

## Effects of preharvest melatonin and boric acid applications on the pomological and biochemical characteristics of highbush blueberry (*Vaccinium corymbosum* cv. 'Camellia')

Volkan OKATAN<sup>1</sup>, Nafiye UNAL<sup>2</sup>, Pinar KALKAN<sup>2</sup>, Melike URFALI<sup>1</sup>,  
Huseyin KALKAN<sup>3</sup>, Tugce SELVI<sup>4</sup>, Hanifeh Seyed HAJIZADEH<sup>5</sup>,  
Halil Ibrahim SAGBAS<sup>6\*</sup>

<sup>1</sup>Eskisehir Osmangazi University, Faculty of Agriculture, Department of Horticulture, Eskisehir, 26160,  
Türkiye; [volkan.okatan@ogu.edu.tr](mailto:volkan.okatan@ogu.edu.tr); [urfalimelike@gmail.com](mailto:urfalimelike@gmail.com)

<sup>2</sup>Akdeniz University, Faculty of Agriculture, Department of Horticulture, Antalya, 07070,  
Türkiye; [nafiye@akdeniz.edu.tr](mailto:nafiye@akdeniz.edu.tr); [bodurpnar@gmail.com](mailto:bodurpnar@gmail.com)

<sup>3</sup>Akdeniz University, Vocational School of Kumluca, Program of Horticulture, Antalya, 07350, Türkiye; [hkalkan@akdeniz.edu.tr](mailto:hkalkan@akdeniz.edu.tr)

<sup>4</sup>Batı Akdeniz Agricultural Research Institute, Republic of Türkiye Ministry of Agriculture and Forestry, Antalya, 07070,  
Türkiye; [tugce.selvi@tarimorman.gov.tr](mailto:tugce.selvi@tarimorman.gov.tr)

<sup>5</sup>University of Maragheh, Faculty of Agriculture, Department of Horticulture, Maragheh, 55136-553, Iran; [hajizade@maragheh.ac.ir](mailto:hajizade@maragheh.ac.ir)

<sup>6</sup>Mugla Sıtkı Kocman University, Fethiye Faculty of Agriculture, Department of Horticulture, Fethiye, Mugla, 48300,  
Türkiye; [halilibrahimsagbas@mu.edu.tr](mailto:halilibrahimsagbas@mu.edu.tr) (\*corresponding author)

### Abstract

The application of boric acid and melatonin in blueberry cultivation has recently gained attention for their potential to enhance fruit quality and biochemical stability. Boric acid and melatonin contribute to cell wall integrity, pollen tube development, and nutrient transport, whereas melatonin, a bioactive compound with strong antioxidant properties, improves stress tolerance and supports fruit quality. In this study, the synergistic effects of foliar applications of boric acid (0, 5, 10, 15 ppm), melatonin (0, 5, 10, 15 ppm), and some combinations (0, 2.5+2.5, 5+5, 7.5+7.5 ppm) at early bloom, full bloom, and post-bloom stages were investigated on the pomological and biochemical traits of highbush blueberry (*Vaccinium corymbosum* cv. 'Camellia'). The combined treatment of 5+5 ppm melatonin + boric acid produced the highest ascorbic acid content, while the 7.5+7.5 ppm combination significantly enhanced antioxidant activity. Boric acid alone at 5 and 10 ppm also improved antioxidant capacity. Although no significant differences were observed in fruit size or firmness, the treatments notably influenced titratable acidity, anthocyanin accumulation, and ascorbic acid content. These findings indicate that preharvest melatonin and boric acid applications can modulate key biochemical attributes, such as ascorbic acid, anthocyanins, and antioxidant activity, thereby improving the nutritional and functional quality of highbush blueberry fruits.

**Keywords:** antioxidant activity; blueberry; preharvest foliar application; nutritional properties; sustainable agriculture

Received: 24 Oct 2025. Received in revised form: 18 Nov 2025. Accepted: 24 Nov 2025. Published online: 19 Dec 2025.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

## Introduction

Blueberries (*Vaccinium spp.*), often referred to as ‘super fruits’, have garnered global attention due to their well-documented health benefits. The fruit peel is particularly rich in anthocyanins, which have been associated with anti-inflammatory, cardioprotective, and blood glucose-lowering properties (Silva *et al.*, 2020; Shi *et al.*, 2021). Numerous phytochemical studies have identified phenolic compounds, particularly anthocyanins, flavonoids, and phenolic acids, as the major bioactive constituents in blueberries (Wang *et al.*, 2015). According to Wang *et al.* (2015), the predominant flavonoids in blueberries include kaempferol, quercetin, and their glycosides. Among various phenolic acids, chlorogenic acids are considered the most abundant, although their levels may vary depending on several biotic and abiotic factors, such as genotype, fertilization regime, climatic conditions, harvest time, soil properties, and water availability (Routray and Orsat, 2011).

Owing to their exceptional taste, high nutritional value, and pharmacological potential, blueberries other berries have been cultivated and consumed as a functional food for decades. Commercial production is primarily concentrated in North and South America, as well as parts of Europe (Retamales and Hancock, 2018; Bozhuyuk *et al.*, 2020; Rivera *et al.*, 2022).

Boron (B) is an essential micronutrient that plays a critical role in plant growth, reproductive development, yield, and overall crop quality (Pereira *et al.*, 2021). Plants have developed efficient uptake and transport mechanisms that function under both deficiency and toxicity conditions; for example, in *Arabidopsis thaliana*, the NIP5;1 channel is upregulated under B deficiency to enhance uptake, while the BOR1 transporter facilitates xylem loading and redistribution (Takano and Tanaka, 2023). The optimal B concentration varies among species but is generally within the range of 10-75 mg kg<sup>-1</sup> in dry leaf tissue; monocots typically require 1-6 mg kg<sup>-1</sup>, whereas dicots may need 20-70 mg kg<sup>-1</sup> (Arunkumar *et al.*, 2018). Boron is crucial for maintaining cell wall structure, regulating the biosynthesis of lignin and ascorbic acid (AsA), and supporting antioxidant defenses (Yang *et al.*, 2023). In addition, B applications have been shown to contribute to fruit quality; for instance, a 0.5% boric acid (BA) treatment in Asian pear increased fruit firmness and soluble solids while helping to maintain phenolic compounds, thereby supporting overall fruit biochemical quality (Khalaj *et al.*, 2017).

