool, geraniol, citronellol, and geranyl formate [39,40], it has high antioxidant activity, antibacterial, and antifungal properties. Therefore, it is generally used in the treatment of hemorrhoids, inflammation, heavy menstrual flows, dysentery, and even cancer [41,42]. Despite the production of geranium by several countries, the essential oil production is lower than the industry requirement [43]. Despite the high global demand for geranium essential oil and its several uses [44], drought stress is negatively affecting the development of this commercial aromatic plant [14]. Although MT application in agriculture for enhancing plant growth and development has been frequently examined, MT's impact on the accumulation of essential oils in aromatic species such as geranium has been poorly studied. To the best of our knowledge, this is the first work to evaluate the impact of this promising molecule on the geranium essential oil profile under drought stress. The objective of this investigation, therefore, was to study the effect of the exogenous application of MT on drought stress tolerance and productivity of geranium plants and its underlying mechanisms.

## 2. Materials and Methods

### 2.1. Experimental site and plant materials

A pot experiment was conducted at the open field of the Faculty of Agriculture, Shebin Elkom, Menoufia University, Egypt (30°33'24.8" N 31°00'51.3" E) during February - July in both 2021 and 2022 seasons. The average day and night temperatures during the study were 30 and 17 ± 1 °C while day and night RH were 34 and 67 ± 5% with an average photoperiod of 13 h. Geranium (*Pelargonium graveolens* L. (Herit) seedlings (one-year-old) were transplanted on the 1<sup>st</sup> of March into 30 cm pots filled with clay soil that consists of 47.52% clay, 38.25% silt, and 14.23% sand. The chemical features of this soil were: OM, 0.16%, EC, 1.31 dsm<sup>-1</sup>, pH, 8.06, Ca<sup>2+</sup>, 42.17 (meqL<sup>-1</sup>), N<sup>+</sup>, 0.19%, PO<sub>4</sub><sup>-3</sup>, 0.031% and K<sup>+</sup>, 0.041%.

### 2.2. Treatments and experimental design

In this research, 64 homogenous plants were divided into 4 equal groups. The first group was subjected to field capacity (FC) at 100% (control), while the second one was subjected to FC at 100% and foliar sprayed with MT at 100 µM. The third group was exposed to FC at 50% (stressed plants) and the last group was subjected to FC at 50% and foliar sprayed with MT at 100 µM. Both groups that were not treated with MT were distilled water sprayed at the same time of MT application. Each treatment had three replicates, and each replicate consisted of 4 pots (12 plants per treatment). A complete randomized design (CRD) was applied to arrange the treatments. MT was attained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was first used for MT dissolving and then the concentration of 100 µM was prepared. Tween-20 at 0.1% (v/v) was applied as a surfactant for MT foliar application. Geranium seedlings were simultaneously exposed to drought stress and MT application one month after transplanting. MT application was applied using a manual pump two times weekly for 3 months. The weighting procedure was used to control the drought stress treatment [45]. For soil dry weight determination, four kg of soil were put in the electric oven at 103°C for 48 h. The pots were then filled with this oven-dried soil. Next, the pots were watered completely for soil saturation. The FC percentage was calculated by the following equation:

$$FC (\%) = [(Wet\ soil\ weight - Dry\ soil\ weight) / Dry\ soil\ weight] \times 100$$

The water amount stored in the FC condition was estimated after deducting the soil dry weight and the pot. Consequently, the 100% FC for control and 50% FC for drought stress were determined.

### 2.3. Growth and herb yield determination

After 12 weeks from the treatments, the growth and herb yield characters were evaluated by measuring plant height (cm), main branch number/plant, and herb fresh weight (g). The samples for the subsequent analysis were also collected since a part of the samples was rapidly frozen in liquid nitrogen and kept at -80 °C, while other samples were oven-dried at 60 °C for further investigations.

### 2.4. Assessment of total chlorophyll content

To extract chlorophyll from geranium leaf samples (0.2 g), acetone (80%) solvent was used. The extract was then centrifuged for 10 min at 15,000 g and evaluated by a spectrophotometer (ST150SA Model 7205, Cole-Parmer Ltd. Stone, Staffs, UK) at 663 and 645 nm. Chlorophyll a and b were measured by the equations of Metzner et al. [46] and expressed as mg g<sup>-1</sup> FW. The total chlorophyll was calculated by combining both values.

### 2.5. Essential oil determination

Fresh weight samples (50 g) of the geranium herb were extracted after 24 h from cutting and the essential oil percentage was determined using hydro distillation units in a Clevenger apparatus for three hours [47] by the following formula:

Essential oil percentage = (volume of oil in the graduated tube/sample fresh weight) × 100

The collected essential oil was dried using sodium sulfate (anhydrous) and stored in dark conditions at -4°C until GC-MS analysis.

### 2.6. Essential oil composition

GC-MS analysis of essential oil was performed using a Varian GC (CP-3800) and MS (Saturn 2200) equipped with a column (film thickness 0.25 µm and ID VF-5ms 30 X 0.25 mm). To detect the GC-MS, a system of electron ionization with an energy of 70 eV was applied. Helium was used as a carrier gas at a 1 mL per minute flow rate. The sample preparation, injection, and program temperature were performed as reported by Ali et al. [44]. The essential oil components of geranium were identified by linking the components' mass spectrum and retention times with standards and the NIST library of the GC-MS system.

