

Preharvest application of biostimulants to improve growth, quality, and yield of blueberry (*Vaccinium corymbosum* L.)

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Abstract

Blueberry production and fruit quality face increasing challenges under current climate change scenarios. Therefore, sustainable strategies such as the use of biostimulants, which have shown beneficial effects that enhance productivity and quality, are needed. This study evaluated the effect of foliar and drench preharvest applications of biostimulants on the growth, yield, and fruit quality of blueberry. The experiment was conducted using plants grown in coconut fiber under greenhouse conditions in a randomized factorial design with three factors: biostimulant, application method, and application dose. The biostimulants tested included melatonin, salicylic acid, glutamic acid, silicon, and yeast extract, applied biweekly from anthesis to fruit maturity. Growth parameters, yield, size, color, and quality traits were measured. The drench application of 100 μ M melatonin resulted in the highest yield and plant height, whereas stem diameter increased mainly with 100 μ M foliar melatonin. Foliar application of 1000 mg L⁻¹ glutamic acid significantly enhanced the SPAD index. Fruit weight, width and height were superior under 4 mM salicylic acid applied via drench and 500 mg L⁻¹ glutamic acid applied foliar. The width/height ratio reached its highest value with 100 μ M foliar melatonin. Foliar silicon at 5 g L⁻¹ increased fruit lightness, while 3 g L⁻¹ yeast extract applied via drench enhanced chroma, with no significant differences in hue. All biostimulants improved fruit firmness; additionally, foliar silicon increased total soluble solids, and 10 g L⁻¹ yeast extract applied foliar enhanced anthocyanin content. Biostimulants applications improved growth, yield, and fruit quality, supporting their potential for sustainable blueberry production.

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Introduction

Blueberry (*Vaccinium corymbosum* L.; Ericaceae) has become one of the most popular berries worldwide due to its fruits' high antioxidant activity, phenolic compounds, organic acids, and elevated vitamin C content (Popova, 2019; Duan *et al.*, 2022). Regular consumption of blueberries is associated with numerous human health benefits, including prevention of diseases such as type 2 diabetes, urinary tract infections, cardiovascular, neurodegenerative, and intestinal disorders (Silva *et al.*, 2020; Onuh *et al.*, 2023; Stull *et al.*, 2024). Besides health benefits, consumers also prefer blueberries with good appearance, firmness, bloom (wax coating), and specific sensory attributes such as a strong sweet-tart flavor (Gilbert *et al.*, 2014). The global blueberry market reached an estimated value of approximately 15 billion USD in 2023, with significant growth expected over the next decade. China, the United States, Peru, Chile, and Mexico are the leading producers supplying fresh consumption markets (Brazelton *et al.*, 2022). Mexico exported around 73,000 tons during the 2023-2024 season, with the United States as its primary destination.

However, blueberry production and quality are challenged by unfavorable conditions linked to climate change, including extreme temperatures, low relative humidity, increased UV levels, high salinity, and drought (Lobos and Hancock, 2015; Bădescu *et al.*, 2016; Tasnim *et al.*, 2021). Therefore, strategies that improve blueberry growth, productivity, and quality are needed. One sustainable alternative is the use of biostimulants, defined as substances or microorganisms applied to plants to enhance nutrient efficiency, stress tolerance, and/or crop quality traits, independent of nutrient content (Du Jardin, 2015). Thus, preharvest application of biostimulants could complement or substitute conventional agronomic practices due to their minimal environmental impact and potential to reduce pesticide and chemical fertilizer use while promoting blueberry productivity and fruit quality.

Some biostimulants, such as melatonin (N-acetyl-5-methoxytryptamine), salicylic acid, glutamate, silicon, and yeast extracts have shown favorable responses when applied to crops. Melatonin at 100 $\mu\text{mol L}^{-1}$ significantly increased root, stem, plant height, and leaf area growth in maize plants (Erdal, 2019). Foliar salicylic acid application improved fruit quality and yield in tomato (Sariñana-Aldaco *et al.*, 2020). Glutamic acid applied to soil or foliage increased yield and quality in carrot crops (Rosa *et al.*, 2023). Silicon dioxide applications in lettuce under water stress improved plant physiology, tolerance, and biofortification with Si (Hidalgo-Santiago *et al.*, 2021). Similarly, yeast application during preharvest enhanced growth and yield in broccoli (Ali and Mutar, 2023).

