



Combined Application of Mannitol and Melatonin Improves Water Retention and Biochemical Parameters of Drought-stressed Tomato Plants

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ABSTRACT

Background: Drought stress causes immense yield losses in plants annually. In recent times, the foliar application of biostimulants has emerged as an effective strategy for enhancing plant stress resilience. Melatonin and mannitol are some of the most important chemical substances that have applied foliar to plants to enhance stress tolerance. However, existing research has mostly focused on the individual application of these substances.

Methods: We examined the impacts of individual and combined melatonin and mannitol on certain physiological and biochemical parameters of tomato plants subjected to drought stress.

Result: Our results showed that plants subjected to combined melatonin and mannitol treatment recorded the highest chlorophyll content under control and moderate drought stress and maintained significantly high ($p \leq 0.05$) amounts of phenol under all the stress levels applied. Detached leaves in the combined treatment from the control and moderate stress groups lost the least amount of water, with the plants recording the lowest wilting rate (27%) under severe drought stress. Combined melatonin and mannitol-treated plants also recorded the highest amount of soluble sugar under moderate and severe drought stress. The highest level of membrane stability was recorded in plants treated with combined mannitol and melatonin under control, moderate and severe drought stress. On the other hand, individual mannitol and melatonin applications proved effective in maintaining high chlorophyll content and ensuring the lowest rate of water loss, respectively, under severe drought stress. Taken together, combined melatonin and mannitol showed potential effectiveness in ameliorating the adverse impacts of drought stress in tomato plants.

Key words: Biostimulants, Drought stress, Mannitol, Melatonin, Water loss.

INTRODUCTION

Abiotic stresses pose a significant challenge to crop production (Laxman and Bhatt, 2017). These stresses represent the most harmful stress factor, severely limiting plant growth and development (Mahajan *et al.*, 2020).

Abiotic stresses induce the accumulation of reactive oxygen species (Kusvuran and Dasgan, 2017), which can cause irreversible damage in plants by hindering cellular redox homeostasis and inducing high oxidative stress levels (Sachdev *et al.*, 2021).

Drought stress is the most destructive of all abiotic stresses, adversely affecting plant growth and development (Khan *et al.*, 2020). The stress is caused by insufficient water availability, leading to a breakdown of metabolic processes in plants (Kumar *et al.*, 2018). Drought stress affects plant growth at the morphological, physiological and molecular level (George *et al.*, 2025).

Tomato (*Solanum lycopersicum*), a member of the Solanaceae family, is among the most widely grown and consumed vegetables globally (Nicola *et al.*, 2009). It is rich in essential nutrients such as vitamins A and C, potassium, folate and antioxidants (Sestito and Palozza, 2019). However, tomato is extremely sensitive to abiotic stresses which can cause up to 70% yield losses depending on stress intensity and duration. Drought stress is the most destructive stress factor in tomato production (Krishna *et al.*, 2022).

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In recent times, the foliar application of biostimulants has emerged as an effective stress mitigation strategy for plants (Mandal *et al.*, 2023). Substances or microorganisms of diverse nutrient characteristics applied to improve plant

nutritional efficiency, resilience to abiotic stress and/or crop quality traits can be classified as biostimulants. Biostimulants come in different forms and perform diverse functions. They may exist as naturally occurring or synthetic substances (du Jardin, 2015).

Melatonin is a key bioactive component in vascular plants (Sheshadri *et al.*, 2018). The potential role of melatonin in plant growth enhancement and regulation has been widely reported (Sun *et al.*, 2021). Melatonin applied exogenously to a variety of crops, mitigated the effects of abiotic stresses in these crops (Colombage *et al.*, 2023). Melatonin also enhanced seed germination and lateral root growth in cucumber plants exposed to drought and cold stress (Zhang *et al.*, 2013).