### 2.7. Determination of H<sub>2</sub>O<sub>2</sub>

The method of Patterson et al. [48] was followed to measure H<sub>2</sub>O<sub>2</sub> production in geranium leaf samples. The leaf extract was prepared by homogenizing a sample of 0.5 g in chilled acetone (6 mL of 100 %) and centrifuged at 12,000 × g for 10 min at 4°C. A volume of 1 mL from the extract was then added to NH<sub>4</sub>OH (0.2 mL) and Ti (SO<sub>4</sub>)<sub>2</sub> (0.1 mL of 5 %) and centrifuged at 3,000 × g for 10 min. After that, 4 mL of 2M from H<sub>2</sub>SO<sub>4</sub> was used to dissolve the pellet. Finally, the absorbance was spectrophotometrically (Cole-Parmer Ltd. ST150SA, Model 7205Stone, Staffs, UK) investigated at 412 nm. To calibrate the absorbance, a standard curve was performed using known several H<sub>2</sub>O<sub>2</sub> concentrations, and the production of H<sub>2</sub>O<sub>2</sub> was recorded in µmol g<sup>-1</sup> FW.

### 2.8. Assessment of lipid peroxidation

Malondialdehyde (MDA) content in fresh leaf samples was measured to evaluate lipid peroxidation as described by Hodges et al. [49]. Each 0.2 g sample was homogenized in 2 mL of 0.1 % trichloroacetic acid and centrifuged for 15 min at 14,000 × g. Next, in 3 mL of 0.5% thiobarbituric acid and 5% trichloroacetic acid, 2 mL of the supernatant was mixed in a water bath at 95 °C for 30 min. The reaction was ended thereafter by cooling on ice and mixture centrifugation was then performed for 15 min at 5,000 × g. Finally, the absorbance of the supernatant was assessed at 450, 532, and 600 nm using 1,1,3,3-tetraethoxy propane as a standard. The content of MDA in µmol mL<sup>-1</sup> was calculated as follows:

$$\text{MDA} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

where A refers to the absorbance at the exact wavelength.

### 2.9. Membrane permeability measurement

The membrane permeability was determined by the method of Yan et al. [50]. Fresh leaf samples were weighed into glass beakers with reverse osmosis water. The beakers containing samples were kept for three hours at 30 ± 1 °C, and the solution conductivity was measured. After that, the samples were boiled for two minutes, and the conductivity was evaluated again after cooling at room temperature. The electrolyte leakage (EC, %) was calculated as follows:

$$\text{EC} = (C_1/C_2) \times 100$$

where C1 and C2 are the conductivities recorded before and after sample boiling.

### 2.10. Determination of glutathione (GSH)

The GSH level in geranium leaves was assessed by the spectrophotometric method reported by Anderson [51] with slight modification [52]. A pure GSH sample was used as a standard for the calibration curve following linear regression analysis.

### 2.11. Proline determination

The method of Bates et al. [53] was followed to estimate the proline content in geranium leaves. Frozen samples (0.5 g) of leaf tissue were homogenized in sulfosalicylic acid (10 mL of 3 %) at 4 °C. Next, the Whatman No. 2 filter paper was used to filter the extract. A mixture of acid-ninhydrin and glacial acetic acid (2 mL each) was added to 2 mL of the resultant filtrate and incubated for one hour at 100 °C, and then the mixture was put on ice to stop the reaction. Four mL of toluene was then used to extract the mixture and finally the solution absorbance was monitored at 520 nm. Pure proline was used to perform a calibration curve and the level of proline was measured as  $\mu\text{mol g}^{-1}$  FW.

### 2.12. Determination of total phenol content

Each powdered sample (1 g) was stirred with methanol (50 mL of 80 %) for two days at room temperature. After removing the methanol, the resultant extract was maintained at 4 °C to determine the total phenols by the method of McDonald et al. [54]. A volume of 0.5 mL of diluted extract ( $1:10 \text{ g mL}^{-1}$ ) or Gallic acid (GA, a standard phenolic compound) was added to 5 mL of diluted Folin-Ciocalteu reagent (1:10) and 4 mL of sodium carbonate (1 M). Finally, the absorbance was investigated at 765 nm and total phenols were estimated as mg GAE  $\text{g}^{-1}$  DW.

### 2.13. Determination of Antioxidant enzyme activities

First, homogenizing a 0.5 g leaf sample in 5 mL sodium phosphate buffer (50 mM and pH 7.5) and 1 mM of PMSF (phenylmethylsulfonyl fluoride). Second, centrifugation of the extract was performed at  $12,000 \times g$  for 20 min at 4 °C and the resultant supernatant was used for enzyme assays. Measuring catalase (CAT, EC 1.11.1.6) activity was conducted following the method of Chandlee and Scandalios [55]. Extract samples (0.04 mL each) were homogenized with potassium phosphate (50 mM) buffer (pH 7.0) and 2.6 mL of  $\text{H}_2\text{O}_2$  (15 mM). Then, the obtained  $\text{H}_2\text{O}_2$  decomposition was estimated by measuring the reduction in absorbance at 240 nm, and CAT activity was expressed as U  $\text{mg}^{-1}$  protein, where 1 U equals a decline of 1 mM  $\text{H}_2\text{O}_2$  per one minute.

Ascorbate peroxidase activity (AXP, EC 1.11.1.11) was assayed by the protocol of Nakano and Asada [56]. Fresh leaf samples (0.1 g) were ground in 0.2 mL of the extraction buffer that consists of Triton X-100 (1 %), polyvinylpyrrolidone 1 % [PVP], EDTA (3.0 mM), Na-phosphate (0.1 M, pH 7.0), and the mixture was then centrifuged for 20 min at  $10,000 \times g$ . A buffer reaction consisting of 0.5 mM ascorbate, 0.1 mM EDTA, 0.1 mM  $\text{H}_2\text{O}_2$ , and 0.05 mL enzyme extract, was organized and the reaction was executed for 5 min at 25 °C. The activity of APX was assessed by measuring the absorbance at 290 nm using a Pharmacia, LKB-Novaspec II spectrophotometer. The activity of APX was calculated using the coefficient ( $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ) of absorbance where one APX enzyme unit can decompose 1.0  $\mu\text{mol}$  of ascorbate in one minute.