The responses induced by these biostimulants may relate to their specific functionalities. Melatonin protects the photosynthetic and stomatal systems and regulates enzymes involved in carbohydrate, lipid, amino acid, nitrogen, sulfur, and phosphorus metabolism (Arnao and Hernández-Ruiz, 2019). Salicylic acid mitigates abiotic stress by acting as a signaling molecule that induces stress-responsive gene expression and participates in defense signaling pathways (Song *et al.*, 2023). Glutamate functions as a signaling molecule involved in seed germination, root architecture, pollen germination, and pollen tube growth (Qiu *et al.*, 2020). Silicon enhances photosynthetic capacity, nutrient uptake, and secondary metabolism by promoting antioxidant compound production (Maghsoudi *et al.*, 2016). Yeast extracts, rich in nucleotides and amino acids from bakery fermentation, stimulate plant metabolism, improving nutrient absorption and resilience to stress factors (Dima *et al.*, 2020).

Research on preharvest biostimulant use in blueberry is limited, and information on the most effective biostimulant types, application methods and doses is needed. Therefore, this study aimed to evaluate the effects of foliar and drench preharvest biostimulant applications on blueberry growth, quality, and yield.

Materials and Methods

Experimental site

The study was conducted at the Department of Horticulture of the Universidad Autonoma Agraria Antonio Narro, located in Saltillo, Coahuila, Mexico. The experiment took place in a tunnel-type greenhouse equipped with fiberglass walls, a heating system, wet wall, and exhaust fans. To monitor environmental conditions inside the greenhouse, a WatchDog 2475 Plant Growth Station mini weather station (Spectrum Technologies, Plainfield, IL 60585, USA) was installed at canopy height. During the experiment, the average maximum and minimum temperatures were 25 °C and 8 °C, respectively (Figure 1). The mean relative humidity was 60%, ranging from 30% to 80%. The average photosynthetically active radiation (PAR) was 555 $\mu\text{mol m}^{-2} \text{s}^{-1}$, fluctuating between 1,490 and 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ depending on sky cloud cover.

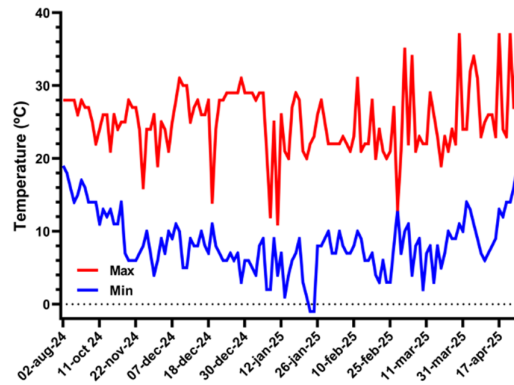


Figure 1. Daily average of maximum and minimum temperatures (°C) inside the greenhouse during the experiment

Crop management and experimental design

First-year highbush blueberry plants (*Vaccinium corymbosum* L.) corresponding to the genotype designated in this study as cultivar S-2525 at BBCH stage 15 (Wichura *et al.*, 2024), were transplanted into 30-L polypropylene pots (PHOTU[®] model, DIDIHU[®], Mexico) filled with coconut fiber-based growing media (DIDIHU[®], Mexico). The substrate physical properties were 75.48% total porosity, 47.27% air-filled porosity, and 28.20% water-holding capacity.

Nutrient management was adjusted according to the phenological stage (Table 1) and supplied via a drip irrigation system with four 15 cm emitters per pot, delivering approximately 1.1-1.4 L per day. Irrigation was applied in short pulses in the morning (7:00 a.m. to 10:00 a.m.) and in the evening (around 5:30 p.m.), when the vapor pressure deficit remained below 1.5 kPa.

Table 1. Ionic concentration of the nutrient solution (in mEq L⁻¹) used at different phenological stages of blueberry (*V. corymbosum*)

Phenological stage	EC (dS m ⁻¹)	pH	NO ₃ ⁻	NH ₄ ⁺	H ₂ PO ₄ ⁻	SO ₄ ²⁻	K ⁺	Ca ²⁺	Mg ²⁺
Vegetative	0.7-0.8	5.5-5.8	2	3	1.0	4.0	2.0	2	1.0
Flower bud differentiation and flowering	0.8-0.9	5.5-5.8	3	1	1.5	4.5	3.5	2	1.5
Fruit production	1.0-1.1	5.5-5.8	3	1	1.5	5.0	4.0	3	1.5

EC: Electrical conductivity; pH: Hydrogen potential; NO₃⁻: Nitrate; NH₄⁺: Ammonium; H₂PO₄⁻: Phosphoric acid; SO₄²⁻: Sulfate; K⁺: Potassium; Ca²⁺: Calcium; Mg²⁺: Magnesium

After two months of establishment (BBCH 19), a formative pruning was performed at 30 cm height, removing leaves and thin shoots from the base of the crown. The experimental setup followed a completely randomized design with a factorial arrangement of three factors: Biostimulant (B) × Application Method (AM) × Dose Application (DA). The combination of these factors resulted in a total of 22 treatments, including a control group treated with distilled water for both foliar and drench applications (Table 2).

