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Kitoplus® and Melatonin Mitigate Drought Stress Effects and Enhance Stevioside and Rebaudioside-A Production in Stevia

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ABSTRACT

Melatonin and growth stimulants are crucial for growth processes and the synthesis of specific metabolites in medicinal plants. This study aimed to assess the impact of melatonin and Kitoplus® on the secondary metabolites of stevia under drought stress conditions. The experiment utilized three concentrations of melatonin (0, 75, and 150 mg L^{-1}) and two treatments of Kitoplus® (a control without Kitoplus® and 1%), arranged in a factorial randomized complete block design with three replications under drought stress. The design involved irrigating at two soil moisture levels: full irrigation and 50% of soil field capacity. The findings indicated that increasing melatonin concentrations (from 0 to 150 mg L^{-1}) significantly enhanced both fresh and dry weights, as well as total carbohydrates. The highest activities of catalase (CAT) and ascorbate peroxidase (APX) were recorded in plants treated with 150 mg L^{-1} of melatonin and 1% Kitoplus®. Furthermore, the highest levels of stevioside and rebaudioside-A were found in plants treated with 50% soil field capacity moisture, combined with 150 mg L^{-1} melatonin and 1% Kitoplus®. Melatonin (75 mg L^{-1}) and Kitoplus® (1%) presented promising alternatives to chemical fertilizers, potentially enhancing yield and improving the quality of secondary metabolites.

Introduction

Stevia (*Stevia rebaudiana* Bert) is a medicinal plant that is a suitable sweetener for diabetic patients (Yadav et al., 2011). Today, the defensive role of secondary metabolites is universally acknowledged, yet investigating the mechanism of environmental stress on their production presents a complex and ambiguous picture. While their levels can increase several times under certain conditions, numerous factors contribute to the variability of this effect. In many cases, stress conditions decrease the production of secondary metabolites (Azzaz et al., 2009). The scarcity of any growth-limiting factor more than photosynthesis enhances secondary metabolite production. Variables such as the timing and duration of stress, frequency of drought, soil characteristics, and rainfall fluctuations influence the plant's drought resistance, resulting in different responses among drought-resistant genotypes from year to year (Ardakani et al., 2007).

Water and nutrients impact the height of flower clusters and stem length, akin to any vegetative or reproductive organ (Erkan et al., 2012). Heightened drought Stress can divert photosynthetic resources towards roots, reducing aerial yield (Shao et al., 2008). Utilizing growth stimulants affecting plant development and growth is a method to bolster yield per unit area and product quality (Gornik et al., 2008; Nahar et al., 2016; Sharifi Rad et al., 2017). Growth stimulants enhance stress resistance and expedite plant development, particularly in roots and leaves. They foster superior seed germination and biological activity (Salwa and Osama, 2014).

The effect of drought stress on the plant depends on the type of plant, species, intensity, and duration

growth stage of the plant. With the onset of drought decrease leaf water and stomatal conduction, and as it intensifies, loses permeability of the leaf cell membrane and damages. On the other hand, Beheshtizadeh et al. (2023) showed that the drought stress had a significant effect on biomass, leaf dry matter, and shoot dry matter in stevia. With the closure of the stomata due to lack of water is difficult and the entry of carbon dioxide into the leaves and in general, photosynthesis is reducing. Plant nutrition in drought conditions is also of special importance and proper nutrition in this situation can increase growth and stress tolerance. This element is the most important component of photosynthetic pigment proteins and has a great effect on leaf size and area (Sheikhalipour et al., 2023; Hayat et al., 2023).

Kitoplus® a natural, organic plant elicitor derived from chitin rich shell fish for growers desiring quality produce and high yields. Torabi Giglou et al. (2023) showed that, combined foliar application of 0.5% Kitoplus® growth stimulant and 10 µM chitosan-coated iron oxide nanoparticles had a greater impact on peppermint yield and quality, especially under drought stress. This treatment compensated for the negative effects of drought stress. Kitoplus® may supply essential amino compounds for plant growth, augmenting total leaf nitrogen or enhancing nitrogen absorption from the soil, possibly due to its chitosan content containing key enzymes. This elevation in nitrogen metabolism activity boosts nitrogen transport within leaves and stems (El-Tanahi, 2012). Furthermore, cytoplasts with chitosan may heighten nutrient availability, absorption, and transport by regulating cellular osmotic pressure, thereby improving plant growth and development, leading to increased leaf count, branching, and surface area, effectively enhancing wet and dry plant weights (Forak and Ramadan, 2012).

Environmental Stress during growth and development stages may diminish leaf area, nutrient absorption, and subsequent yield. Yield typically declines with increasing stress levels, as the plant allocates most photosynthetic resources towards producing osmotic regulators like proline and glycine betaine and sugar compounds such as sucrose, fructose, and fructan, reducing cell water potential (Najafvand et al., 2008; Torabi Giglou et al., 2023). These compounds in high-stress conditions are costly for the plant, offsetting yield. Research indicates that under study conditions, the Kitoplus® application enhances growth through improved nutrient availability and increasing yield (Torabi Giglou et al., 2020; Heydarnajad Giglou et al., 2024). The growth increment, from application to final yield, is attributed to synergistic growth enhancement and antagonistic effects on reducing factors. Other studies reported an 8.2% increase in yield compared to control treatments, noting a 40% increase in tomato, corn, wheat, and barley yield with growth stimulants (Zahir et al., 2004; Jangao et al., 1991).