Glutathione reductase enzyme (GR, EC 1.6.4.2) was determined following the procedure of Foyer and Halliwell [57] and the modification of Rao [58]. Leaf samples (0.5 g) were extracted in 2.0 mL of buffer consisting of 1.0 % Triton X-100, 3.0 mM EDTA (0.1 % PVP), and 1 M Na-phosphate (pH 7). The obtained mixture was then centrifuged for 10 min at  $10,000 \times g$  and the resulting supernatant was investigated at 340 nm for GR activity, following NADPH glutathione-dependent oxidation. The reaction mixture was composed of 0.2 NADPH, 0.05 mL of enzyme extract, and 0.5 mM glutathione disulfide, and was reserved for 5 min at 25 °C. To overcome glutathione disulfide oxidation, the correction was performed without NADPH. Finally, the activity of GR was measured using the absorbance coefficient of  $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$ , since one GR unit can decompose 1.0  $\mu\text{mol}$  per minute of NADPH.

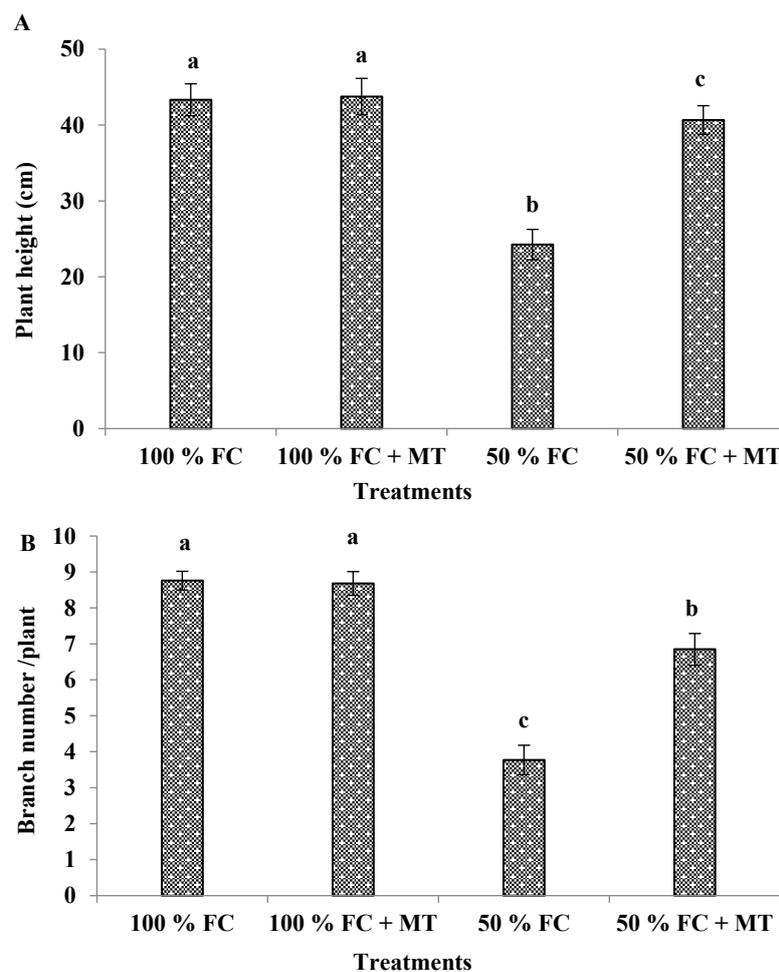
### 2.14. Statistical analysis

A combined analysis was performed on the results of both seasons and the results were pooled. The ANOVA test was performed using the SPSS program (13.3 versions), and the separation of means was conducted using the Duncan multiple range test [59] at  $P \leq 0.05$ .