Table 2. Treatments with biostimulants, application method, and dosage applied to blueberry (*Vaccinium corymbosum* L.)

Treatments	Biostimulants	Application method	Dosage application
T1	Distilled water	Foliar	SD
T2	Distilled water	Drench	SD
T3	Melatonin (MT)	Foliar	50 µM
T4	Melatonin (MT)	Foliar	100 µM
T5	Melatonin (MT)	Drench	50 µM
T6	Melatonin (MT)	Drench	100 µM
T7	Salicylic acid (SA)	Foliar	2 mM
T8	Salicylic acid (SA)	Foliar	4 mM
T9	Salicylic acid (SA)	Drench	2 mM
T10	Salicylic acid (SA)	Drench	4 mM
T11	Glutamic acid (GA)	Foliar	500 mg L ⁻¹
T12	Glutamic acid (GA)	Foliar	1000 mg L ⁻¹
T13	Glutamic acid (GA)	Drench	500 mg L ⁻¹
T14	Glutamic acid (GA)	Drench	1000 mg L ⁻¹
T15	Silicon (Si)	Foliar	5 g
T16	Silicon (Si)	Foliar	10 g
T17	Silicon (Si)	Drench	2 g
T18	Silicon (Si)	Drench	4 g
T19	Yeast extract (YE)	Foliar	5 g
T20	Yeast extract (YE)	Foliar	10 g
T21	Yeast extract (YE)	Drench	3 g
T22	Yeast extract (YE)	Drench	6 g

The biostimulants used were: melatonin (MT) at 98% purity (M5250, Sigma-Aldrich), salicylic acid (SA) at 99% (Jalmek), glutamic acid (GA) at 99% (Jalmek), silicon dioxide (SI) at 92% (Diatomix), and yeast extract (YE) (*Saccharomyces cerevisiae* - Nevada). All biostimulants were applied every two weeks during anthesis (open flower stage) and fruit development (BBCH 60 to 89).

Evaluated parameters

Yield, growth, and SPAD index

Yield per plant was determined by counting the number of ripe fruits per plant. Fruits were weighed by treatment using a precision electronic scale (Esnova SE-2000, ±0.1 g) (Esnova, Mexico City). For growth quantification, after harvest, plant height of the longest shoot (cm) was measured from the base of the stem to the apex using a measuring tape (Trupper, Mexico), and stem diameter (mm) was recorded at the base of the plant using a caliper (Stereon HER-411, Stereon, Mexico). Additionally, SPAD index (Soil Plant Analysis Development) were recorded by taking four random readings per plant from fully expanded young leaves at midday using a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Osaka, Japan).

Fruit weight and size

Individual fruit weight (g) was recorded using a precision electronic balance (Esnova SE-2000, ± 0.1 g). Fruit width (equatorial diameter) (mm) and height (polar diameter) (mm) were measured with a manual caliper (Scienceware H134160001, Scienceware, Wayne, NJ, USA) with 0.1 mm accuracy. The width/height ratio was estimated by dividing the two values. For weight and size measurements, eight mature, freshly harvested, and damage-free fruits were selected per treatment.

Fruit skin color

Fruit skin color was assessed using a compact colorimeter (CR-300, Konica Minolta, Osaka, Japan). Measurements were expressed in CIE L^* , a^* , and b^* coordinates, with eight readings per treatment. The L^* value represents lightness, on a scale from 0 (black) to 100 (white). From L^* , a^* , and b^* values, the hue angle ($h^\circ = \arctan [b/a]$) and chroma index ($C = \sqrt{a^2 + b^2}$) were calculated, following McGuire (1992). Hue angle describes perceived color tone on a circular scale from 0° to 360° , where 0° and 360° indicate red-purple, 90° yellow, 180° bluish green, and 270° blue (Spinardi *et al.*, 2019). Chroma reflects color intensity, indicating how saturated or pure the color is compared to neutral gray.

Fruit quality

Fruit firmness (kg/cm^2) was measured in eight fruits per treatment using a manual penetrometer (GY-2) with a range of 0.2 - 4 kg/cm^2 . Total soluble solids (TSS) ($^\circ\text{Brix}$) were individually measured in eight fruits using a handheld refractometer (Atago ATC-1E, Atago, Tokyo, Japan) with a range of 0 - 32 $^\circ\text{Brix}$.