Melatonin can be metabolized into various biologically active compounds by enzymes, pseudo-enzymes, or non-enzymatic compounds, such as (5-methoxytryptamine) and OHM3c hydroxymelatonin. Studies have elucidated melatonin's multifaceted roles in plants, including scavenging reactive oxygen species (ROS), bolstering resistance to biotic and abiotic stresses such as pathogen attack, extreme temperatures, salinity, drought, waterlogging, and low sulfur levels (Liu et al., 2019; Giglou et al., 2023). The interaction between melatonin and ROS underscores melatonin's efficacy as an antioxidant through direct and indirect mechanisms, modulating ROS-mediated signaling such as hydrogen peroxide balance. Melatonin is a potent antioxidant, surpassing ascorbic acid in reducing reactive oxygen species levels (Bahcesular et al., 2020). Besides enzymatic antioxidant systems, non-enzymatic antioxidants play pivotal roles in reducing ROS toxicity. Melatonin is a signaling molecule regulating downstream defense genes encoding numerous non-enzymatic antioxidants (Cao et al., 2017). Several studies identify melatonin as an activator of the plant antioxidant system.

Under drought stress conditions and water deficiency, secondary metabolite production escalates due to intracellular oxidation prevention; any growth-limiting deficiency surpassing photosynthesis augments secondary metabolite production (Hermes and Matson, 1992). Moreover, since different Kitoplus® biological fertilizer treatments increase essential oil levels, essential oils being terpenoid compounds necessitating ATP, NADPH, CO_2, and glucose for biosynthesis, are directly correlated with photosynthesis and photosynthetic product levels (Asgaripour Rafiei, 2011). Kitoplus® biological fertilizer, akin to nitroxin biological fertilizer, boosts photosynthetic tissue levels by aiding nitrogen, phosphorus, and sulfur absorption, vital for chlorophyll production and supplying necessary plant enzymes, thereby elevating essential oil levels in peppermint plants (Martin et al., 2006).

Numerous nanotechnology applications in agriculture have been proposed and the use of iron oxide nanoparticles and chitosan coating is a new way of providing the elements needed by the plant. Few studies have been conducted on the application of melatonin and Kitoplus® in medical plants such as stevia, which necessitates research in this field. Therefore, due to the economic importance and increasing demand for mint and its widespread use of this medicinal plant, in this study, the effect of melatonin and plant growth stimulants on reducing the effects drought stress was assessed.

Material and methods

Plant growth and treatments

This study followed as factorial design within a randomized complete block framework, incorporating three replications in Mahabad University at 2022. One square meter was saturated with water and subsequently covered with plastic to determine the field capacity moisture percentage (the moisture content retained in the soil post-gravitational water discharge). Post-irrigation and subsequent drainage, soil moisture was assessed at 6 h intervals at a depth of 30 cm, corresponding to the root development zone, utilizing a hygrometer (Giglou et al., 2022). This procedure was repeated until the moisture measurements stabilized across several consecutive checks. The established moisture percentage was deemed equivalent to the field capacity moisture, serving as the basis for the irrigation treatments, which were calculated as a percentage of this soil moisture value. To perform a soil assessment, sampling was done in a zigzag pattern from three points and at three depths up to a depth of 30 cm. The soil samples from root growth depths were sent to the laboratory of the Soil and Water Research Institute, Rad Kimia soil. The soil test results are presented in Table 1.

Table 1. Results of experimental farm soil test results. Mixed sampling from a depth of 0-30 cm. Parameters show percentages of clay, silt, sand, acidity, salinity, field capacity, and permanent wilting point.

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Acidity (pH)	Salinity (EC_e)	Field capacity (%)	PWP (%)
0-30	29	43	28	7.39	2.75	29.7	17.46

Salinity (EC_e): the electrical conductivity (EC) of a solution or soil and water mix in the field or laboratory. EC_e is the estimated electrical conductivity of the extract from a saturated soil paste. PWP: permanent wilting point.

The drought stress was administered at two soil moisture levels: full irrigation (control) and 50% of soil field capacity moisture. Melatonin treatments were applied at three concentrations: 0 (control), 75, and 150 mg L^{-1}, Additionally, Kitoplus®, a growth stimulant produced by Kimia Sabzavar, Iran, was used at two concentrations: 0 and a 1% solution (Torabi Giglou et al., 2023).

Stevia (*Stevia rebaudiana* Bertoni) seedlings, sourced from Urmia Zarin Cooperative, were acclimatized in greenhouse conditions for one week before being transplanted to the main experimental field. The onset of drought stress (First, drought stress was applied) and foliar applications commenced 25 d post-transplantation. Irrigation within each block was individually managed to maintain the designated soil moisture levels (Full Irrigation and 50% of soil field capacity moisture), with a uniform volume of 300 liters per block until the desired moisture level was achieved. Melatonin and Kitoplus® treatments were administered in equal volumes across blocks, reflecting the specified concentrations, and were applied four times at 20 d intervals.

Two weeks following the final foliar application, various traits, including fresh weight (FW), dry weight (DW), total phenol content (TPC), total flavonoid content (TFC), and antioxidant enzyme activities were assessed for each sample immediately prior to the last irrigation.

Yield

Fresh weight (FW) and dry weight (DW) of the plants were quantified to determine yield. Sampling was conducted randomly twice from an area of 0.5 m^2 in each plot's center. The plant material was dried at 40 °C for 96 h before being weighed to measure the dry matter content.

Measuring phenols (TPC), flavonoids (TFC), and antioxidants (TAC)

The plants after harvest at the end of study, were dried in an oven at 40 °C for 96 h, after which the dried material was milled. Extraction was performed using the method described by Pourmarad et al. (2006).

Total phenol content (TPC)

For the TPC assay, 2 mL of a 2% sodium carbonate solution, 2.8 mL of distilled water, and 100 µL of Folin-Ciocalteu's phenol reagent (50%) were added to 100 µL of the plant extract. The absorbance was then measured at a wavelength of 720 nm relative to the control, with the absorbance being recorded at half the initial time interval. Gallic acid (GA) was used as a standard for constructing a standard curve. The total phenol content of the extracts was expressed as milligrams of GA equivalent per gram of plant dry weight (Meda et al., 2004).