Melatonin (MT) (N-acetyl-5-methoxytryptamine), a compound synthesized from tryptophan, is naturally present in both animal and plant systems and functions as a powerful antioxidant and free radical scavenger in plants (Arnao and Hernández-Ruiz, 2018). Beyond its antioxidative properties, MT acts as a signaling molecule similar to phytohormones, regulating key physiological processes such as fruit ripening, senescence, circadian rhythms, photosynthesis, seed germination, and stress responses (Arnao and Hernández-Ruiz, 2018). Numerous studies have demonstrated the beneficial role of MT in improving fruit physiological and biochemical quality. In sweet cherry, MT enhanced antioxidant systems (Carrión-Antolí *et al.*, 2022); in mango, banana, peach, and nectarine it delayed fruit softening and promoted the accumulation of bioactive compounds (Hu *et al.*, 2017; Liu *et al.*, 2020; Bal, 2021); and in apricot and pomegranate it improved overall fruit quality and increased antioxidant capacity (Lorente-Mento *et al.*, 2021). Similar effects have been reported in strawberry and tomato, where MT promoted phenolic and anthocyanin accumulation, enhancing the biochemical quality of the fruits (Liu *et al.*, 2018; Sharafi *et al.*, 2019). Additionally, MT improves antioxidant enzyme activities in several fruits, reduces browning in fresh-cut pear and lychee, enhances disease resistance in citrus and apple, and suppresses ethylene biosynthesis in apple, pear, and mango (Jannatizadeh, 2019). Collectively, these findings highlight MT as an effective bioregulator with strong potential to preserve fruit quality. This study aimed to assess the effects of foliar applications of varying concentrations of MT, BA, and their combinations on the phytochemical composition and fruit quality traits of highbush blueberry (*V. corymbosum* cv. ‘Camellia’) under non-stress, soilless cultivation conditions. Although the individual roles of

BA and MT in plant physiology and stress modulation are well documented, studies exploring their combined preharvest application in fruit crops, particularly during the active growth phase of blueberry, remain limited. Addressing this research gap, the current investigation focuses on the potential synergistic effects of MT and BA on bioactive compound accumulation and pomological quality, contributing to the development of sustainable and nutritionally enhanced fruit production strategies.

## Materials and Methods

### *Experimental site and plant material*

This study was conducted during the 2023-2024 growing season on three-year-old southern highbush blueberry (*Vaccinium corymbosum* cv. 'Camellia') bushes cultivated under soilless conditions in the central district of Antalya, Türkiye. The plant material was supplied by "Bilen Agro," located in Sariabalı village, Serik district, Antalya. Plants were grown in 20-liter plastic pots equipped with drainage holes. The substrate consisted of a 70:30 (v/v) mixture of sphagnum peat and perlite, offering high water-holding capacity and effective drainage, ideal for acidophilic species.

Pots were arranged in rows within a managed orchard and placed on black polyethylene ground cover, which allowed water drainage while inhibiting light penetration and weed growth. All plants were grown under open-field conditions using a soilless cultivation system with natural climate exposure. Irrigation was applied via a drip system and scheduled based on seasonal conditions and soil moisture. During spring and summer, irrigation occurred 2-3 times per week, and daily during hot, dry periods. In autumn and winter, irrigation frequency was adjusted according to plant needs.

Fertilization practices were adjusted according to the phenological stages of the crop. Nitrogen-based fertilizers were applied during early vegetative growth, followed by balanced NPK applications throughout flowering and fruit development. In later stages, a potassium-enriched formulation was used to support fruit quality. To prevent micronutrient deficiencies, foliar iron chelate was applied periodically, and irrigation water was acidified with phosphoric acid to maintain an optimal substrate pH. Additionally, pine bark mulch was used to reduce evaporation and suppress weed growth.

### *Treatment applications*

Melatonin (MT) was applied at 5, 10, and 15 ppm; BA at 5, 10, and 15 ppm; and combined treatments of MT + BA were applied at 2.5 + 2.5, 5 + 5, and 7.5 + 7.5 ppm. As reported by Fernández *et al.* (2013), foliar nutrient absorption reaches its maximum under conditions of lower temperature and reduced light intensity. Therefore, in our study, foliar applications were carried out in the late afternoon, shortly before sunset. The MT (5, 10, 15 ppm) and BA (5, 10, 15 ppm) concentrations were selected based on previous studies reporting effective and safe foliar doses in horticultural crops (Okatan *et al.*, 2023; Mansouri *et al.*, 2023; Yousef and Nasef, 2023). Considering the sensitivity of blueberry to boron, moderate concentrations were chosen to avoid phytotoxicity. Combined MT + BA treatments (2.5 + 2.5, 5 + 5, and 7.5 + 7.5 ppm) were designed to match the total dose of single applications to allow evaluation of synergistic effects without exceeding safe limits. All treatments were administered via foliar spray at three phenological stages: early blooming, full bloom, and post-bloom. The experimental design was a randomized complete block with three replications per treatment, each consisting of 10 plants.

### *Determination of pomological characteristics*

Pomological traits such as fruit length (mm), fruit width (mm), fruit weight (g), soluble solids content (SSC, %), titratable acidity (TEA, %), and firmness ( $\text{kg cm}^{-2}$ ) were evaluated following the methods described by Balbontín *et al.* (2013). Fruit color was measured on 20 randomly selected berries per replicate using a digital

colorimeter (Konica Minolta, Japan). Surface color attributes were recorded as L\* (lightness), a\* (green-red axis), b\* (blue-yellow axis), chroma (color intensity), and hue angle (h°).

#### *Determination of ascorbic acid content (AsA)*

Blueberry samples were first pureed and filtered to obtain juice. For analysis, the juice was centrifuged, and 400 µL of 0.4% oxalic acid and 4.5 mL of 2,6-dichlorophenolindophenol (DCPIP) solution were added to the supernatant. AsA content was measured spectrophotometrically using a Shimadzu UV-1800 spectrophotometer (Japan) at a wavelength of 520 nm, following the protocol of Bor *et al.* (2006).

#### *Determination of antioxidant activity (AA)*

To determine antioxidant activity, 5 g of blueberry fruit samples were extracted with 30 mL of methanol using ultrasonic-assisted extraction for 40 minutes, followed by centrifugation at 13,000 × g for 15 minutes. The supernatant was used for the antioxidant assay. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined according to the method described by Sun *et al.* (2014). Absorbance was measured at 517 nm, and the scavenging activity (%) was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_c - A_s}{A_c} * 100$$

In this equation,  $A_c$  is the DPPH radical absorption of the control sample and  $A_s$  is the DPPH absorption of the sample.