Mannitol is an osmolyte found in higher plants that aids in the defense against a host of abiotic stresses (Hema *et al.*, 2014). It is produced as a primary photo-synthetic product in certain plant species (Mitoi *et al.*, 2009). Mannitol plays a vital role in plant physiology, serving as a carbon storage mechanism and providing defense against environmental stress (Patel and Williamson, 2016).

Drought stress mitigation methods, such as extensive irrigation and genetic modification, have limitations in cost, sustainability and acceptance. There is a need to find alternative and more efficient means to mitigate the effects of drought stress in plants. The foliar application of mannitol and melatonin has been reported to enhance drought stress tolerance in plants (Zhang *et al.*, 2013; Mahmoud *et al.*, 2024). Although the individual effects of melatonin and mannitol on stress amelioration have been studied in plants (Altaf *et al.*, 2022; Mahmoud *et al.*, 2024), limited studies have investigated the impact of combined melatonin and mannitol on drought tolerance in tomato plants under drought stress. This study aims to examine how mannitol, melatonin and their combination can affect drought-stressed tomato plants, comparing their responses to those treated with water to determine which treatment is most effective at mitigating the effects of drought stress.

MATERIALS AND METHODS

Experimental site and design

The experiments were carried out in the screen house of the Department of Plant and Environmental Biology, University of Ghana from March to October, 2024. Seeds of the tomato variety Roma Savana were used for this experiment. The experiment was set up using the split-plot design. Three main plots were established based on stress severity: Severe drought stress, moderate drought stress and no stress. Each main plot was further divided into 4 subplots/blocks according to the foliar treatments applied: Mannitol, melatonin, mannitol + melatonin and a control.

Watering regime and treatment application

Watering was carried out once every five days in plants under severe drought stress and once every three days for those under moderate drought stress. Plants in the control

group were watered every other day. For foliar treatment, 2% mannitol and 0.1 mM melatonin were used. The prepared solutions of mannitol, melatonin and their combination were carefully and uniformly applied to the leaves of tomato seedlings once a week. Plants in the control group received a similar foliar spray with distilled water.

Chlorophyll content

Total chlorophyll content was analysed according to the methodology by Arnon (1949). Filtration was carried out following the grinding of leaf samples from various treatments with 10 mL of acetone. Afterwards, the absorbance of the filtrate was determined at wavelengths of 645 nm and 663 nm with a UV-visible spectrophotometer. Total chlorophyll content was calculated with the formula:

Total chlorophyll (mg/g) =

$$20.2(A_{645}) + 8.02(A_{663}) \times (V/1000 \times W)$$

Rate of water loss from leaves

The rate of water loss in leaf samples was determined following the method outlined in Wang *et al.*, (2011). Leaves were dehydrated at room temperature for six (6) hours. Three replicates of fresh weight measurements (LWt) were made at 60-minute intervals. Following exposure, the relative water loss (RWL) in leaves was calculated as follows:

$$\frac{FW-LWt}{FW} \times 100$$

Where,

FW = Fresh leaf weight before desiccation.

LWt = Leaf weight t hours after desiccation.

Rate of plant wilting

Wilting rate was determined in tomato plants after 7 weeks of exposure to stress treatment by counting the total number of wilted leaves in each treatment group and expressing it as a percentage of the total number of leaves.

Phenol content

Phenol content was analysed in triplicate following the method outlined by Blainski *et al.* (2013). 0.7 g of fresh leaves was ground in 10 mL of methanol and left overnight. The homogenate was filtered and diluted with 100 ml of water. A test tube was filled with approximately 200 µL of the diluted solution, 1.4 mL of distilled water and 0.1 mL of 50% Folin-Ciocalteu phenol reagent. After three minutes, 23% (w/v) of sodium carbonate was added to the mixture. The resulting mixture was gently vortexed after two hours and the absorbance was observed at 765 nm. Gallic acid was used as a standard to determine total phenolic content.