Total flavonoids content (TFC)

To determine the TFC, 1.5 mL of 80% methanol, 100 µL of a 10% $AlCl_2$ solution, 100 µL of a 1 mM sodium acetate solution, and 2.8 mL of distilled water were added to 500 µL of each extract. The absorbance of the solution was measured at a wavelength of 415 nm, relative to the control, over 40 min. The blank solution contained all the components above but with 80% methanol substituting the extract. Quercetin was used as a standard for the construction of the standard curve. The total flavonoid content of the extracts was reported in milligrams of quercetin equivalent per gram of plant dry weight (Mita et al., 1997).

Total carbohydrates (TC)

The TC content was quantified using the phenol-sulfuric acid method. Absorbance was measured with a spectrophotometer at a wavelength of 485 nm. Standard curves were prepared using solutions with glucose concentrations ranging from 0 to 10 mg 100 mL^{-1}. Considering the dry weight of the samples, the TC content was calculated as milligrams per gram of sample dry weight (Irigoyen et al., 1992).

Measurement of catalase (CAT), ascorbate peroxidase (APX) activity, and peroxidase enzyme activity (POD)

To quantify the activities of antioxidant enzymes, 0.1 g of fresh leaves (after harvesting within a week after the last stage of foliar application) was homogenized in 1.5 mL of phosphate-buffered saline (PBS) at pH 7.4, containing 1 g of PVP, 0.8 g of KCl, 0.8 g of NaCl, 0.14 g of Na_2HPO_4, and 0.02 g of KH_2PO_4 on ice. The mixture was ground in a mortar and pestle and then centrifuged at 45 °C at 10,000 rpm for 10 min. The supernatant was subsequently used for measuring CAT and APX activities.

CAT activity was measured using the method described by Boominathan and Doran (2002). This involved monitoring the decrease in absorbance at a wavelength of 240 nm for 1 min using a spectrophotometer (Uvi Light XS 5 SECOMAM), from which CAT activity was calculated.

APX activity was assessed following the method Boominathan and Doran (2002) outlined. The activity of APX was determined by measuring the absorbance at 290 nm wavelength for 1 min using a spectrophotometer (Uvi Light XS 5 SECOMAM). Subsequently, the APX activity was calculated.

POD activity was determined according to the method outlined by Upadhyaya et al. (1985). The reaction mixture comprised 2.5 mL of 50 mM phosphate buffer (pH = 7) with 1 mL of 1% guaiacol, 1 mL of 1% H_2O_2, and 0.1 mL of the extract. POD activity was quantified using a spectrophotometer based on the increase in absorbance at 420 nm over 1 min.

Extraction of stevioside and rebaudioside-A

Dried plant material was finely ground for the analysis of Stevioside and Rebaudioside-A. Then, 300 mg of this powder was mixed with 30 mL of a methanol solution (HPLC grade) of 60% methanol and 40% deionized water (ddH_2O) in 15 mL test tubes. This mixture underwent ultrasonic treatment in a water bath for 15 min, a process that was repeated three times. After ultrasonication, the mixture was centrifuged at 2,000 rpm for 10 min, and the supernatant fractions were collected; this step was repeated twice. The collected supernatants were evaporated using a rotary evaporator at 45 °C under vacuum to yield dry extracts. To these dry extracts, 800 µL of methanol was added, and vortexing completely dissolved the solution. The extracts were then filtered through a 0.45 µm filter before being stored at -20 °C for subsequent analysis via high-performance liquid chromatography (HPLC), following the methodology described by Jakabova et al. (2012).

HPLC

The HPLC analysis employed an NH_2 column. The mobile phase consisted of acetonitrile and purified water in an 80:20 ratio, utilizing Merck licrosalo solvents. Measurements were conducted at an ultraviolet wavelength of 210 nm, with the column temperature maintained at a specific, unmentioned degree Celsius and a flow rate of 1.2 mL min^{-1}, as per Woelwer-Rieck et al. (2010).

Statistical analysis

Experimental data from a factorial experiment utilizing a randomized complete block design were analyzed using SAS 9.3 (SAS Institute Inc., North Carolina, USA) at a 5% probability level.

Results

Yield

Applying melatonin and Kitoplus® under stress and non-stress conditions significantly enhanced the fresh weight (FW) and dry weight (DW) of stevia plants. Moreover, an incremental trend in FW and DW was observed with rising melatonin concentrations, reaching a peak with Kitoplus® administration. The maximal FW (21.96 g) was recorded in plants treated with 150 mg L^{-1} melatonin and 1% Kitoplus® under optimal irrigation conditions (Fig. 1a). Similarly, the investigation into the effects of drought Stress and Kitoplus® on DW revealed the highest value under full irrigation and with 1% Kitoplus® concentration (Fig. 1b). The maximal DW was noted under full irrigation conditions with 150 mg L^{-1} melatonin (Fig. 1c).