#### *Determination of anthocyanin*

Total anthocyanin content of blueberry fruit samples was determined using the pH differential spectrophotometric method described by Giusti and Wrolstad (2003). Diluted fruit extracts were prepared in two buffer systems: pH 1.0 (hydrochloric acid-potassium chloride) and pH 4.5 (sodium acetate). Absorbance values were recorded at 531 and 700 nm. The total anthocyanin content was calculated based on the difference in absorbance between the two pH values using the formula:

$$\text{Anthocyanin} = (A_{531} - A_{700})_{\text{pH}1.0} - (A_{531} - A_{700})_{\text{pH}4.5}$$

Values were expressed as mg cyanidin-3-glucoside equivalents per 100 g fresh weight (mg 100 g<sup>-1</sup> FW), using a molar extinction coefficient of 28,000 L mol<sup>-1</sup> cm<sup>-1</sup>.

#### *Determination of total phenolic content (TPC)*

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965), with minor modifications. Briefly, 1 mL of fruit extract was mixed with 60 mL of distilled water, followed by the addition of 5 mL Folin-Ciocalteu reagent. After 7.5 minutes, 15 mL of 20% sodium carbonate solution was added, and the mixture was diluted to 100 mL with distilled water. The mixture was vortexed and incubated in the dark at 25 °C for 2 hours. After centrifugation at 15,000 × g for 10 minutes, absorbance was measured at 750 nm using a UV-Vis spectrophotometer. Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE g<sup>-1</sup> DW), based on a gallic acid calibration curve.

#### *Determination of total flavonoids content (TFC)*

Total flavonoid content was measured using a modified aluminum chloride colorimetric method according to Chang *et al.* (2002). In brief, 50 µL of fruit extract was added to a test tube containing 950 µL methanol, 4 mL distilled water, and 300 µL of 5% sodium nitrite (NaNO<sub>2</sub>) solution. After 5 minutes of incubation, 300 µL of 10% aluminum chloride (AlCl<sub>3</sub>) was added and the mixture was allowed to stand for 6 minutes. Subsequently, 2 mL of 1 M sodium hydroxide (NaOH) was added and the volume was adjusted to 10 mL with distilled water. After standing for 15 minutes, absorbance was measured at 510 nm. TFC values were

calculated based on a standard curve of quercetin and expressed as mg quercetin equivalents per gram dry weight (mg QE g<sup>-1</sup> DW).

### Statistical analysis

Statistical analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was conducted for all measured pomological and biochemical variables, and mean separations were determined using Duncan's multiple range test at the  $p < 0.05$  significance level. In addition to ANOVA, Pearson correlation coefficient analysis was performed using R version 3.4.3 ([www.r-project.org](http://www.r-project.org)) to evaluate the relationships among quality parameters. Furthermore, multivariate analyses, including Principal Component Analysis (PCA), were carried out to explore sample clustering and the contribution of individual variables. Correlation heatmaps, PCA biplots, and hierarchical cluster dendrograms were generated using R software (version 4.1.2, R Foundation for Statistical Computing).

## Results

### Fruit pomology, pH, soluble solids content (SSC), titratable acidity (TEA) and fruit firmness

Data on fruit length, fruit width, fruit weight, pH, SSC, TEA, and fruit firmness are presented in Table 1. Among these traits, fruit weight, width, and length are considered primary indicators of fruit quality. The results revealed that none of the treatments induced statistically significant differences in fruit weight, width, or length ( $p > 0.05$ ). Similarly, there were no significant effects observed on pH, SSC, or firmness between the treatments (Table 1).

**Table 1.** Effect of different treatments on fruit length, width, weight, pH, SSC, TEA and firmness of blueberry

Treatments	Fruit length (mm)	Fruit width (mm)	Fruit weight (g/10 fruit)	pH	SSC (%)	TEA (%)	Fruit firmness (kg cm <sup>-2</sup> )
Control	14.91 a	20.45 a	25.00 a	3.76 a	12.78 a	0.24 a	5.93 a
MT (5 ppm)	15.08 a	20.87 a	25.33 a	3.71 a	11.60 a	0.19 ab	5.61 a
MT (10 ppm)	15.19 a	20.85 a	25.67 a	3.71 a	11.97 a	0.13 b	5.53 a
MT (15 ppm)	14.51 a	20.95 a	25.00 a	3.74 a	10.83 a	0.18 ab	5.42 a
BA (5 ppm)	14.99 a	19.84 a	25.33 a	3.81 a	12.77 a	0.19 ab	5.75 a
BA (10 ppm)	14.75 a	20.57 a	27.00 a	3.73 a	10.63 a	0.23 a	5.33 a
BA (15 ppm)	14.81 a	19.91 a	26.33 a	3.79 a	12.00 a	0.23 a	4.81 a
MT+BA (2.5+2.5 ppm)	14.46 a	20.16 a	21.67 a	3.83 a	13.83 a	0.19 ab	4.91 a
MT+BA (5+5 ppm)	14.87 a	20.26 a	24.33 a	3.84 a	12.40 a	0.20 ab	5.47 a
MT+BA (7.5+7.5 ppm)	14.58 a	20.37 a	24.00 a	3.88 a	13.97 a	0.17 ab	5.13 a

Different letters indicate significant differences in each trait according to Duncan's test at  $p < 0.05$  (SSC: soluble solids content; TEA: titratable acidity; MT: melatonin; BA: boric acid)

However, TEA was significantly influenced by the treatments. The highest TEA value was recorded in the control group (0.24%), while the lowest was observed in the MT (10 ppm) treatment, with a value of 0.13%. Among all treatments, only MT at 10 ppm resulted in a statistically significant reduction in TEA compared to the control ( $p < 0.05$ ). Although slight decreases in TEA were noted in other MT and BA treatments, these differences were not statistically significant.

Overall, both MT and BA applications tended to reduce TEA relative to the untreated control, indicating a potential acid-suppressing effect of these compounds on blueberry fruits.