Anthocyanin content was determined using the differential pH method as described by Giusti and Wrolstad (2001). For this, 50 mg of lyophilized fruit tissue were weighed and 5 mL of ethanol acidified with 1% hydrochloric acid were added. The mixture was centrifuged at 4000 rpm for 15 minutes at 0°C . Two reaction phases were prepared: in the first, 400 μL of the extract were mixed with 1600 μL of 0.025 M potassium chloride (KCl) solution adjusted to pH 1.0; in the second, 400 μL of the extract were mixed with 1600 μL of 0.4 M sodium acetate solution adjusted to pH 4.5. Absorbance of both mixtures was measured at 520 and 700 nm using a UV-visible spectrophotometer (Jenway 6320D, Staffordshire, UK). Anthocyanin concentration was calculated using Equation S3 and expressed as $\text{mg } 100 \text{ g}^{-1}$ dry weight.

Statistical analysis

Data were tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test). A three-way ANOVA (B^*AM^*DA) was performed ($p < 0.05$), followed by Tukey's multiple comparison test ($p < 0.05$), using InfoStat software (v2020, National University of Córdoba, Córdoba, Argentina). A correlation matrix in heat map format was generated using GraphPad Prism version 9.3.0 (San Diego, California, USA), applying Pearson's correlation coefficient ($p < 0.05$). The results were color-coded, with red indicating negative correlations (-1), blue for positive correlations (+1), and white for neutral correlations (0).

Results

Yield, growth, and SPAD index

Highly significant differences were detected among biostimulant (B) treatments for yield ($p < 0.001$; Table 3). All biostimulants significantly outperformed the control. Melatonin (MT) produced the highest yield increase, achieving 41.0% above the untreated control, followed by yeast extract (YE, 38%), silicon (SI, 34%), glutamic acid (GA, 33%) and salicylic acid (SA, 31%). Regarding the application method (AM), drench application was slightly more effective than foliar spray, with a 1.3% increase. Significant differences were also observed between doses, where dose B produced 5.0% higher yield than dose A. Significant interactions were found for B^*AM , B^*DA , and B^*AM^*DA . In the three-way interaction, the best treatment was T6 (100 μM MT) applied via drench, with a mean yield of 362.65 grams per plant (Table 4).

Table 3. Analysis of variance and mean comparison for yield, plant height, stem diameter, and SPAD index in blueberry plants as affected by biostimulants (B), application method (AM), and dose application (DA), including their interactions

Source of variation	Yield (g)	Plant height (cm)	Stem diameter (mm)	SPAD index
Biostimulants (B)				
Control (C)	246.53 ± 2.70 e	65.23 ± 0.15 c	16.80 ± 0.09 d	58.58 ± 0.10 e
Melatonin (MT)	347.49 ± 3.63 a	81.40 ± 1.30 a	23.48 ± 0.39 a	63.33 ± 0.68 c
Salicylic Acid (SA)	323.12 ± 1.86 d	79.69 ± 0.87 b	22.34 ± 0.26 b	64.06 ± 0.89 c
Glutamic Acid (GA)	328.47 ± 3.10 c	80.03 ± 1.45 ab	21.35 ± 0.20 c	65.39 ± 0.69 b
Silicon (SI)	330.65 ± 2.35 c	79.35 ± 1.10 b	23.08 ± 0.27 a	66.86 ± 0.39 a
Yeast Extract (YE)	340.16 ± 1.95 b	80.14 ± 0.55 ab	22.18 ± 0.23 b	61.76 ± 0.47 d
Application Method (AM)				
Foliar (F)	317.35 ± 5.30 b	76.57 ± 0.91 b	21.57 ± 0.37 a	62.85 ± 0.58 a
Drench (D)	321.46 ± 4.92 a	78.71 ± 1.05 a	21.51 ± 0.34 a	63.15 ± 0.59 a
Dose application (DA)				
Dose A	311.60 ± 4.84 b	77.67 ± 0.99 a	21.52 ± 0.34 a	63.10 ± 0.56 a
Dose B	327.20 ± 5.15 a	77.61 ± 1.00 a	21.55 ± 0.37 a	62.90 ± 0.61 a
Significance ^(x)				
B	***	***	***	***
AM	***	***	ns	ns
DA	***	ns	ns	ns
B*AM	***	***	***	***
B*DA	***	***	***	ns
AM*DA	ns	***	ns	***
B*AM*DA	***	***	**	***
C.V (%)	3.17	1.84	3.16	1.95

^xEach value represents the mean of eight observations \pm standard error ($n = 4$). ^xSignificance level: ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. C.V. = coefficient of variation. Means followed by the same letter within a column are not significantly different according to Tukey's test ($p < 0.05$)

Plant height also showed highly significant differences among biostimulant treatments ($p < 0.001$, Table 3). All biostimulants increased plant height relative to the control, with MT again producing the greatest increase, 24.8% above the control. Regarding application method, drench was more effective than foliar application, with a 2.8% difference. Although no significant differences were detected between doses, significant interactions were found for B*AM, B*DA, AM*DA, and B*AM*DA. In the three-way interaction, treatment T6 (100 μ M MT, drench) resulted in the greatest plant height, reaching 87.28 cm (Table 4).