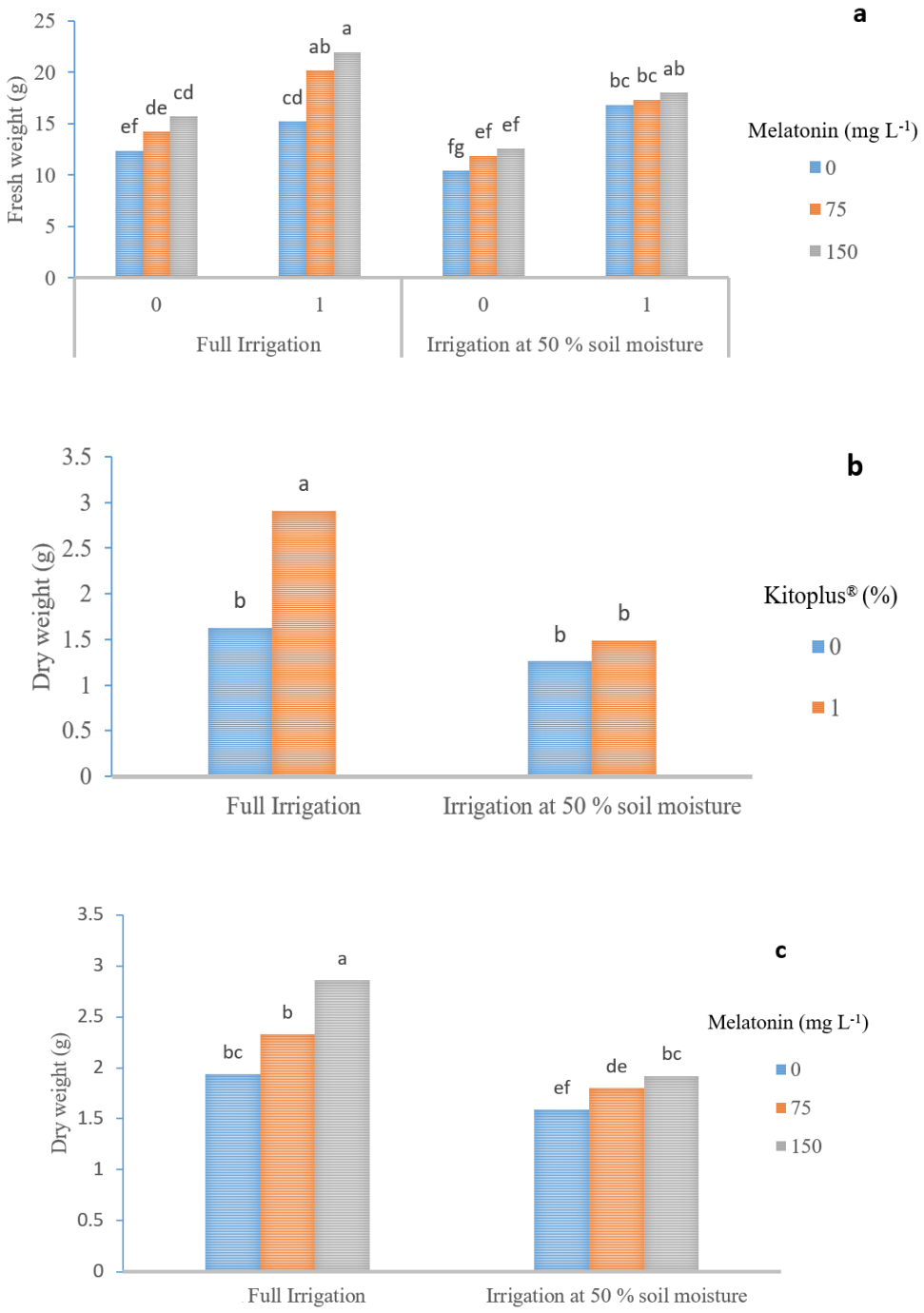

Fig. 1. Effects of melatonin and Kitoplus® on fresh and dry weights of stevia under stress and non-stress conditions. (**a**) Fresh weight (FW) of stevia plants under varying melatonin concentrations and Kitoplus® application, showing peak FW at 150 mg L^{-1} melatonin and 1% Kitoplus® under full irrigation; (**b**) Dry weight (DW) trends under drought stress and Kitoplus® application, with the highest DW recorded under full irrigation and 1% Kitoplus®; (**c**) Maximum DW observed under full irrigation with 150 mg L^{-1} melatonin.

Total phenol (TPC) and total flavonoid (TFC) content

TPC levels were elevated under stress conditions and with the use of melatonin and Kitoplus®. The combination of Kitoplus® and melatonin under full irrigation and at 50% soil field capacity moisture significantly increased TPC levels. The highest TPC concentration (57.86 mg g^{-1}) was observed under drought stress at 50% soil field capacity moisture

with 1% Kitoplus® (Fig. 2a) and with the application of 150 mg L^{-1} melatonin (Fig. 2b).

The application of Kitoplus® at 50% soil field capacity moisture led to a reduction in TFC, whereas the combination of melatonin and irrigation at the same moisture level resulted in an upward trend in this index, peaking with 150 mg L^{-1} melatonin (Fig. 3).