*Color values*

Color is a critical quality attribute in blueberry fruits, influencing both consumer perception and nutritional value. The effects of the treatments on color parameters are presented in Table 2. Statistical analysis revealed no significant differences among the MT, BA, or combined MT+BA treatments in terms of color parameters ( $p > 0.05$ ).

**Table 2.** Effect of different treatments on color parameters of blueberry

Treatments	L*	a*	b*	Chroma	h°
Control	45.65 a	-0.15 a	-7.06 a	7.18 a	271.24 a
MT (5 ppm)	45.50 a	0.18 a	-6.72 a	6.80 a	273.20 a
MT (10 ppm)	46.51 a	1.01 a	-6.83 a	7.48 a	283.65 a
MT (15 ppm)	42.83 a	0.85 a	-6.40 a	6.71 a	282.45 a
BA (5 ppm)	46.35 a	0.13 a	-7.92 a	8.23 a	271.95 a
BA (10 ppm)	44.84 a	-0.03 a	-6.70 a	6.94 a	270.44 a
BA (15 ppm)	46.07 a	0.50 a	-6.65 a	6.94 a	279.82 a
MT+BA (2.5+2.5 ppm)	42.60 a	-0.15 a	-6.65 a	6.71 a	270.60 a
MT+BA (5+5 ppm)	45.66 a	0.00 a	-6.48 a	6.95 a	241.57 a
MT+BA (7.5+7.5 ppm)	44.26 a	0.66 a	-6.40 a	6.64 a	277.66 a

Different letters indicate significant differences in each trait according to Duncan's test at  $p < 0.05$  (L\*: lightness; a\*: green-red axis; b\*: blue-yellow axis; chroma: color intensity; h°: hue angle; MT: melatonin; BA: boric acid)

Despite the lack of statistical significance, some trends were observed. The highest values for lightness ( $L^*$ ) and redness ( $a^*$ ) were recorded in the MT (10 ppm) treatment. The MT (15 ppm) treatment yielded the highest yellowness ( $b^*$ ) value. Regarding chroma, the highest value was observed in the BA (5 ppm) treatment, while the hue angle was greatest in the MT (10 ppm) application.

Although these differences were not statistically significant, high chroma values are of particular importance as they are associated with richer pigmentation, which in turn is linked to higher concentrations of health-promoting compounds such as anthocyanins.

*Bioactive components*

Significant differences were observed in several phytochemical attributes of blueberry fruits depending on the treatment applied. AsA content ranged from 13.65 to 24.96 mg/100 g fresh weight (FW). The highest AsA level was recorded in the combined MT+BA treatment at 5+5 ppm, which was significantly greater than all other treatments ( $p < 0.05$ ). In contrast, the lowest AsA concentration was observed in the MT (15 ppm) treatment, while the control group (17.14 mg/100 g FW) exhibited intermediate values, showing no significant difference from several other treatments (Table 3).

Anthocyanin content also varied significantly among treatments ( $p < 0.05$ ). The control group exhibited the lowest value (146.94 mg/100 g FW), whereas the MT+BA (2.5+2.5 ppm) treatment produced the highest anthocyanin content (197.51 mg/100 g FW). Notably, several BA and MT+BA combinations led to a statistically significant increase in anthocyanin concentration compared to the control.

In contrast, TFC and TPC showed no statistically significant differences among treatments ( $p > 0.05$ ), with TFC ranging between 3.58 and 3.81 mg quercetin equivalents (QE)  $g^{-1}$  dry weight (DW), and TPC ranging from 236.00 to 412.00 mg gallic acid equivalents (GAE)  $g^{-1}$  DW.

Antioxidant activity ranged from 25.78% to 26.60%. The highest antioxidant activity was observed in the MT+BA (7.5+7.5 ppm) treatment, which was significantly higher than the MT (5 ppm) treatment ( $p < 0.05$ ). All other treatments, including the control, showed statistically similar antioxidant activity levels.

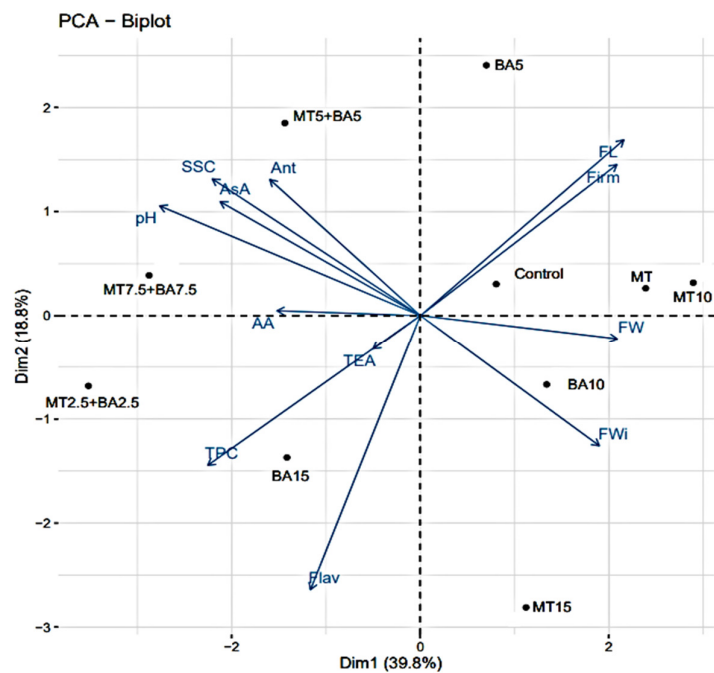
**Table 3.** Effect of different treatments on AsA, TFC, anthocyanin, TPC, and antioxidant activity of blueberry

Treatments	AsA (mg/100g)	Flav (mg QE g <sup>-1</sup> DW)	Ant (mg 100 g <sup>-1</sup> FW)	TPC (mg GAE g <sup>-1</sup> DW)	AA (%)
Control	17.14 bc	3.65 a	146.94 c	337.22 a	26.19 ab
MT (5 ppm)	14.79 bc	3.62 a	181.98 ab	309.67 a	25.78 b
MT (10 ppm)	15.55 bc	3.68 a	157.70 bc	236.00 a	25.99 ab
MT (15 ppm)	13.65 c	3.77 a	144.16 c	352.67 a	26.10 ab
BA (5 ppm)	15.44 bc	3.58 a	195.23 a	270.67 a	25.83 ab
BA (10 ppm)	17.34 bc	3.70 a	195.51 a	287.67 a	25.92 ab
BA (15 ppm)	19.16 b	3.81 a	184.24 ab	380.00 a	25.98 ab
MT+BA (2.5+2.5 ppm)	19.27 b	3.75 a	197.51 a	412.00 a	25.94 ab
MT+BA (5+5 ppm)	24.96 a	3.60 a	192.42 a	335.00 a	26.21 ab
MT+BA (7.5+7.5 ppm)	19.84 b	3.73 a	191.18 a	326.33 a	26.60 a