Determination of membrane stability index

Membrane stability index was determined in triplicate following the method reported by Sairam, (1994). Following this method, 100 mg of leaves were put into well-labeled boiling tubes containing 20 mL of double-distilled water.

After 30 minutes incubation at 40°C, the initial electrical conductivity (C1) was taken with an electrical conductivity meter. The samples were then heated to 100°C, cooled and the final electrical conductivity (C2) re-recorded. The membrane stability index (MSI) was then calculated for each treatment as follows:

$$\text{MSI} = 1 - \frac{C1}{C2} \times 100$$

Soluble sugar estimation

The amount of sugar in each sample was estimated in triplicate with the protocol outlined by Bryant and Overell, (1951) based on a phenol-sulphuric acid method. 50 mg of leaf sample from each treatment group was put into a boiling tube and 20 mL of 80% ethanol was added. After 60 minutes of heating on a hot plate at 50°C, the resulting extract was cooled and transferred with deionized water into a 50 mL flask. This was followed by the addition of 2 mL of 18% phenol and 5 mL of 3% conc H₂SO₄. Finally, the flask was made up to the mark with deionized water. Absorbance readings were taken at 490 nm and the sugar content was determined using a standard curve.

Data analysis

Analysis of variance (ANOVA) was performed on the collected data with the Minitab software (Version 17). Means were compared using Tukey-pairwise comparison analysis to determine significant differences at a probability level of 5%.

RESULTS AND DISCUSSION

Chlorophyll content

Chlorophyll content in tomato plants decreased with increasing drought stress (Fig 1), highlighting the harmful effects of drought on the photosynthetic systems, as also observed by Oguz *et al.* (2022). Under all stress levels, the chlorophyll content of water-treated plants was significantly

lower ($p \leq 0.05$) than that of plants treated with mannitol, melatonin and combined mannitol and melatonin. This finding suggests that the exogenous application of these compounds confers protective effects on chlorophyll retention under drought stress, consistent with the observations of Wei *et al.* (2022). According to research by Adrees *et al.* (2015), mannitol reduces the harmful effects of abiotic stress by improving the antioxidant machinery of plants. Similarly, melatonin has been reported to induce stress resilience in plants through its antioxidant activity, stomatal conductance regulation and modulation of hormone signaling pathways (Ikram *et al.*, 2024). Under control and moderate drought conditions, plants treated with combined mannitol and melatonin exhibited the highest chlorophyll content, whereas mannitol-treated plants demonstrated the highest chlorophyll content under severe drought stress. This observation may be due to the fact that severe drought stress may have induced intense osmotic stress in tomato plants, thereby enhancing mannitol's role in maintaining osmotic balance and reducing chlorophyll degradation.

Phenol content

The biosynthesis of phenolic compounds is an important physiological response to drought stress due to their pivotal roles in mitigating stress (Park *et al.*, 2023). Plants treated with combined melatonin and mannitol maintained significantly high ($p \leq 0.05$) amounts of phenol under all stress levels (Fig 2). The increase in phenol content indicates that the combined treatment enhances the biosynthesis of phenols and may improve the tolerance of plants under both stressed and non-stressed conditions. Under the control, tomato plants recorded a 30.17, 37.16 and 139.29% increase in phenol accumulation for mannitol, melatonin and combined mannitol and melatonin treatments, respectively, relative to plants treated with water. Under moderate drought stress, plants treated with the melatonin-mannitol combination recorded the highest percentage