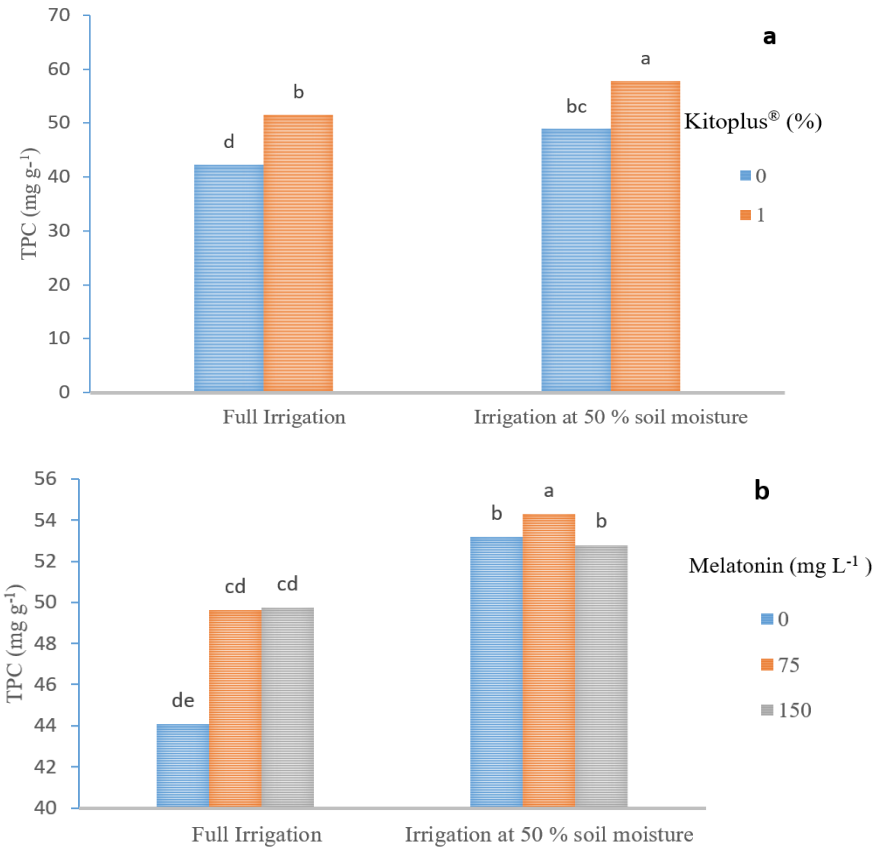

Fig. 2. Effects of melatonin and Kitoplus® on total phenolic content (TPC) under stress and non-stress conditions. (**a**) TPC levels under varying moisture conditions and Kitoplus® application, showing the highest concentration (57.86 mg g^{-1}) under drought stress at 50% soil field capacity moisture with 1% Kitoplus®; (**b**) TPC levels in response to melatonin application, peaking at 150 mg L^{-1} under drought stress at 50% soil field capacity moisture.

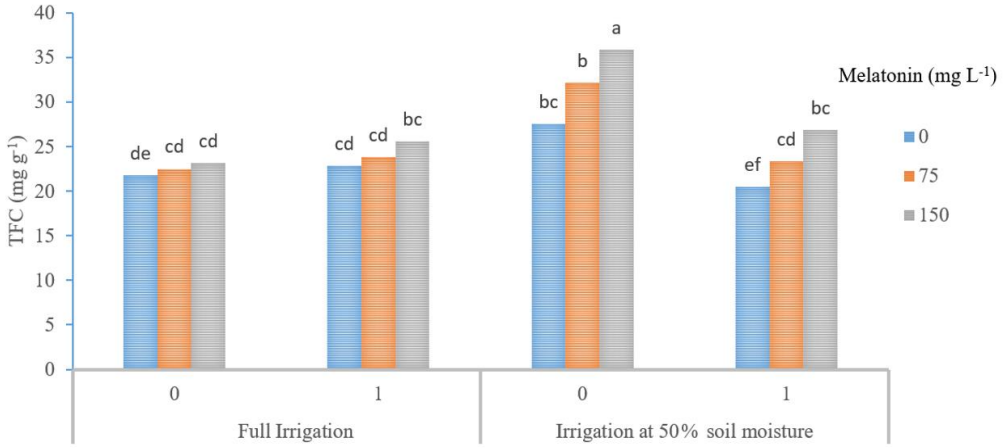

Fig. 3. Effects of melatonin and Kitoplus® on total flavonoid content (TFC) under different moisture conditions. TFC levels decreased with Kitoplus® application at 50% soil field capacity moisture, while the combination of melatonin and irrigation at the same moisture level increased TFC, reaching its highest value with 150 mg L^{-1} melatonin.

Activity of antioxidant enzymes

The activities of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) enzymes were highest under drought stress conditions at 50% soil field capacity moisture. Kitoplus® enhanced the activity of CAT, SOD, and APX enzymes in both irrigation scenarios. A medium concentration of melatonin (75 mg L^{-1}) was more effective in increasing the activity of these antioxidant enzymes under full irrigation than a higher concentration (150 mg L^{-1}). However, under irrigation conditions at 50% soil field capacity moisture, the enzyme activities of CAT, SOD, and APX peaked at a melatonin concentration of 150 mg L^{-1}. Consequently, the highest activity levels of CAT (Fig. 4), SOD (Fig. 5), and APX (Fig. 6) enzymes were observed in plants treated with 150 mg L^{-1} melatonin and 1% Kitoplus® under 50% soil field capacity moisture.

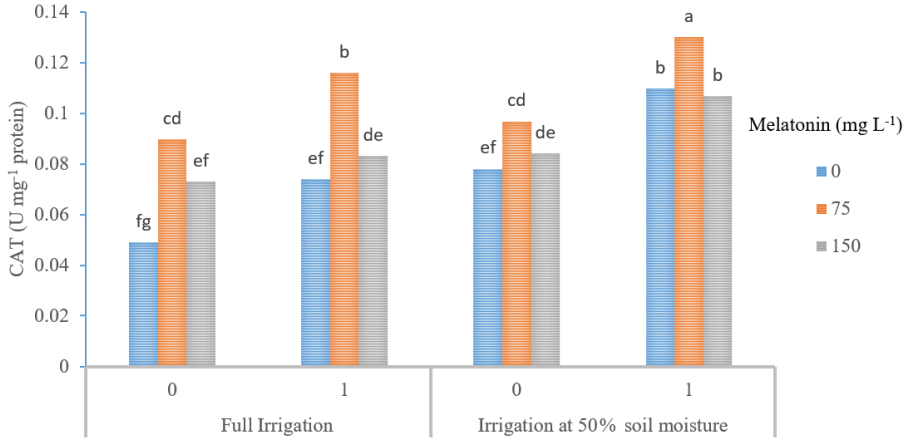

Fig. 4. Catalase enzyme activity in plants treated with Kitoplus® and melatonin under stress conditions. Catalase activity was highest under drought stress at 50% soil field capacity moisture with 150 mg L^{-1} melatonin and 1% Kitoplus®.