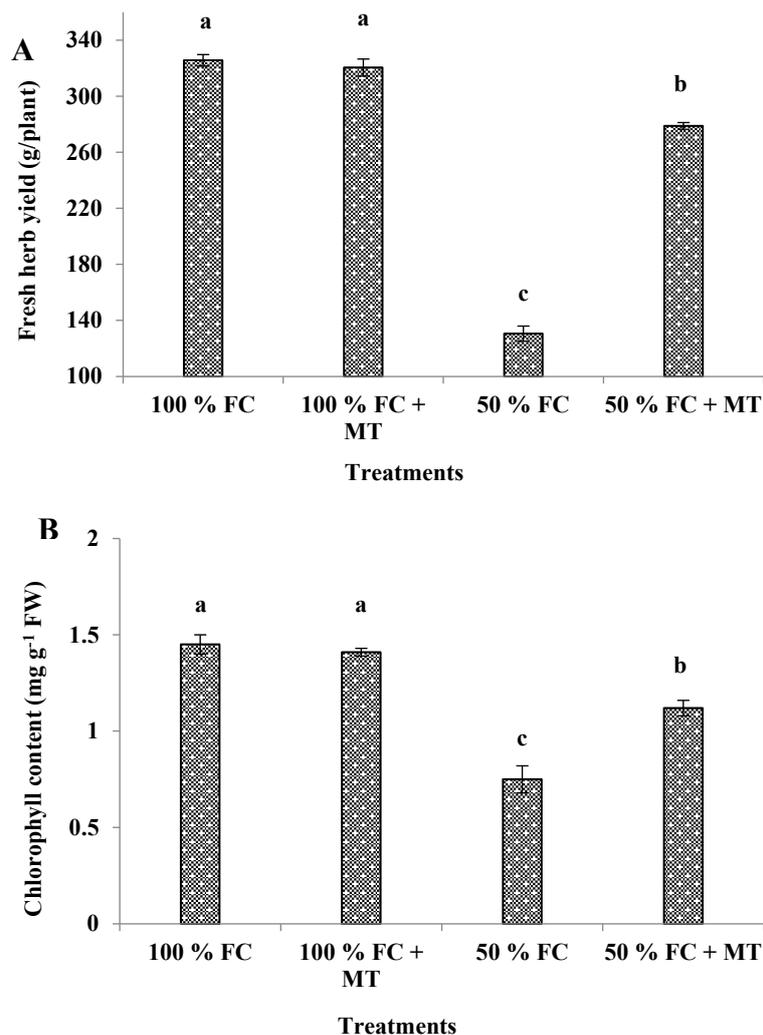
### 3. Results

#### 3.1. Growth characters and herb yield

Drought stress (50% FC) significantly reduced the plant height and branch number of geranium (Figure 1), resulting in the herb yield reduction (Figure 2A) as compared with unstressed plants. Contrarily, MT application markedly increased the growth and yield relative to unstressed plants. Plant height was reduced by 44.01% in comparison with unstressed plants while this reduction was only 6.14% with MT application. Also, the herb yield of stressed plants was reduced by 59.91% while this reduction was only 14.38% when MT was applied relative to unstressed plants (100% FC). Notably, MT treatment had no effect when it was applied to unstressed plants (100% FC).



**Figure 1.** Impact of MT treatment on plant height (A) and branch number (B) of drought-stressed *Pelargonium graveolens* L. Her. Plants were exposed to 50 or 100 FC and foliar sprayed with 100  $\mu$ M MT. Results are means  $\pm$  SE of three replications pooled from two seasons. Different letters represent significant statistical differences between the treatments at  $p \leq 0.05$ .



**Figure 2.** Impact of MT treatment on herb yield (A) and chlorophyll content (B) of drought-stressed *Pelargonium graveolens* L. Her. Plants were exposed to 50 or 100 FC and foliar sprayed with 100  $\mu$ M MT. Results are means  $\pm$  SE of three replications pooled from two seasons. Different letters represent significant statistical differences between the treatments at  $p \leq 0.05$ .

### 3.2. Essential oil content

Essential oil accumulation in geranium leaves has markedly elevated under drought stress (50% FC) compared with the control (100% FC). A slight and insignificant increase in essential oil percentage was noticed due to MT application whether in stressed or non-stressed plants. On a percentage basis, MT enhanced the oil percentage in the stressed plants by 33% compared to well-watered plants (Table 1). In contrast, the essential oil yield was decreased in response to drought stress in comparison with non-stressed plants, whereas MT foliar spray considerably enhanced oil yield in drought-stressed plants by 123% relative to plants that received only drought stress (Table 1).

**Table 1.** Impact of MT treatment on essential oil percentage and oil yield of drought-stressed *Pelargonium graveolens* L. Her.

Treatments	Essential oil (%)	Essential oil yield (mL/plant)
100 % FC	0.18 ± 0.03b	0.59 ± 0.04b
100 % FC + MT	0.19 ± 0.02b	0.61 ± 0.06b
50 % FC	0.23 ± 0.03a	0.30 ± 0.04c
50 % FC + MT	0.24 ± 0.01a	0.67 ± 0.03a

Plants were exposed to 50 or 100% FC and foliar sprayed with 100 µM MT. Results are means ± SE of three replications pooled from two seasons. Different letters represent significant statistical differences between the treatments at  $p \leq 0.05$ .

### 3.3. Essential oil composition

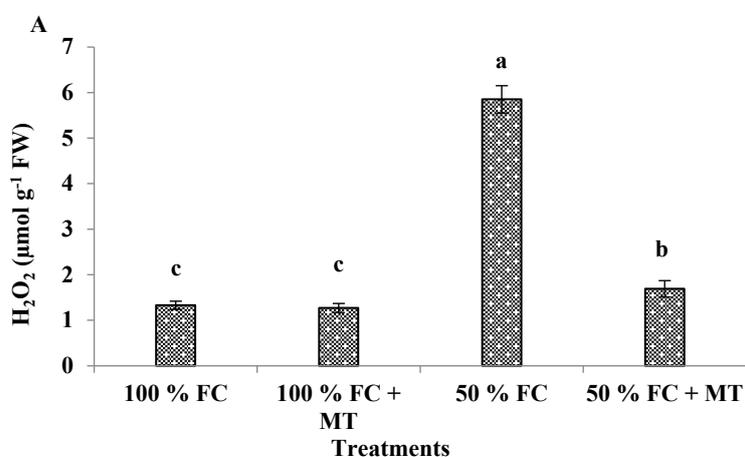
The essential oil composition obtained in the current work indicated that the main oil components were citronellol, geraniol, linalool, citronellyl formate, and geranyl formate. Among them, citronellol showed higher than 26% of the essential oil (Table 2). Generally, drought stress treatment enhanced the essential oil components compared with well-watered plants, which were further elevated by MT foliar spray, more so when applied under stress.