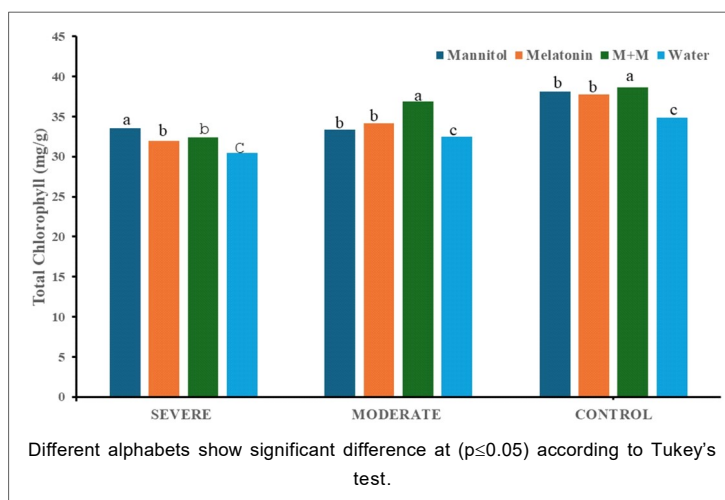


Fig 1: Variation in mean chlorophyll content of biostimulant-treated tomato plants under drought stress.

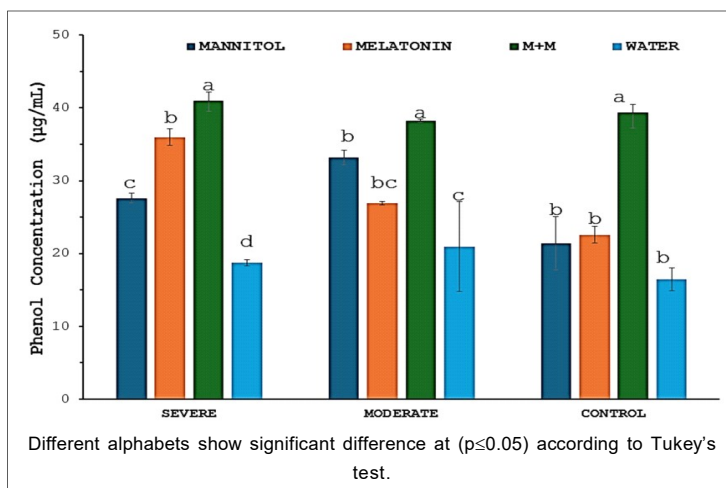


Fig 2: Variation in mean phenol concentration of biostimulant-treated tomato plants under drought stress.