Stem diameter exhibited highly significant differences among biostimulant treatments ($p < 0.001$). MT produced the largest stem diameter, corresponding to a 39.8% increase relative to the control. Neither application method nor dose showed significant main effects. However, significant interactions were detected for B*AM, B*DA, and B*AM*DA. In the three-way interaction, the best treatment was T4 (100 μ M MT, foliar), which produced a stem diameter of 25.65 mm (Table 4).

Table 4. Means values and Tukey's multiple comparison test for blueberry yield, plant height, stem diameter and chlorophyll index (SPAD) for the triple interaction among biostimulants, application method, and dose application

Treatment	Yield (g)	Plant height (cm)	Stem diameter (mm)	SPAD index
C Foliar	246.01 k	65.16 j	16.86 g	58.76 k
C Drench	247.05 k	65.30 j	16.74 g	58.66 k
MT 50 μ M Foliar	335.90 def	73.98 i	23.28 bc	63.70 defghi
MT 100 μ M Foliar	356.93 a	81.53 cdef	25.65 a	60.28 j
MT 50 μ M Drench	334.50 defg	82.83 bcde	22.50 bcdef	63.33 defghi
MT 100 μ M Drench	362.65 a	87.28 a	22.47 bcdef	66.03 abcde
SA 2mM Foliar	318.05 ij	84.43 abc	22.83 bcde	66.65 abcd
SA 4mM Foliar	333.58 defg	77.15 ghi	23.20 bcd	68.15 ab
SA 2mM Drench	315.40 ij	80.33 defg	21.95 cdef	60.83 hij
SA 4mM Drench	325.45 fghi	76.85 ghi	21.38 ef	60.60 ij
GA 500mg L ⁻¹ Foliar	321.53 hij	76.13 hi	20.95 f	64.30 cdefg
GA 1000mg L ⁻¹ Foliar	335.83 def	73.40 i	20.90 f	62.28 fghij
GA 500mg L ⁻¹ Drench	312.95 j	84.93 abc	21.45 def	66.28 abcde
GA 1000mg L ⁻¹ Drench	343.58 cde	85.68 ab	22.10 cdef	68.73 a
SI 5g L ⁻¹ Foliar	320.68 ij	82.98 bcde	22.28 cdef	68.65 ab
SI 10g L ⁻¹ Foliar	324.75 ghi	76.68 ghi	22.15 cdef	65.43 bcdef
SI 2g L ⁻¹ Drench	332.88 efg	73.95 i	23.65 bc	65.83 abcde
SI 4g L ⁻¹ Drench	344.30 cd	83.80 abcd	24.23 ab	67.53 abc
YE 5g L ⁻¹ Foliar	331.70 fgh	83.45 bcde	22.98 bcde	60.40 j
YE 10g L ⁻¹ Foliar	347.45 bc	78.70 fgh	21.00 f	61.20 ghij
YE 3g L ⁻¹ Drench	333.85 defg	78.63 fgh	22.80 bcde	64.05 defgh
YE 6g L ⁻¹ Drench	347.65 bc	79.78 efgh	21.93 cdef	61.40 ghij

^aEach value represents the mean of four observations per treatment (n = 4). Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05). C= Control, MT= Melatonin, SA= Salicylic Acid, GA=Glutamic Acid, SI= Silicon and YE= Yeast Extract

The SPAD index also varied significantly among biostimulant treatments (p < 0.001). Silicon (Si) induced the highest SPAD value, with a 14.1% increase over the control. No significant effects were observed for application method or dose. Significant interactions were detected for B*AM, AM*DA, and B*AM*DA (Table 3), the highest SPAD index was observed in T14 (1000 mg L⁻¹ GA), applied via drench, with an average of 68.73 (Table 4).

Fruit weight and size

Highly significant differences (p < 0.001) were observed in fruit weight among the biostimulant treatments (Table 5). The highest fruit weight was obtained with glutamic acid, representing a 37.6% increase compared to the control. Regarding the application method (AM), no significant differences were found between foliar and drench applications. Similarly, the application doses (DA) did not show significant effects. The analysis of variance detected significant interactions among all factors and their combinations, in the three-way interaction (Table 6), the highest fruit weight was recorded in T11 (500 mg L⁻¹ GA), applied via foliar spray, with an average of 4.05 g.