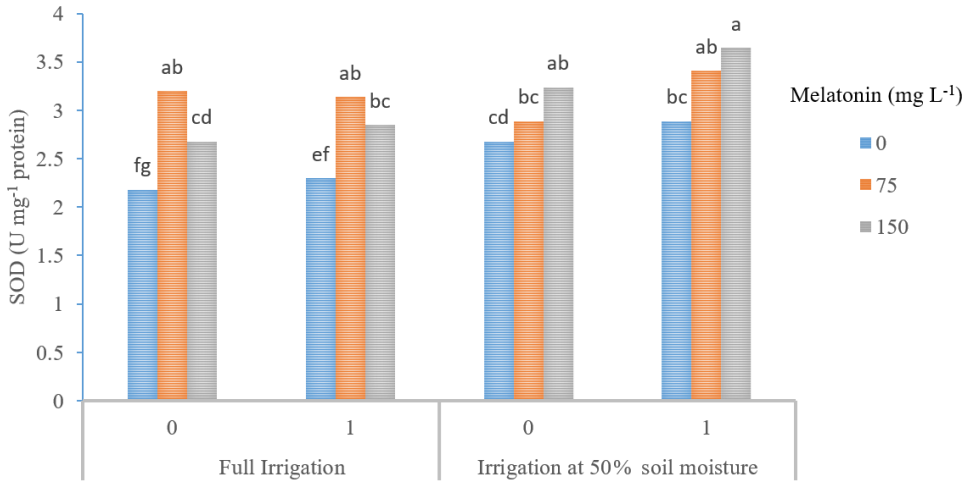

Fig. 5. Superoxide dismutase activity in plants treated with Kitoplus® and melatonin under stress conditions. Superoxide dismutase activity peaked under drought stress at 50% soil field capacity moisture with 150 mg L^{-1} melatonin and 1% Kitoplus®, while a medium melatonin concentration (75 mg L^{-1}) was more effective under full irrigation.

Total carbohydrate content (TC)

The administration of melatonin at concentrations of 75 and 150 mg L^{-1}, combined with the use of Kitoplus® at 1%, significantly increased the total carbohydrate (TC) content in stevia plants across both irrigation regimes. TC levels peaked under irrigation at 50% soil field capacity moisture. The highest TC concentration (5.67 mg g^{-1}) was observed in plants treated with 150 mg L^{-1} melatonin and 1% Kitoplus® under these conditions (Fig. 7).

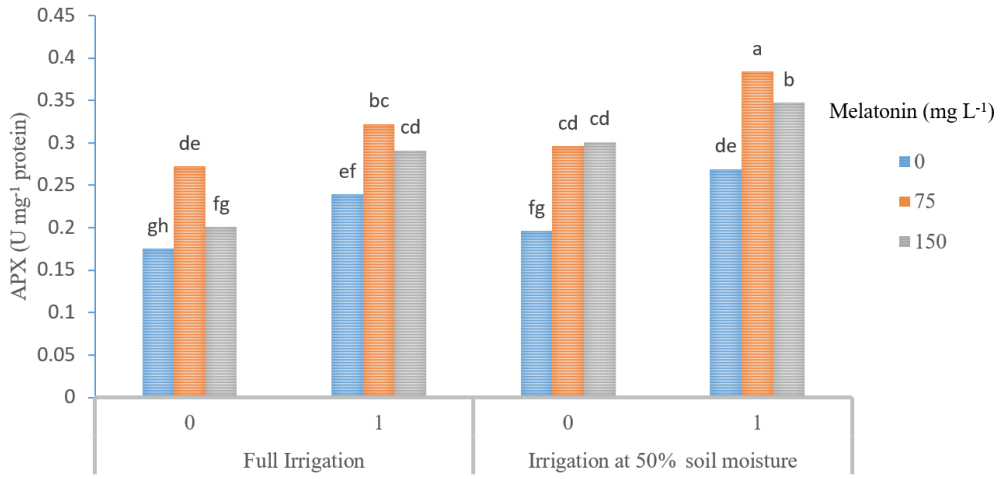

Fig. 6. Ascorbate peroxidase activity in plants treated with Kitoplus® and melatonin under stress conditions. Ascorbate peroxidase activity peaked under drought stress at 50% soil field capacity moisture with 150 mg L^{-1} melatonin and 1% Kitoplus®.

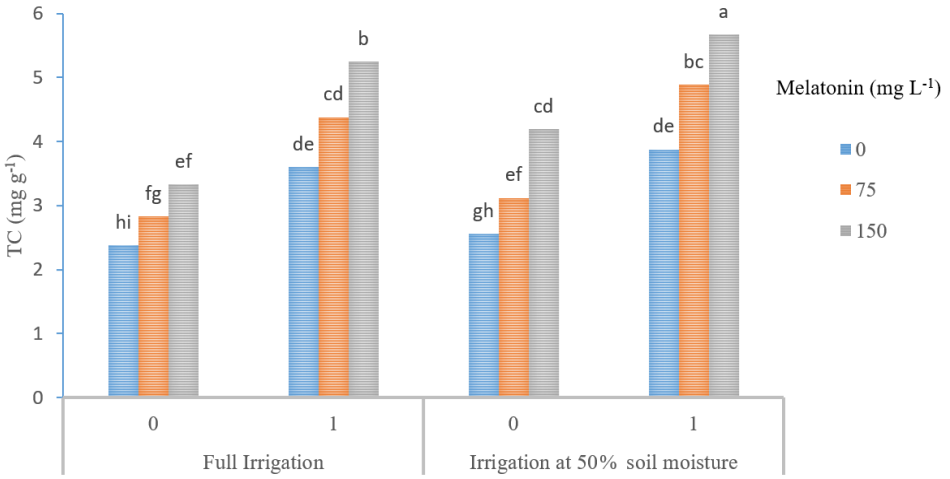

Fig. 7. Total carbohydrate changes in stevia plants treated with Kitoplus® and melatonin under stress conditions. Total carbohydrate content significantly increased with 75 and 150 mg L^{-1} melatonin combined with 1% Kitoplus® under both irrigation regimes, peaking at 5.67 mg g^{-1} under 50% soil field capacity moisture with 150 mg L^{-1} melatonin and 1% Kitoplus®.