Different letters indicate significant differences in each trait according to Duncan's test at  $p < 0.05$  (AsA: ascorbic acid; Flav: flavanoid; Ant: anthocyanin; TPC: total phenolic content; AA: antioxidant activity; MT: melatonin; BA: boric acid)

#### Principal component analysis, Pearson correlation and dendrogram clustering

To evaluate the relationships and correlations among treatments and measured variables, Principal Component Analysis (PCA) was conducted, and the results are presented in Figure 1. The PCA revealed that two principal components accounted for a substantial proportion (58.6%) of the total variance among the data.

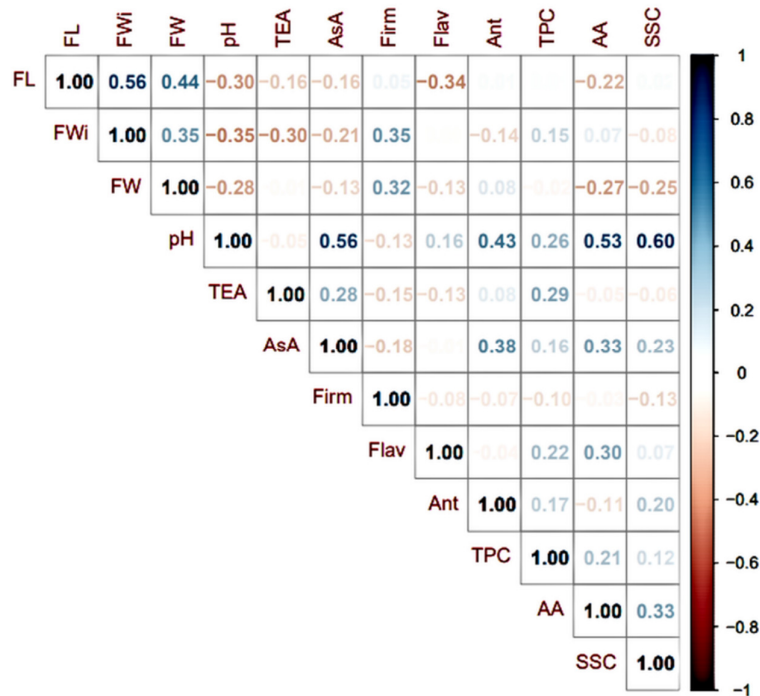
**Figure 1** PCA analysis for applications properties

FL: fruit length; FWI: fruit width; FW: fruit weight; TEA: titratable acidity; AsA: ascorbic acid; Firm: Firmness; Flav: Flavanoid; Ant: Anthocyanin; TPC: total phenolic content; AA: antioxidant activity; SSC: soluble solids content

Among the treatments, MT (10 ppm) and MT+BA (7.5+7.5 ppm) were associated with the highest values for several chemical parameters, whereas the control group exhibited the lowest values in this regard. The PCA biplot also illustrated a strong positive correlation between fruit width and fruit weight, as well as a negative correlation between total flavonoid content and fruit length.

These findings indicate that specific treatments-particularly the combined applications-may influence biochemical traits more distinctly than pomological ones, and that certain quality parameters are interrelated.

Figure 2 presents the correlation matrix illustrating the statistical relationships among the evaluated quality parameters. A strong positive correlation was observed between fruit length (FL) and both fruit width (FWi) ( $r = 0.56$ ) and fruit weight (FW) ( $r = 0.44$ ), indicating that larger fruits in length also tend to be wider and heavier.



**Figure 2** Correlation matrix based on Pearson's correlation coefficients among the quality parameters  
 FL: fruit length; FWi: fruit width; FW: fruit weight; TEA: titratable acidity; AsA: ascorbic acid; Firm: Firmness; Flav: Flavanoid; Ant: Anthocyanin; TPC: total phenolic content; AA: antioxidant activity; SSC: soluble solids content

Moderate positive correlations were detected between pH and AsA content ( $r = 0.56$ ), antioxidant activity ( $r = 0.53$ ), and SSC ( $r = 0.60$ ), suggesting that fruits with higher pH values also exhibit improved nutritional quality. AsA content was positively correlated with anthocyanin concentration ( $r = 0.38$ ), TPC ( $r = 0.33$ ), and antioxidant activity ( $r = 0.33$ ), reinforcing its role in enhancing the antioxidant capacity of the fruit.

Additionally, TFC exhibited a positive correlation with both TPC ( $r = 0.30$ ) and anthocyanin content ( $r = 0.22$ ), indicating that these bioactive components tend to accumulate in parallel.

Conversely, a very weak negative correlation was found between TEA and pH ( $r = -0.05$ ). While this inverse relationship aligns with the expected acid-base chemistry of fruit, the correlation was not statistically significant in this dataset.

Overall, the correlation analysis highlights meaningful interactions among pomological and biochemical traits, particularly emphasizing the contribution of AsA, anthocyanins, and phenolic compounds to the antioxidant properties of blueberry fruits.

## Discussion

Melatonin (MT) is a phytohormone-like signaling molecule that regulates multiple physiological processes in plants, including germination, photosynthesis, fruit ripening, senescence, and stress tolerance (Arnao and Hernández-Ruiz, 2018; Xu *et al.*, 2019). Kumari *et al.* (2023) further highlighted its roles in vegetative and reproductive development, nutrient uptake, ion transport, and hormonal regulation. MT also functions as a potent antioxidant by scavenging reactive oxygen species (ROS), stimulating antioxidant enzyme activity, and reducing lipid peroxidation through the suppression of LOX activity and MDA accumulation, thereby protecting membrane integrity and bioactive compounds (Shang *et al.*, 2021). Although many previous studies have shown that MT can delay softening and preserve bioactive compounds, most of these responses were reported under stress conditions. In contrast, the present experiment was conducted under non-stressful, soilless cultivation, offering new insights into MT activity in optimal growing environments.