Table 5. Analysis of variance and mean comparison for fruit weight, width, height, and width/height ratio of blueberry fruits as affected by biostimulants (B), application method (AM), dose application (DA), and their interactions

Source of variation	Fruit weight (g)	Width (mm)	Height (mm)	Width/height ratio
Biostimulants (B)				
Control (C)	2.50 ± 0.02 d	17.20 ± 0.05 d	12.38 ± 0.09 d	1.39 ± 0.01 cd
Melatonin (MT)	3.18 ± 0.08 b	19.29 ± 0.26 ab	13.12 ± 0.17 c	1.47 ± 0.02 a
Salicylic Acid (SA)	3.13 ± 0.14 bc	18.61 ± 0.32 c	13.11 ± 0.21 c	1.42 ± 0.01 bc
Glutamic Acid (GA)	3.44 ± 0.11 a	19.68 ± 0.22 a	13.61 ± 0.13 ab	1.45 ± 0.01 ab
Silicon (SI)	3.03 ± 0.03 c	18.62 ± 0.13 c	13.45 ± 0.10 b	1.38 ± 0.01 cd
Yeast Extract (YE)	3.09 ± 0.04 bc	18.88 ± 0.16 bc	13.82 ± 0.17 a	1.37 ± 0.02 d
Application Method (AM)				
Foliar (F)	3.04 ± 0.06 a	18.85 ± 0.17 a	13.23 ± 0.11 a	1.43 ± 0.01 a
Drench (D)	3.08 ± 0.06 a	18.57 ± 0.16 b	13.27 ± 0.10 a	1.40 ± 0.01 b
Dose Application (DA)				
Dose A	3.08 ± 0.06 a	18.62 ± 0.16 b	13.30 ± 0.10 a	1.40 ± 0.01 b
Dose B	3.04 ± 0.06 a	18.81 ± 0.17 a	13.20 ± 0.11 a	1.43 ± 0.01 a
Significance ^(x)				
B	***	***	***	***
MA	ns	***	ns	***
DA	ns	*	ns	***
B*MA	***	***	***	*
B*DA	***	***	***	**
MA*DA	***	***	***	ns
B*MA*DA	***	**	***	***
C.V (%)	3.97	2.10	2.05	2.58

^xEach value represents the mean of eight observations ± standard error (n = 8). ^{*}Significance level: ns = not significant, * p < 0.05, ** p < 0.01, *** p < 0.001. C.V. = coefficient of variation. Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05)

Fruit diameter (width) also showed significant differences (p < 0.001) among treatments, with the greatest width observed in the AG treatment, showing a 14.4% increase over the control. The application method had a significant effect (p < 0.001), with foliar application resulting in larger fruit width. Significant differences were also found for application dose. Interactions B*AM, B*DA, AM*DA, and B*AM*DA were all significant, in the three-way interaction, the best treatments for increasing fruit width were T14 and T12, with mean values of 20.68 and 20.58 mm (Table 6), respectively.

Fruit height showed significant differences (p < 0.001) among biostimulants, with the highest value recorded in the yeast treatment, representing an 11.6% increase. No significant differences were observed for application method or dose. However, the interactions B*MA, B*DA, AM*DA, and B*AM*DA were significant, in the three-way interaction (Table 6), the most effective treatment for increasing fruit height was T3 (50 µM melatonin), applied via foliar spray, with an average of 14.43 mm.

Table 6. Means and Tukey's comparison of blueberry fruit weight and size for the triple interaction of biostimulants, application method, and dose application