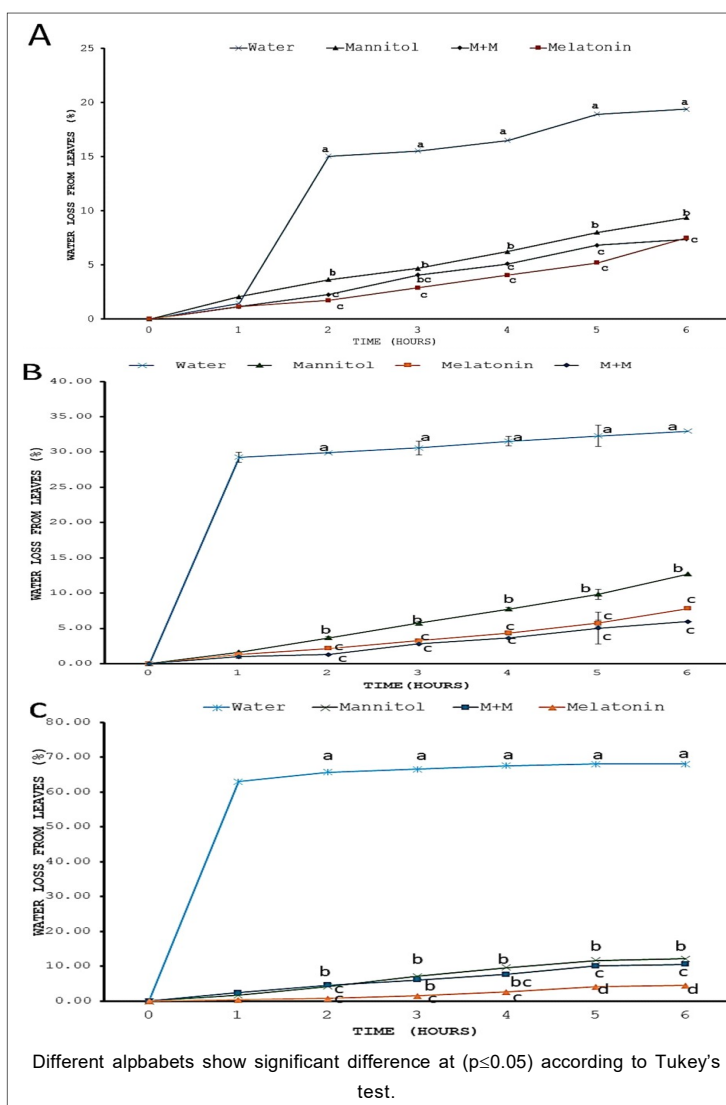


Fig 3: Variations in mean rate of water loss in leaves of biostimulant-treated tomato plants under A: No drought stress B: Moderate drought stress C: Severe drought stress.

increase in phenol accumulation of 82.65%, followed by plants treated with mannitol (58.57%) and melatonin (28.66%).

Rate of water loss from leaves

Detached leaves of water-treated plants in the control group consistently lost significantly more ($p \leq 0.05$) water than leaves treated with biostimulants (Fig 3A). At the end of a 6-h period, water-treated plants had lost 17% of their water content, compared with 7.39%, 7.51% and 9.37% of water loss in detached leaves of combined melatonin and mannitol-treated plants and individual melatonin and mannitol-treated plants, respectively. Under moderate drought stress, leaves of water-treated plants recorded the highest water loss rate of 29.25% after an hour, peaking at 32.95% after 6 hours (Fig 3B). Combined melatonin mannitol-treated leaves showed the lowest rate of water loss of 5.96% after 6 hours. Detached leaves of water-treated plants under severe drought lost more than 60% percent of their water content after 1-hr of dehydration. The leaves continued to lose water, losing 68% of their water content by the end of the experiment (Fig 3C). Detached leaves of mannitol-treated plants, on the other hand, lost 12.13% of their water content, while plants subjected to melatonin and combined treatments lost 4.46% and 10.53% of their water content respectively. The enhanced water retention capability in biostimulant treated plants suggests that single and combined application of melatonin and mannitol improved water retention and overall plant water-use efficiency in plants.

Rate of wilting in tomato plants

Under control and moderate drought, plants did not show significant difference ($p \leq 0.05$) in the mean percentage rate of wilting (Fig 4). However, under severe drought stress, the mean percentage rate of wilting in water-treated plants was about 61%, which was significantly higher ($p \leq 0.05$)

than that of biostimulant-treated plants. Plants treated with combined melatonin and mannitol recorded a significantly lower ($p \leq 0.05$) rate of wilting (27%) compared to those treated with mannitol alone (33%) and melatonin alone (35%). The observed trends in wilting under severe drought stress highlight the potential effectiveness of the combined melatonin and mannitol treatment in enhancing water retention and reducing drought-induced physiological damage. The ability of the combined melatonin and mannitol treatment to maintain turgor pressure and minimize wilting emphasizes their dual role in osmoprotection (Ikram *et al.*, 2024) and oxidative stress reduction (Afzal *et al.*, 2023), making this combination a highly effective strategy for enhancing drought tolerance in tomato plants.

Soluble sugar content

In response to drought stress, plants accumulate soluble sugars (sucrose, glucose and fructose) as an adaptation mechanism (Amoah *et al.*, 2024). Sugars are involved in stress defense mechanisms such as stomatal closure, energy production, reactive oxygen species (ROS) scavenging and the protection of cellular structures. Under moderate and severe drought stress, plants treated with combined mannitol and melatonin recorded the highest amount of soluble sugar, followed by single treatments of mannitol and melatonin, respectively, indicating enhanced stress tolerance in the biostimulant treated plants.