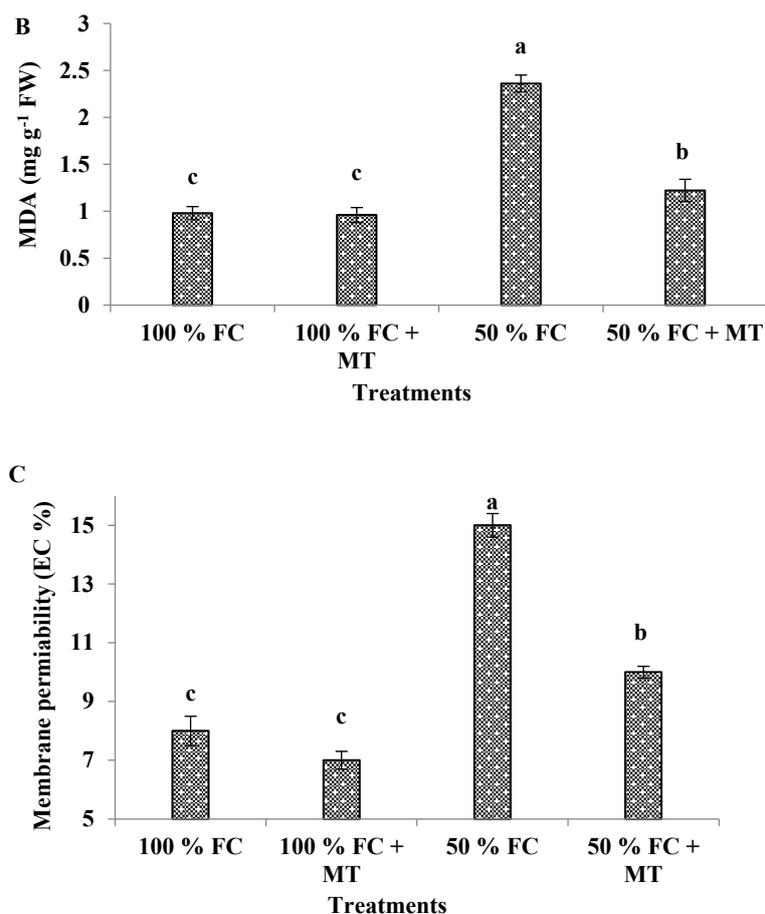
### 3.4. Chlorophyll content

The leaf chlorophyll content was adversely affected by drought stress, whereas MT application significantly improved the chlorophyll content in 50% FC-stressed plants (Figure 2B). The chlorophyll content in plants exposed to 50% FC was reduced by 48.28%, while it was reduced only by 22.76% with MT application. Under 100% FC condition, MT application had no effect on the chlorophyll content (Figure 2B).

### 3.5. H<sub>2</sub>O<sub>2</sub> production

H<sub>2</sub>O<sub>2</sub> content in drought-stressed plants was markedly increased (4.40-fold) compared with well-watered plants (Figure 3A). However, MT foliar spray significantly reduced H<sub>2</sub>O<sub>2</sub> content relative to 50% FC-stressed plants, which recorded 1.27-fold in comparison to plants exposed to 100% FC. Well-watered plants treated with MT resulted in insignificant H<sub>2</sub>O<sub>2</sub> production compared with those that received no MT application (Figure 3A).





**Figure 3.** Impact of MT treatment on H<sub>2</sub>O<sub>2</sub> production (A), malondialdehyde (MDA) content (B), and membrane permeability (C) of drought-stressed *Pelargonium graveolens* L. Her. Plants were exposed to 50 or 100 FC and foliar sprayed with 100 μM MT. Results are means ± SE of three replications pooled from two seasons. Different letters represent significant statistical differences between the treatments at  $p \leq 0.05$ .

### 3.6. MDA content

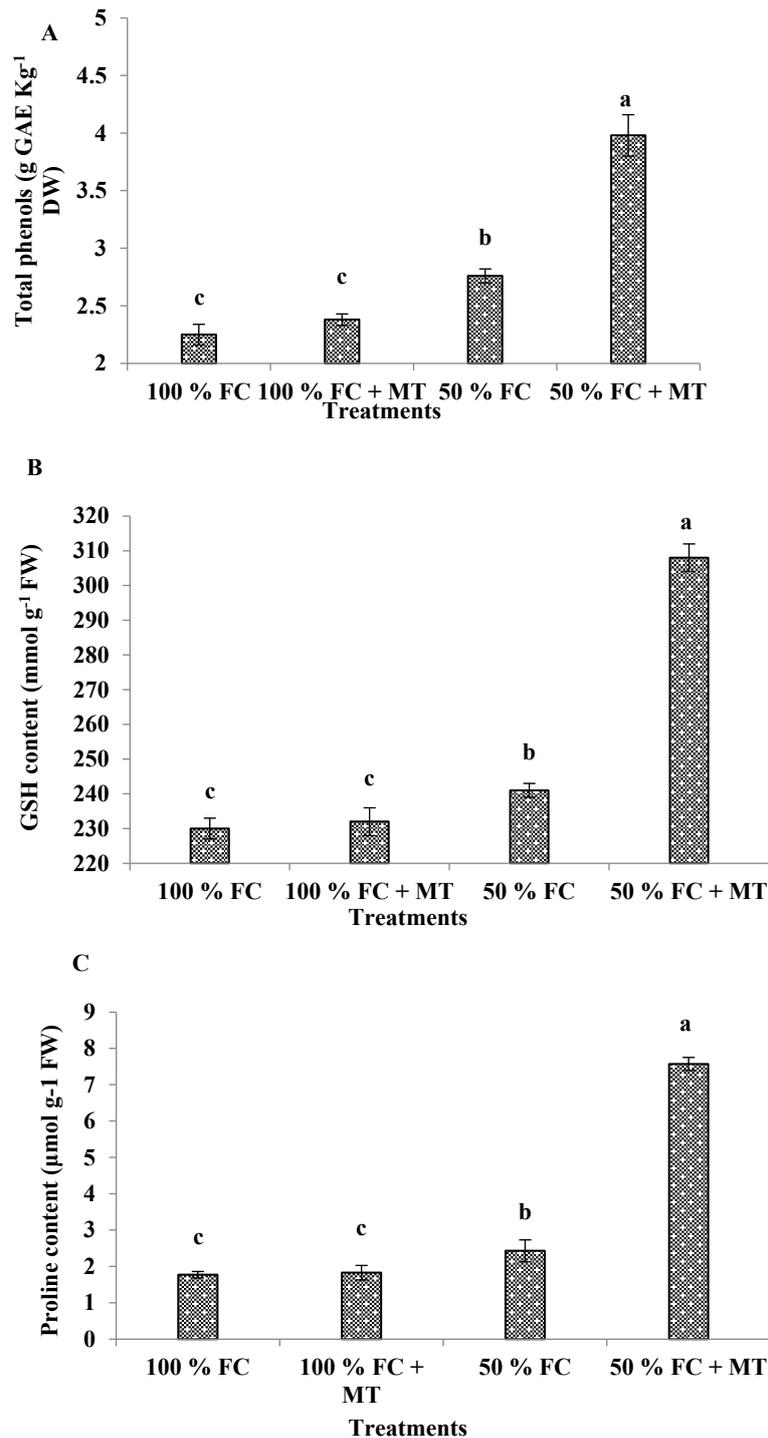
Lipid peroxidation, as detected by MDA accumulation, was considerably elevated in geranium-stressed plants under 50% FC condition, while these plants when foliar sprayed with MT had a significantly lower value of MDA (Figure 3B). In stressed plants that received MT application, MDA content was 1.24-fold while it was 2.41-fold in absence of MT treatment, compared with 100% FC treatment (Figure 3B).