Treatment	Fruit weight (g)	Width (mm)	Height (mm)	Width/height ratio
C Foliar	^a 2.43 i	17.05 k	12.25 g	1.39 cdefg
C Drench	2.57 i	17.35 k	12.51 g	1.38 cdefg
MT 50 μ M Foliar	3.68 b	20.33 ab	14.23 a	1.43 bcde
MT 100 μ M Foliar	3.05 cdefg	19.83 abcd	12.68 fg	1.56 a
MT 50 μ M Drench	3.10 cdefg	19.20 cdef	12.75 fg	1.51 ab
MT 100 μ M Drench	2.88 fg	17.80 hijk	12.83 efg	1.39 cdefg
SA 2mM Foliar	2.80 gh	18.33 efghi	12.83 efg	1.43 bcde
SA 4mM Foliar	2.88 fg	17.85 ghijk	12.38 g	1.44 bcde
SA 2mM Drench	2.80 gh	17.58 ijk	12.80 efg	1.38 defg
SA 4mM Drench	4.05 a	20.68 a	14.43 a	1.43 bcde
GA 500mg L ⁻¹ Foliar	4.05 a	20.58 a	14.25 a	1.44 bcde
GA 1000mg L ⁻¹ Foliar	2.98 efg	19.38 bcde	13.13 def	1.48 abc
GA 500mg L ⁻¹ Drench	3.35 bcd	18.70 efgh	13.30 cdef	1.41 bcdef
GA 1000mg L ⁻¹ Drench	3.38 bc	20.05 abc	13.75 abcd	1.46 abcd
SI 5g L ⁻¹ Foliar	2.98 efg	18.23 fghij	13.95 abc	1.31 fg
SI 10g L ⁻¹ Foliar	3.00 efg	19.18 cdef	13.28 cdef	1.44 bcde
SI 2g L ⁻¹ Drench	3.15 cdef	18.63 efghi	13.50 bcde	1.38 cdefg
SI 4g L ⁻¹ Drench	2.98 efg	18.45 efghi	13.08 defg	1.41 bcde
YE 5g L ⁻¹ Foliar	3.08 cdefg	19.25 bcdef	13.15 def	1.46 abcd
YE 10g L ⁻¹ Foliar	3.00 efg	18.93 defg	14.10 ab	1.34 efg
YE 3g L ⁻¹ Drench	3.03 defg	18.20 fghij	14.05 ab	1.30 g
YE 6g L ⁻¹ Drench	3.28 cde	19.15 cdef	13.98 abc	1.37 defg

^aEach value represents the mean of eight observations per treatment (n = 8). Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05). C= Control, MT= Melatonin, SA= Salicylic Acid, GA=Glutamic Acid, SI= Silicon and YE= Yeast Extract

The fruit width/height ratio showed highly significant differences among biostimulants, application methods, and doses (p < 0.001). The highest value was observed with melatonin (MT). Foliar application promoted a more balanced width/height ratio compared to drench. Regarding dose, Dose B slightly outperformed Dose A. All interactions were significant, except for AM*DA (Table 5).

Fruit skin color

As shown in Table 7, the luminosity value (L^*) showed significant differences among treatments for the B factor. All biostimulant treatments increased fruit brightness compared to the control, with the SI treatment presenting the highest value. Regarding the application method (AM), no significant differences were detected, while the application dose (DA) showed statistical differences (p < 0.05), with dose B being superior. Significant interactions were observed for B*AM, B*DA, and B*AM*DA, the treatment with the highest L^* value was T15 (5 g silicon), applied via foliar spray, with a value of 41.53 (Table 8).

Table 7. Analysis of variance and mean comparison for the skin color of blueberry fruits as a function of biostimulants (B), application method (AM), application dose application (DA), and their interactions

Source of variation	L^*	C^*	b°
Biostimulants (B)			
Control (C)	^a 35.82 \pm 0.18 d	5.93 \pm 0.07 c	281.61 \pm 2.25 a
Melatonin (MT)	37.92 \pm 0.19 c	7.10 \pm 0.10 b	285.28 \pm 14.17 a
Salicylic Acid (SA)	38.93 \pm 0.23 b	7.39 \pm 0.07 b	280.94 \pm 1.71a

Glutamic Acid (GA)	37.70 ± 0.44 c	7.27 ± 0.07 b	293.07 ± 4.24 a
Silicon (SI)	40.31 ± 0.39 a	7.89 ± 0.12 a	270.83 ± 12.55 a
Yeast Extract (YE)	39.37 ± 0.37 b	7.80 ± 0.12 a	293.23 ± 3.65 a
Application Method (AM)			
Foliar (F)	38.25 ± 0.29 a	7.16 ± 0.11 b	280.62 ± 4.61 a
Drench (D)	38.43 ± 0.26 a	7.29 ± 0.11 a	287.70 ± 4.80 a
Dose Application (DA)			
Dose A	38.12 ± 0.29 b	7.23 ± 0.12 a	284.94 ± 4.88 a
Dose B	38.56 ± 0.25 a	7.23 ± 0.10 a	283.38 ± 4.59 a
Significance ^(x)			
B	***	***	ns
MA	ns	*	ns
DA	*	ns	ns
B*MA	***	***	ns
B*DA	***	ns	ns
MA*DA	ns	ns	ns
B*MA*DA	*	**	ns
C.V (%)	2.28	4.14	11.05

^xEach value represents the mean of eight observations ± standard error (n = 8). ^xSignificance level: ns = not significant, * p < 0.05, ** p < 0.01, *** p < 0.001. C.V. = coefficient of variation. Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05)