Membrane stability index

Membrane integrity is crucial for the survival of cells under stress; once the membrane is compromised, cells will lose their ions and sugars, leading to metabolic disorder (Patel and Williamson, 2016). The highest levels of membrane stability were recorded in plants treated with the combined mannitol and melatonin under control, moderate and severe drought stress (Table 1) thus showing consistently better membrane integrity compared with those

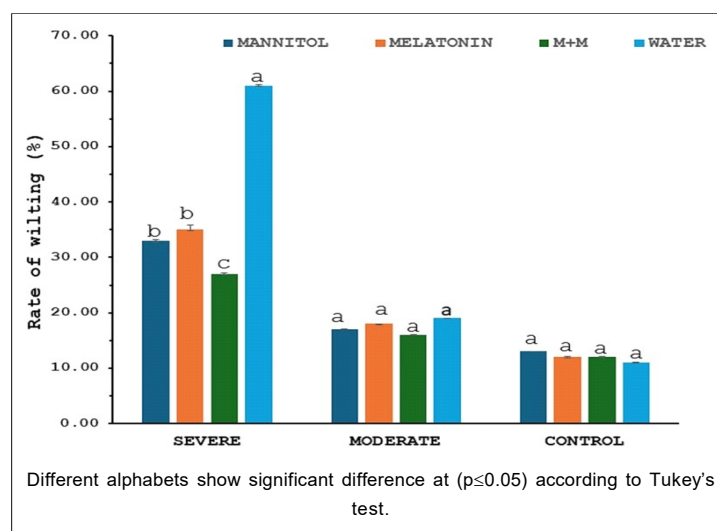


Fig 4: Variation in mean rate of wilting of biostimulant-treated tomato plants under drought stress.

Table 1: Variation in mean soluble sugar content and membrane stability indices of biostimulant-treated tomato plants under drought stress conditions.

Treatment blocks	Soluble sugar content ($\mu\text{g/mL}$)			Membrane stability index (%)		
	Control	Moderate	Severe	Control	Moderate	Severe
Mannitol	0.24 $\pm$ 0.0b	0.39 $\pm$ 0.3b	0.34 $\pm$ 0.0b	82.3 $\pm$ 0.0a	69.3 $\pm$ 1.2b	47.2 $\pm$ 0.4b
Melatonin	0.27 $\pm$ 0.0a	0.34 $\pm$ 0.00c	0.31 $\pm$ 0.0c	75.6 $\pm$ 1.2b	70.0 $\pm$ 0.3b	43.0 $\pm$ 0.1c
M+M	0.26 $\pm$ 0.4a	0.43 $\pm$ 0.0a	0.43 $\pm$ 0.0a	80.2 $\pm$ 0.6a	78.3 $\pm$ 0.0a	51.5 $\pm$ 0.3a
Water	0.28 $\pm$ 0.3a	0.24 $\pm$ 0.24d	0.24 $\pm$ 0.02d	75.0 $\pm$ 0.0b	51.3 $\pm$ 0.2c	27.1 $\pm$ 0.6d

Different alphabets show significant difference at ($p \leq 0.05$) according to Tukey's test.

treated individually with mannitol or melatonin. Plants treated with water recorded the lowest membrane stability indices under all drought conditions (Table 1), indicating greater susceptibility to cellular damage induced by drought.

CONCLUSION

In this work, we have confirmed that the application of mannitol and melatonin, either singly or in combination, is effective in imparting drought tolerance to tomato plants under stress. It was, however, showed that the combined effects of the two compounds were effective in drought stress mitigation. The foliar application of combined melatonin and mannitol to tomato plants under drought stress improved chlorophyll content, reduced the rate of water loss as well as wilting and enhanced membrane stability and soluble sugar accumulation. The foliar application of combined melatonin and mannitol represents a potential means of ensuring resilience in plants exposed to drought stress.

Conflict of interest

The authors declare no conflict of interest.

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