### 3.7. Membrane permeability

Drought-stressed geranium showed a remarkable increase in membrane permeability, while MT-treated plants significantly reduced the permeability of the cell membranes (Figure 3C). Compared with the well-watered plants, the increase in membrane permeability of the stressed plants was 87.5%, but it was only 25% when the stressed plants were sprayed with MT (Figure 3C). MT application to 100% FC condition had no impact on the membrane permeability.

### 3.8. Total phenols

A significant increase in the total phenols was observed in drought-stressed plants in comparison with well-watered ones (Figure 4A). However, geranium-stressed plants that foliar sprayed with MT accumulated significantly higher total phenols compared with those exposed only to 50% FC without MT treatment (Figure 4A).



**Figure 4.** Impact of MT treatment on the contents of total phenols (A), glutathione (B), and proline (C) of drought-stressed *Pelargonium graveolens* L. Her. Plants were exposed to 50 or 100 FC and foliar sprayed with 100  $\mu$ M MT. Results are means  $\pm$  SE of three replications pooled from two seasons. Different letters represent significant statistical differences between the treatments at  $p \leq 0.05$ .

Plants exposed to 50% FC with or without MT application increased total phenols by 76.89% and 22.67%, respectively, compared with well-watered plants. On the other hand, a slight but not significant increase in the total phenols was recorded due to MT application under 100% FC (Figure 4A).

### 3.9. Glutathione (GSH) content

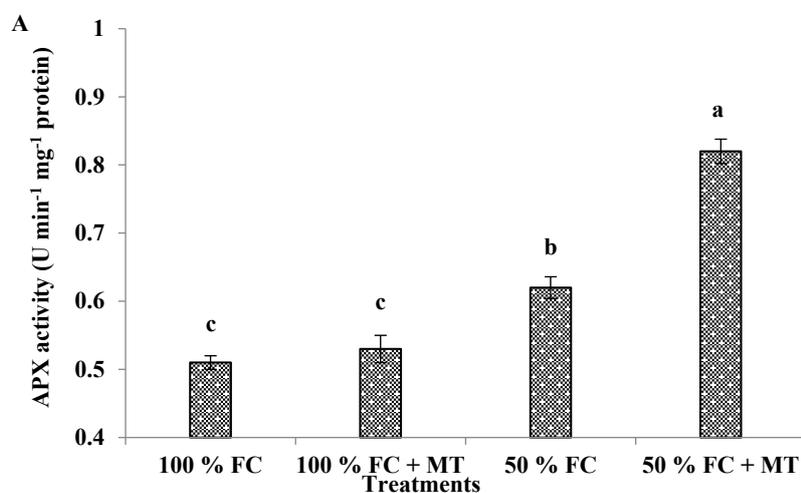
Drought stress considerably elevated the GSH content in geranium relative to well-watered plants (Figure 4B). Plants exposed to 50% FC showed a significant increase in GSH content compared with those received 100% FC; this increase in GSH was further promoted by MT application in drought-stressed geranium. Compared to well-watered plants, GSH content was induced by 4.78 and 33.91% in drought-stressed plants without or with MT application, respectively (Figure 4B).