Boron (BA) is essential for cell wall stabilization via pectin cross-linking and plays important roles in meristematic growth, carbohydrate transport, nitrogen metabolism, and phytohormone synthesis (Shireen *et al.*, 2018). It promotes pollen tube elongation, flowering, and fruit formation. Blueberries are particularly sensitive to boron availability, with both deficiency and toxicity negatively affecting growth and fruit quality. Optimal leaf tissue levels range between 20-60 ppm, while root-zone concentrations of 0.5-1 ppm are considered adequate; however, boron mobility is often limited in acidic substrates preferred by blueberries (García-Sánchez *et al.*, 2020).

Several studies have shown that combined MT and BA treatments can enhance yield and improve disease resistance, likely due to the complementary contributions of BA to cell wall stability and nutrient transport, and MT to oxidative stress mitigation (Sider *et al.*, 2022). Additionally, synergistic interactions between MT and BA have been demonstrated under abiotic stress conditions such as drought and salinity (Hussain *et al.*, 2020; Zhang *et al.*, 2020; Liu *et al.*, 2021). BA mainly affects cell wall-associated processes and enzyme activity, whereas MT modulates antioxidant and hormonal processes, offering complementary physiological functions (Ahmad *et al.*, 2021).

In the present study, however, MT and BA applications did not significantly affect fruit size, firmness, SSC, or external color attributes. This limited response may be attributed to several factors. First, MT activity is highly dose-dependent, and both insufficient and excessive concentrations may fail to elicit physiological changes (Carrión-Antolí *et al.*, 2022). Second, the timing of application is critical, and suboptimal phenological timing may suppress expected MT effects. MT can also interact with ethylene biosynthesis, potentially accelerating ripening in some species, although responses vary by fruit type and cultivar (Verde *et al.*, 2022). Environmental factors such as temperature, light intensity, and humidity further influence MT efficacy, and optimal conditions may reduce the activation of antioxidant pathways (Liu *et al.*, 2018).

Findings from Zhang *et al.* (2017) on 'Brigitta' blueberry support our results. Their study reported that high MT doses (100-300 mg·L<sup>-1</sup>) increased fruit weight and TEA content while having no effect on SSC, consistent with our observations. Similarly, Sun *et al.* (2014) noted that MT accelerated tomato ripening and improved flavor and color traits, though concentrations  $\geq 200$  mg·L<sup>-1</sup> negatively affected quality. These results emphasize the importance of optimizing both dose and timing.

The most prominent treatment effects in our study were observed in AsA, anthocyanins, and antioxidant activity. Combined MT+BA treatments produced the highest values in all three parameters. AsA content peaked under MT+BA (5+5 ppm), whereas anthocyanin content increased across all combination treatments (2.5+2.5, 5+5, 7.5+7.5 ppm). The highest antioxidant activity occurred under MT+BA (7.5+7.5 ppm). BA alone at 5 and 10 ppm also significantly enhanced anthocyanins, while the control consistently showed the lowest levels. These results indicate a synergistic interaction between MT and BA in enhancing or stabilizing bioactive compounds, likely through complementary effects on antioxidant metabolism and cell wall

function (Dou *et al.*, 2022; Qu *et al.*, 2022). Liu *et al.* (2018) also reported MT-induced increases in anthocyanins and flavor compounds in strawberries.

Previous studies have also shown that MT alleviates boron toxicity by reinforcing cell wall polysaccharides and regulating membrane permeability (Li *et al.*, 2013; Moussa and Algamal, 2017; Al-Huqail *et al.*, 2020). Unlike those studies, the BA concentrations used here were non-toxic, and MT+BA treatments exhibited synergistic rather than antagonistic effects.

Wenfei *et al.* (2023) demonstrated that preharvest MT and chitosan treatments improved the nutraceutical quality of blueberries by enhancing anthocyanins and total phenolics, findings consistent with our results. PCA analysis (Figure 1) further supported these biochemical patterns, showing strong positive correlations between fruit width and weight, a weak negative association between flavonoids and fruit length, and moderate positive relationships among pH, AsA, anthocyanins, and antioxidant activity. These associations indicate that specific biochemical traits co-vary in response to MT and BA treatments.

Overall, this study provides novel evidence that MT and BA applications can enhance the bioactive quality of blueberries even under non-stress, soilless conditions. The observed synergistic effects, particularly in combined treatments, contribute valuable insights into the regulation of fruit biochemical traits in optimal growing environments.

## Conclusions

This study demonstrated that preharvest foliar applications of melatonin (MT) and boric acid (BA) influenced key biochemical quality attributes of southern highbush blueberry cv. 'Camellia' grown under soilless conditions. While MT and BA alone did not significantly affect pomological traits, combined treatments produced clear synergistic effects, particularly on antioxidant-related parameters. The MT + BA application at 5 + 5 ppm resulted in the highest ascorbic acid (AsA) content, whereas anthocyanin concentration and antioxidant activity were maximized under the 7.5 + 7.5 ppm treatment. BA alone at 5 and 10 ppm also enhanced anthocyanin accumulation. These findings highlight the potential of MT and BA combinations to improve nutritionally important bioactive compounds in blueberries under non-stress conditions. Future studies should focus on optimizing doses and application timing and investigating the associated physiological and molecular mechanisms to better understand how MT and BA regulate bioactive compound synthesis and antioxidant capacity.

## Authors' Contributions

Conceptualization: VO and HIS; Data curation: NU and HK; Formal analysis: NU and HK; Investigation: VO, PK, and TS; Methodology: PK; Resources: MU; Software: NU; Supervision: VO and HIS; Visualization: MU; Writing - original draft: HSH; Writing - review and editing: VO, NU, PK, MU, HK, TS, HSH, and HIS.

All authors read and approved the final manuscript.

## Acknowledgements

This study was not supported by any funding agency or institution.