Stevioside and rebaudioside-a

The levels of stevioside and rebaudioside-a were positively correlated with the concentration of melatonin and the application of Kitoplus®, particularly under irrigation stress. This enhancement was more pronounced with Kitoplus® at 1% concentration and irrigation at 50% soil field capacity moisture than control conditions. The data revealed the highest concentration of stevioside (2838.27 ppm) in plants treated with 150 mg L^{-1} melatonin (Fig. 8), and the peak level of Rebaudioside-A (638.88 ppm) was achieved with 75 mg L^{-1} melatonin and 1% Kitoplus® under irrigation at 50% soil field capacity moisture (Fig. 9).

Discussion

The data presented in this study illustrate a decline in plant yield attributable to drought stress. Conversely, the foliar application of Kitoplus® and melatonin, particularly at a concentration of 150 mg L^{-1}, markedly enhanced yield parameters in stevia plants. These findings align with previous research on the efficacy of natural growth promoters (Khan et al., 2002; Gornik et al., 2008). The chitosan in Kitoplus® potentially supplies amino acids, thereby increasing total leaf nitrogen (N) content or enhancing the plant's N absorption capacity from the soil. Zhang et al. (2017) indicated that chitosan-treated wheat seedlings showed improved N reduction and assimilation. Furthermore, Kitoplus® may enhance the availability, uptake, and transportation of essential nutrients by modulating cellular osmotic

pressure, thereby augmenting leaf count, shoot growth, and leaf area, consequently boosting plant growth and development (Farouk and Ramadan, 2012). This observation is supported by Rahman et al. (2018) who noted a positive impact of chitosan treatment on fresh and dry biomass production of strawberry plants.

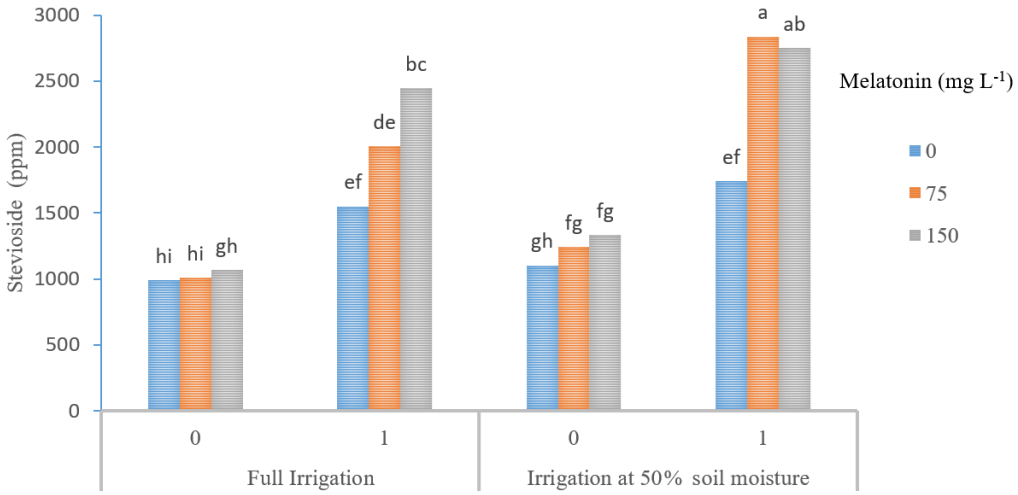

Fig. 8. Stevioside changes in stevia plants treated with Kitoplus® and melatonin under stress conditions. Stevioside levels increased with melatonin and Kitoplus® application, peaking at 2838.27 ppm in plants treated with 150 mg L^{-1} melatonin under 50% soil field capacity moisture.

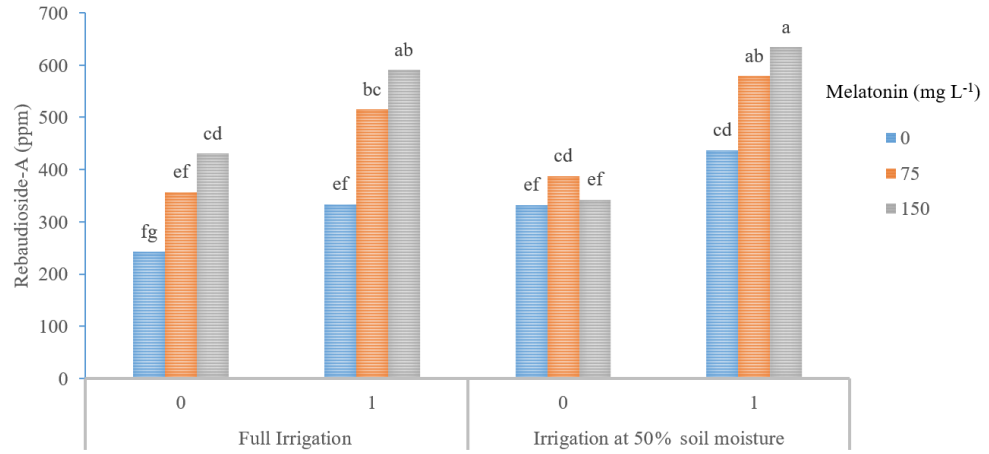

Fig. 9. Rebaudioside-A changes in stevia plants treated with Kitoplus® and melatonin under drought stress. Rebaudioside-a content was highest (638.88 ppm) with 75 mg L^{-1} melatonin and 1% Kitoplus® under irrigation at 50% soil field capacity moisture.