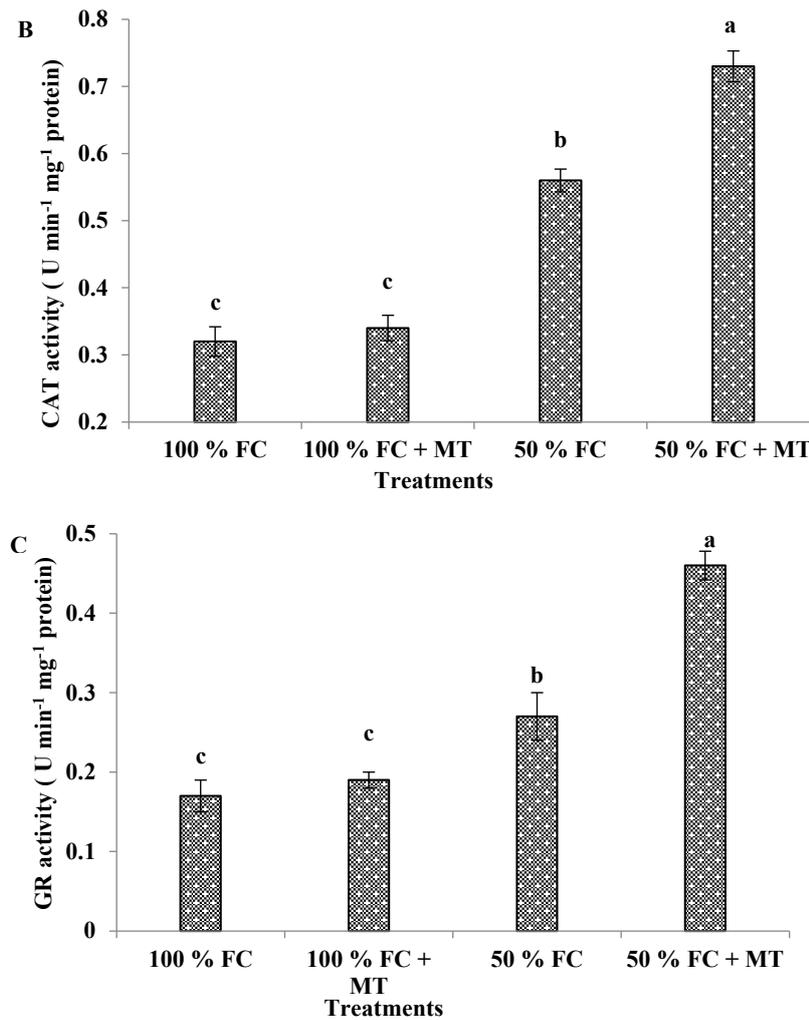
### 3.10. Proline

Geranium plants exposed to 50% FC accumulated significantly higher levels of proline in comparison to unstressed (100% FC) plants (Figure 4 C). Under drought stress, the proline increase was about 1.37-fold higher than that obtained in non-stressed geranium plants. MT application to the stressed plants stimulated proline accumulation by about 4.14-fold compared with 100% FC condition (Figure 4 C). On the contrary, under non-stressful conditions, the MT application did not show any considerable increase in the proline content.

### 3.11. APX, CAT, and GR enzyme activities

The activity of APX, CAT, and GR enzymes was markedly increased under drought stress relative to 100% FC condition, and further increases in the three enzymes' activity were observed with MT application (Figure 5). The stimulation in APX, CAT, and GR activities of drought-stressed geranium was further increased by foliar application of MT. Compared with well-watered plants, the APX, CAT, and GR activities were increased by 61, 128, and 171%, respectively, when plants were exposed to 50% FC and sprayed with MT (Figure 5). On the other hand, well-watered plants showed the lowest activities in the tested enzymes.





**Figure 5.** Impact of MT treatment on ascorbate peroxidase (A), catalase (B), and glutathione reductase (C) enzyme activities of drought-stressed *Pelargonium graveolens* L. Her. Plants were exposed to 50 or 100 FC and foliar sprayed with 100  $\mu$ M MT. Results are means  $\pm$  SE of three replications pooled from two seasons. Different letters represent significant statistical differences between the treatments at  $p \leq 0.05$ .

#### 4. Discussion

Water resources are limited especially in arid and semi-arid areas, which might worsen in the future. Therefore, maximizing plant productivity per water unit is a crucial goal of scientists worldwide. The reduction in geranium growth and yield by drought stress observed in the current work was previously demonstrated in *Ocimum* [62] and lemon verbena [36]; these studies indicated adversely affected growth, leaf expansion, plant height, and biomass production by drought stress. Drought stress-induced reduction in geranium growth and herb yield is most probably attributed to a reduction in relative water content leading to leaf turgor reduction and hence limits plant growth [7] and to a photosynthetic reduction declining the accumulation of dry matter [8]. Also, stomatal closure has been observed under drought stress which limits the diffusion of CO<sub>2</sub> into the carboxylation site and thus reduces the photosynthetic rate and in turn plant growth and yield [61]. Further, the decrease in drought-stressed plant growth may be due to drought-induced damage to chlorophyll synthesis, which leads to photosynthesis activity reduction which is reflected in growth and yield decline [17]. The drought-enhanced oxidative injury damages the cellular proteins, lipids, and pigments of the chloroplast under stressful conditions may also contribute to declined growth and yield [3]. However, Ali et al. [4] reported that the growth reduction in response to drought stress is presumably an adaptive mechanism to cope with the negative impacts of drought.

The decline in the chlorophyll content observed in this research may be interpreted by the drought-stimulated oxidative damage [3] and drought-impaired grana lamella of the chloroplasts [60], which reduces the photosynthesis efficiency and plant growth. In this study, MT application counterbalanced the drought's adverse impacts and enhanced the growth of geranium which was associated with enhanced chlorophyll content; we propose this to be a possible strategy by which MT might augment photosynthesis efficiency and hence plant growth rate. In agreement, earlier reports have confirmed the promotional effect of MT on plant growth under drought stress [33,36,60,63], which was ascribed to MT action as a growth enhancer improving the growth and protecting the plants from abiotic stresses. Two works revealed that this MT protection against drought stress is dose-dependent [64,65]. The impact of MT on preserving the chlorophyll content under drought is consistent with the work of Sadak et al. [63] who illustrated maintained chlorophyll content by MT treatment in moringa under drought stress. MT-preserved chlorophyll content might be due to MT stimulating the synthesis of metabolites involved in chlorophyll biosynthesis and reducing the rate of its decomposition [66].

This investigation is the first to study the effect of MT on the essential oil content in drought-stressed geranium. Golkar et al. [67] indicated that the secondary metabolites are induced as a defense strategy under abiotic stress, which might be the situation in our work. The high essential oil percentage produced under drought stress was also enhanced in other aromatic species in response to abiotic stresses [13,17,68]. Contrarily, drought conditions had a negative impact on the essential oil percentage of *Ocimum basilicum* 'Genovese' and *Ocimum americanum*, whereas no change was observed in *Ocimum x africanum* species [62]. Therefore, we assume that the effect of drought stress on the essential oil percentage seems to be species dependent. Interestingly, a further increase was obtained by MT application in well-watered or stressed geranium plants, more so in stressed ones (50% FC), which is consistent with results in *Salvia* [17], *Citrus* [19], and lemon verbena [36]. The reduction in the essential oil yield, in contrast to the increase in oil percentage observed in stressed plants could be ascribed to a decrease in their herb yield. This response was similarly observed in other species under water shortage [17,33]. We, therefore, propose that the MT-enhanced oil yield reported here may be due to MT-improved geranium growth and biomass production.