## Conflict of Interests

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

## References

- Ahmad S, Muhammad I, Wang GY, Zeeshan M, Yang L, Ali I, Zhou XB (2021). Ameliorative effect of MT improves drought tolerance by regulating growth, photosynthetic traits and leaf ultrastructure of maize seedlings. *BMC Plant Biology* 21(1):368. <https://doi.org/10.1186/s12870-021-03160-w>
- Al-Huqail AA, Khan MN, Ali HM, Siddiqui MH, Al-Huqail AA, AlZuaibr FM, ... Al-Humaid LA (2020). Exogenous MT mitigates boron toxicity in wheat. *Ecotoxicology and Environmental Safety* 201:110822. <https://doi.org/10.1016/j.ecoenv.2020.110822>
- Arnao MB, Hernández-Ruiz J (2018) MT and its relationship to plant hormones. *Annals of Botany* 121(2):195-207. <https://doi.org/10.1093/aob/mcx114>
- Arunkumar BR, Thippeshappa GN, Anjali MC, Prashanth KM (2018). Boron: A critical micronutrient for crop growth and productivity. *Journal of Pharmacognosy and Phytochemistry* 7(2):2738-2741.
- Bal E (2021). Effect of melatonin treatments on biochemical quality and postharvest life of nectarines. *Journal of Food Measurement and Characterization* 15(1):288-295. <https://doi.org/10.1007/s11694-020-00636-5>
- Balbontín C, Ayala H, M Bastías R, Tapia G, Ellena M, Torres C, ... Silva H (2013). Cracking in sweet cherries: A comprehensive review from a physiological, molecular, and genomic perspective. *Chilean Journal of Agricultural Research* 73(1):66-72. <http://dx.doi.org/10.4067/S0718-58392013000100010>
- Bor JY, Chen HY, Yen GC (2006). Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. *Journal of Agricultural and Food Chemistry* 54(5):1680-1686. <https://doi.org/10.1021/jf0527448>
- Bozhuyuk MR, Ercisli S, Ayed RB, Jurikova T, Fidan H, Ilhan G, ... Sagbas HI (2020). Compositional diversity in fruits of rowanberry (*Sorbus aucuparia* L.) genotypes originating from seeds. *Genetika* 52(1):55-65. <https://doi.org/10.2298/GENSR2001055B>
- Carrión-Antolí A, Martínez-Romero D, Guillén F, Zapata PJ, Serrano M, Valero D (2022). Melatonin pre-harvest treatments leads to maintenance of sweet cherry quality during storage by increasing antioxidant systems. *Frontiers in Plant Science* 13:863467. <https://doi.org/10.3389/fpls.2022.863467>
- Chang CC, Yang MH, Wen HM, Chern JC (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 10(3):3. <https://doi.org/10.38212/2224-6614.2748>
- Dou J, Wang J, Tang Z, Yu J, Wu Y, Liu Z, ... Tian Q (2022). Application of exogenous melatonin improves tomato fruit quality by promoting the accumulation of primary and secondary metabolites. *Foods* 11(24):4097. <https://doi.org/10.3390/foods11244097>
- Fernández V, Sotiropoulos T, Brown PH (2013). Foliar fertilization: Scientific principles and field practices (1st ed). International Fertilizer Industry Association (IFA). Paris, France. <https://www.fertilizer.org/resource/foliar-fertilization-scientific-principles-and-field-practices/>
- García-Sánchez F, Simón-Grao S, Martínez-Nicolás JJ, Alfosea-Simón M, Liu C, Chatzissavvidis C, ... Cámara-Zapata JM (2020). Multiple stresses occurring with boron toxicity and deficiency in plants. *Journal of Hazardous Materials* 5(397):122713. <https://doi.org/10.1016/j.jhazmat.2020.122713>
- Giusti MM, Wrolstad RE (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal* 14(3):217-225. [https://doi.org/10.1016/S1369-703X\(02\)00221-8](https://doi.org/10.1016/S1369-703X(02)00221-8)
- Hu W, Yang H, Tie W, Yan Y, Ding Z, Liu Y, ... Jin Z (2017). Natural variation in banana varieties highlights the role of melatonin in postharvest ripening and quality. *Journal of Agricultural and Food Chemistry* 65:9987-9904. <https://doi.org/10.1021/acs.jafc.7b03354>
- Hussain M, Ali Q, Nasir F (2020). Synergistic effects of boron and melatonin in improving plant tolerance to abiotic stress. *Journal of Plant Nutrition and Soil Science* 183(4):587-597. <https://doi.org/10.1002/jpln.201900607>