Generally, the data regarding yield parameters (fresh and dry weight) indicate vigorous plant growth under drought-stress conditions. Conversely, applying melatonin and Kitoplus® through foliar spraying, particularly at a concentration of 1%, resulted in a significant increase in most cases. The findings are consistent with previous studies demonstrating that using chitosan compounds and melatonin enhances plant growth indicators and total chlorophyll content (Verma et al., 2010; Zhang et al., 2017; Giglou et al., 2022). On other hand Sheikhalipour et al. (2023) showed that, growth and photosynthetic pigments of the plants decreased with the severity of the stress but the relevant traits (growth and photosynthetic pigments) were improved with the applications of melatonin and TiO_2 NPs under mid and severe stress. In another study, Simalt et al. (2023) showed that in salinity conditions (50 mM NaCl), 500 µM of melatonin had the most favorable effect on the synthesis of phenolic acids. The phenolic acids in that case reached a level three-times higher than that in the samples with the same melatonin concentration but without NaCl. We also found that the content of phenolic compounds varied depending

on the age of the leaves. To the best of our knowledge, this is the first study to describe the effect of melatonin and NaCl on the synthesis on phenolic acids and flavonoids in stevia.

Previous reports indicated that vegetative growth parameters such as plant height, number of internodes, internode distance, fresh and dry weight of the entire plant, stem, and overall plant biomass decreased with increasing drought stress intensity. This decline can be attributed to a significant decrease in plant branching due to drought stress. Moreover, fluctuations in soil moisture significantly impact plant fresh and dry weights, leading to decreased chlorophyll content and reduced photosynthetic efficiency (Cavatte et al., 2012). This decrease may also result from stomatal closure and inhibition of rubisco enzyme activity (Lawlor and Cornic, 2002). Nevertheless, the stress induced by drought stress suppresses photosynthetic efficiency to a certain extent.

Kitoplus®, owing to its chitosan content, potentially enhances critical enzyme activity involved in nitrogen metabolism, facilitating nitrogen transport within leaves and stems (El-Tanahy et al., 2012). Additionally, Kitoplus® containing chitosan may augment the availability, absorption, and transport of essential nutrients by regulating cellular osmotic pressure. Consequently, this improvement in plant growth and development, including increased leaf count, branching, leaf surface area, and overall leaf area, contributes to enhanced fresh and dry plant weight (Farouk and Ramadan, 2012).

Developing effective defense mechanisms in plants, including the activity of antioxidant enzymes such as POD, CAT, and APX, is crucial for mitigating oxidative damage (Ma et al., 2021; Shankramm et al., 2016). The application of melatonin in conjunction with Kitoplus® significantly enhanced the activity of these antioxidant enzymes in peppermint compared to untreated plants. Different combinations of Kitoplus® and melatonin levels improved the activity of these enzymes differently. Specifically, CAT and APX activities increased with 75 mg L^{-1} melatonin and 1% Kitoplus® under drought conditions. CAT activity, for instance, increased by 62.35% and 15.62% with 75 mg L^{-1} melatonin and 1% Kitoplus®, compared to control and stressed plants, respectively. This finding aligned with Giglou et al. (2023), who reported increased antioxidant enzyme activities such as CAT and APX due to chitosan and melatonin treatment.

Under drought conditions, the highest levels of stevioside and rebaudioside-A were obtained in response to 150 mg L^{-1} and 75 mg L^{-1} melatonin, respectively, when Kitoplus® was used at 1% concentration. The findings are consistent with other studies highlighting the positive impact of melatonin on enhancing specific secondary metabolites in plants (Tarchoune et al., 2013; Bahcesular et al., 2020; Haydari et al., 2019; Torabi Giglou et al., 2023). Giglou et al. (2022) suggested that the increase in secondary metabolites was likely due to the enhanced availability of melatonin and Kitoplus®. Melatonin has been reported to mitigate oxidative damage to crucial biomolecules such as DNA, proteins, and lipids by inhibiting Fenton's reaction. These growth regulators can be effectively applied together to reduce oxidative stress, as Hu et al. (2016) and Wei et al. (2017) demonstrated.

Conclusions

This study evaluated the ramifications of the foliar application of melatonin and Kitoplus® on stevia plants in response to drought stress. The findings illustrated that treatment with 150 mg L^{-1} melatonin and 1% Kitoplus® under drought conditions enhanced plant yields, outperforming the control group. Notably, the concentrations of stevioside and rebaudioside-A were augmented in response to the increased treatment concentrations during drought stress. Stevia plants treated with 150 mg L^{-1} melatonin and Kitoplus® exhibited significant enhancements in antioxidant enzymes CAT and APX activities. The research posits that employing melatonin at 150 mg L^{-1} alongside Kitoplus® as growth stimulants can bolster the production of TPC, TFC, and TC in medicinal plants, thereby fostering sustainable agricultural practices. The implications of this study for sustainable agriculture are profound, especially in the cultivation of medicinal plants like stevia. Melatonin and Kitoplus® appeared as viable alternatives to chemical fertilizers by increasing yield and augmenting the quality of secondary metabolites. This innovative approach offers farmers a sustainable option to reduce reliance on chemical fertilizers, which potentially carry adverse environmental impacts.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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