GC-MS analysis showed that the main constituents of essential oil were citronellol, geraniol, linalool, citronellyl formate, and geranyl formate; their percentages were increased in drought-stressed plants compared with well-watered plants (Table 2). Our result is consistent with those of Bidabadi et al. [17] who report an increase in the essential oil components of two *Salvia* species under drought stress. MT application significantly improved these components, more so when applied under water deficit, which agrees with the data that exogenous MT enhanced several compounds in volatile oil in *Salvia* [17] and bitter orange [69]. However, the MT's role in the essential oil biosynthesis of aromatic plants is poorly understood. We suggest that this promotional effect may be related to the fact that MT has an auxin-like activity [70], that has been found to promote volatile oil synthesis in several aromatic species [71,72]. It is worth mentioning that the effect of MT in enhancing the essential oil content in drought-stressed geranium reported in this study is novel.

ROS can contribute to cellular signaling only when they exist at lower levels in plant cells [73]. As abiotic stress, drought perturbs cellular oxygen metabolism, leading to ROS overproduction that triggers harmful effects on several cellular organelles [74]. Here, we report that drought exposure to geranium plants induced a considerable  $H_2O_2$  accumulation, which was associated with a concomitant increment in MDA, indicating drought stress-induced oxidative damage resulting in cellular lipid peroxidation. The result is in accordance with previous works in *Salvia* [17], lemon verbena [36], and moringa [63], showing elevated ROS production and lipid peroxidation under water deficit conditions. Lipid peroxidation promoted by drought stress results in a disturbance of membrane integrity probed by impaired membrane permeability [4]. This is also supported by the data that ROS has an adverse impact on membrane lipids and proteins causing their damage [9,21]. Our study revealed that MT foliar spray caused a significant reduction in both  $H_2O_2$  and MDA accumulation, and restored membrane stability (measured by low membrane permeability) of drought-stressed geranium leaves, suggesting MT scavenging of oxidative injury by keeping a steady

state of intracellular ROS levels [75]. The result is consistent with the previous reports in *Salvia* [17] and *Moringa* [63], indicating MT application detoxifies the ROS hazardous impact under drought conditions.

Stressful conditions-triggered oxidative stress in plants is eliminated by evolving oxygen scavenging machinery to decline ROS, which comprises antioxidant enzymes and non-enzymatic antioxidants [20,52,76,77]. As for the antioxidant defense system, geranium-stressed plants generated higher total phenols, GSH, and proline compared to well-watered plants. However, it seems that the motivation of the antioxidant system in stressed geranium was not adequate to cope with the overproduction of H<sub>2</sub>O<sub>2</sub> and MDA, and therefore led to the detected oxidative injury. In contrast, the application of MT enhanced both non-enzymatic and enzymatic machinery in drought-stressed geranium: higher elevation of APX, CAT, and GR activities and total phenols, GSH, and proline contents in treated plants are seemingly pointing to their participation in oxidative stress amelioration which inhibits the adverse oxidative impact of drought stress. In support, it is reported that MT is a potent antioxidant playing a crucial role in mitigating oxidative stress and enhancing plant resilience [36]. Also, polyphenols have been shown to be important non-enzymatic antioxidants in response to stress conditions [19,78]. Proline accumulation under adverse conditions has been reported to have multiple roles that help plants cope with the injurious effects and enhance stress resilience [4,9,79]. In agreement with our results, accumulation of proline was observed in lemon verbena and improved tolerance to drought [36]. Additionally, increasing GSH levels in drought-stressed-geranium in this study is in accordance with the investigation in *Salvia* [17] grown under drought stress. The functions of GSH and phenols as non-enzymatic antioxidants that participate in H<sub>2</sub>O<sub>2</sub> and MDA reductions under MT treatment have been documented [80]. Furthermore, MT has been found to regulate the synthesis and accumulation of GSH and phenolic compounds, indicating a potential interplay among them in mitigating stress-induced damage [52]. MT-enhanced phenols, GSH, and proline accumulation in drought-stressed geranium shown here is first reported.

The enhanced activities of APX, CAT, and GR enzymes observed in the current investigation under drought were further increased with MT application, indicating that MT effectively provided a defense against oxidative injury under drought stress. Consistent with the current results, inducing the antioxidant enzymes under water scarcity has been observed in several aromatic species [3,7,36]. We, therefore, indicate that MT application can enhance the enzymatic antioxidant system, which is able to effectively scavenge ROS, preserve membrane function, and finally induce resistance against water scarcity. Our interpretation is supported by the previously published works on *Salvia* species [17] and *moringa* [63] reporting MT function in minimizing intracellular ROS, reducing their damage, and stimulating tolerance to drought imposition.

## 5. Conclusions

MT foliar spray has significantly alleviated drought-induced oxidative damage via the induction of nonenzymatic and enzymatic antioxidant mechanisms and thus enhanced geranium growth, herb yield, essential oil content, chlorophyll content, reduced H<sub>2</sub>O<sub>2</sub> and MDA accumulation, and eventually drought stress tolerance. This report is the first to reveal the vital role of exogenous MT in enhancing drought stress tolerance in geranium due to the aforementioned responses. These findings, therefore, indicate that MT could be a safe and suitable alternative for other synthetic materials to attenuate the adverse impact of water scarcity and enhance the plant tolerance to drought in geranium and most probably in other aromatic species.

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