- Jannatizadeh A (2019). Exogenous melatonin applying confers chilling tolerance in pomegranate fruit during cold storage. *Scientia Horticulturae* 27(246):544-549. <https://doi.org/10.1016/j.scienta.2018.11.027>
- Khalaj K, Ahmadi N, Souri MK (2017). Improvement of postharvest quality of asian pear fruits by foliar application of boron and calcium. *Horticulturae* 3(1):15. <https://doi.org/10.3390/horticulturae3010015>
- Kumari A, Singh SK, Mathpal B, Verma KK, Garg VK, Bhattacharyya M, Bhatt R (2023). The biosynthesis, mechanism of action, and physiological functions of melatonin in horticultural plants: A review. *Horticulturae* 9(8):913. <https://doi.org/10.3390/horticulturae9080913>
- Li C, Feng J, Huang WY, An XT (2013). Composition of polyphenols and antioxidant activity of rabbiteye blueberry (*Vaccinium ashei*) in Nanjing. *Journal of Agricultural and Food Chemistry* 61(3):523-31. <https://doi.org/10.1021/jf3046158>
- Liu C, Zheng H, Sheng K, Liu W, Zheng L (2018). Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology* 139:47-55. <https://doi.org/10.1016/j.postharvbio.2018.01.016>
- Liu S, Huang H, Huber DJ, Pan Y, Shi X, Zhang Z (2020). Delay of ripening and softening in 'Guifei' mango fruit by postharvest application of melatonin. *Postharvest Biology and Technology* 163:111136. <https://doi.org/10.1016/j.postharvbio.2020.111136>
- Liu Y, Zhang M, Zhang Z (2021). The role of melatonin in enhancing plant growth and stress tolerance. *Frontiers in Plant Science* 12:697315. <https://doi.org/10.3389/fpls.2021.697315>
- Lorente-Mento JM, Guillén F, Castillo S, Martínez-Romero D, Valverde JM, Valero D, Serrano M (2021). Melatonin treatment to pomegranate trees enhances fruit bioactive compounds and quality traits at harvest and during postharvest storage. *Antioxidants* 10(6):820. <https://doi.org/10.3390/antiox10060820>
- Mansouri S, Koushesh Saba M, Sarikhani H (2023). Exogenous melatonin delays strawberry fruit ripening by suppressing endogenous ABA signaling. *Scientific Reports* 13:14209. <https://doi.org/10.1038/s41598-023-41311-1>
- Moussa HR, Algamal SM (2017). Does exogenous application of melatonin ameliorate boron toxicity in spinach plants?. *International Journal of Vegetable Science* 23(3):233-245. <https://doi.org/10.1080/19315260.2016.1243184>
- Okatan V, Aşkın MA, Polat M, Bulduk I, Çolak AM, Güçlü SF, ... Caruso G (2023). Effects of melatonin dose on fruit yield, quality, and antioxidants of strawberry cultivars grown in different crop systems. *Agriculture* 13(1):71. <https://doi.org/10.3390/agriculture13010071>
- Pereira GL, Siqueira JA, Batista-Silva W, Cardoso FB, Nunes-Nesi A, Araújo WL (2021). Boron: More than an essential element for land plants? *Frontiers in Plant Science* 14(11):610307. <https://doi.org/10.3389/fpls.2020.610307>
- Qu G, Wu W, Ba L, Ma C, Ji N, Cao S (2022). MT enhances the postharvest disease resistance of blueberries fruit by modulating the jasmonic acid signaling pathway and phenylpropanoid metabolites. *Frontiers in Chemistry* 22(10):957581. <https://doi.org/10.3389/fchem.2022.957581>
- Retamales JB, Hancock JF (2018). *Blueberries: Crop production science in horticulture* (2nd ed.). CABI. Boston, MA, USA.
- Rivera S, Giongo L, Cappai F, Kerckhoffs H, Sofkova-Bobcheva S, Hutchins D, East A (2022). Blueberry firmness-a review of the textural and mechanical properties used in quality evaluations. *Postharvest Biology and Technology* 192:112016. <https://doi.org/10.1016/j.postharvbio.2022.112016>
- Routray W, Orsat V (2011). Blueberries and their anthocyanins: factors affecting biosynthesis and properties. *Comprehensive Reviews in Food Science and Food Safety* 10(6):303-320. <https://doi.org/10.1111/j.1541-4337.2011.00164.x>
- Shang F, Liu R, Wu W, Han Y, Fang X, Chen H, Gao H (2021). Effects of melatonin on the components, quality and antioxidant activities of blueberry fruits. *LWT – Food Science and Technology* 147:111582. <https://doi.org/10.1016/j.lwt.2021.111582>
- Sharafi Y, Aghdam MS, Luo Z, Jannatizadeh A, Razavi F, Fard JR, Farmani B (2019). Melatonin treatment promotes endogenous melatonin accumulation and triggers GABA shunt pathway activity in tomato fruits during cold storage. *Scientia Horticulturae* 254:222-227. <https://doi.org/10.1016/j.scienta.2019.04.056>
- Shi C, Zhang J, Jia Z, Yang X, Zhou Z (2021). Intelligent pH indicator films containing anthocyanins extracted from blueberry peel for monitoring tilapia fillet freshness. *Journal of the Science of Food and Agriculture* 101(5):1800-1811. <https://doi.org/10.1002/jsfa.10794>

- Shireen F, Nawaz MA, Chen C, Zhang Q, Zheng Z, Sohail H, ... Bie Z (2018). Boron: functions and approaches to enhance its availability in plants for sustainable agriculture. *International Journal of Molecular Sciences* 19(7):1856. <https://doi.org/10.3390/ijms19071856>
- Sider J, Smith A, Lee T (2022). Effects of boron and melatonin on plant growth and stress tolerance. *Journal of Agricultural Science* 58(3):124-138. <https://doi.org/10.1016/j.jags.2022.01.023>
- Silva S, Costa EM, Veiga M, Morais RM, Calhau C, Pintado M (2020). Health promoting properties of blueberries: A review. *Critical Reviews in Food Science and Nutrition* 60(2):181-200. <https://doi.org/10.1080/10408398.2018.1518895>
- Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3):144-58. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Sun Q, Zhang N, Wang J, Zhang H, Li D, Shi J, Zhao B ... Guo YD (2014). Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. *Journal of Experimental Botany* 66(3):657-668. <https://doi.org/10.1093/jxb/eru332>
- Takano J, Tanaka M (2023). Boron-sensing mechanisms involved in boron transport regulation in plants. *Journal of Plant Nutrition and Soil Science* 1-8. <https://doi.org/10.1002/jpln.202300029>
- Verde A, Míguez JM, Gallardo M (2022). Role of MT in apple fruit during growth and ripening: possible interaction with ethylene. *Plants* 11(5):688. <https://doi.org/10.3390/plants11050688>
- Wang LJ, Wu J, Wang HX, Li, SS, Zheng XC, Du H, ... Wang LS (2015). Composition of phenolic compounds and antioxidant activity in the leaves of blueberry cultivars. *Journal of Functional Foods* 16:295-304. <https://doi.org/10.1016/j.jff.2015.04.027>
- Wenfei J, Xiaoqiong W, Jingheng M, Lixin W, Linyu L, Jinying L, ... Lin W (2023). Exogenous application of melatonin improves the growth and physiological properties of blueberry seedlings under salt stress. *Biotechnology & Biotechnological Equipment* 37(1):2202781. <https://doi.org/10.1080/13102818.2023.2202781>
- Xu T, Chen Y, Kang H (2019). Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. *Frontiers in Plant Science* 10:1388. <https://doi.org/10.3389/fpls.2019.01388>
- Yang W, Yang H, Ling L Chun C, Peng L (2023). Tolerance and physiological responses of *Citrus* rootstock cultivars to boron toxicity. *Horticulturae* 9(1):44. <https://doi.org/10.3390/horticulturae9010044>
- Yousef EAA, Nasef I (2023). Effect of boron foliar application and different fruit-set orders on summer squash seed yield and quality. *Egyptian Journal of Horticulture* 50(1):85-95. <https://doi.org/10.21608/ejoh.2023.167396.1216>
- Zhang L, Liu Z, Wang Y (2020). Effects of boron and melatonin on plant growth and stress resistance. *Plant Growth Regulation* 92(2):241-252. <https://doi.org/10.1007/s10725-020-00523-6>
- Zhang XC, Zhu YQ, Wang YN Luo C, Wang X (2017). Effects of different plant growth regulators on blueberry fruit quality. In: *IOP Conference Series: Earth and Environmental Science*. IOP Publishing p 012038. <https://doi.org/10.1088/1755-1315/81/1/012038>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



**License** - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

**Notes:**

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.