Table 8. Means and Tukey's comparison of blueberry fruit color for the triple interaction of biostimulants, application method, and dose application

Treatment	<i>L*</i>	<i>C*</i>	<i>b^o</i>
C Foliar	35.53 h	5.89 fg	2.81.61 a
C Drench	36.11 gh	5.97 f	2.82.67 a
MT 50µM Foliar	38.18 bcdef	6.61 ef	309.62 a
MT 100µM Foliar	37.13 efgh	6.95 de	232.50 a
MT 50µM Drench	38.16 bcdef	7.50 abcde	292.83 a
MT 100µM Drench	38.23 bcdef	7.32 cde	306.19 a
SA 2mM Foliar	37.95 cdefg	7.10 de	278.54 a
SA 4mM Foliar	39.70 abcd	7.49 bcde	283.25 a
SA 2mM Drench	39.31 abcde	7.40 cde	284.23 a
SA 4mM Drench	38.75 bcde	7.56 abcd	277.74 a
GA 500mg L ⁻¹ Foliar	36.13 fgh	7.26 cde	278.24 a
GA 1000mg L ⁻¹ Foliar	38.25 bcdef	7.09 de	288.18 a
GA 500mg L ⁻¹ Drench	36.29 fgh	7.25 cde	318.94 a
GA 1000mg L ⁻¹ Drench	40.13 abcd	7.47 bcde	286.93 a
SI 5g L ⁻¹ Foliar	41.53 a	8.40 ab	273.61 a
SI 10g L ⁻¹ Foliar	41.06 a	8.05 abc	276.70 a
SI 2g L ⁻¹ Drench	40.20 abc	7.61 abcd	244.12 a
SI 4g L ⁻¹ Drench	38.44 bcdef	7.49 bcde	288.88 a
YE 5g L ⁻¹ Foliar	37.85 defg	7.36 cde	290.69 a
YE 10g L ⁻¹ Foliar	39.65 abcd	7.80 abcd	292.91 a
YE 3g L ⁻¹ Drench	40.27 ab	8.42 a	285.28 a
YE 6g L ⁻¹ Drench	39.71 abcd	7.62 abcd	304.07 a

^xEach value represents the mean of four eight observations per treatment (n = 8). Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05). C= Control, MT= Melatonin, SA= Salicylic Acid, GA=Glutamic Acid, SI= Silicon and YE= Yeast Extract

For color saturation (C^*), significant differences were also recorded among biostimulants, all outperforming the control, with SI and L showing the highest values. The application method had a significant effect, with the drench application being superior; no differences were detected between doses. Significant interactions were identified for B*AM and B*AM*DA (Table 8), the highest chroma (C^*) value was recorded in Treatment 21 (3 g YE), applied via drench, with a C^* value of 8.42.

Regarding color hue (b^o), no statistical differences were observed among treatments, and no significant interactions were detected for this variable. Thus, the results indicate that fruits from all treatments exhibited a uniform blue coloration.

Fruit quality

Fruit firmness showed significant differences ($p < 0.001$) among the biostimulant (B) treatments; all biostimulants increased firmness compared to the control, with glutamic acid (AG) showing the highest value (Table 9). The application method (AM) and application dose (DA) showed no significant differences; however, significant interactions were observed among B*AM*DA (Table 10).

Table 9. Analysis of variance and mean comparison for firmness, total soluble solids (TSS), and anthocyanins of blueberry fruits based on biostimulants (B), application method (AM), and application dose application (DA) and their interactions

Source of variation	Firmness (kg/cm ²)	TSS (°Brix)	Anthocyanins (mg 100g ⁻¹ DW)
Biostimulants (B)			
Control (C)	3.65 ± 0.02 b	11.85 ± 0.18 c	123.77 ± 1.44 e
Melatonin (MT)	3.83 ± 0.02 a	12.68 ± 0.16 b	157.91 ± 4.00 d
Salicylic Acid (SA)	3.78 ± 0.07 a	13.28 ± 0.20 a	161.91 ± 9.89 d
Glutamic Acid (GA)	3.87 ± 0.01 a	12.49 ± 0.11 b	175.83 ± 9.67 c
Silicon (SI)	3.84 ± 0.02 a	13.64 ± 0.29 a	211.55 ± 12.88 b
Yeast Extract (YE)	3.86 ± 0.01 a	12.56 ± 0.22 b	235.92 ± 19.82 a
Application Method (AM)			
Foliar (F)	3.81 ± 0.01 a	12.84 ± 0.15 a	193.35 ± 9.41 a
Drench (D)	3.80 ± 0.03 a	12.65 ± 0.13 a	162.28 ± 6.35 b
Dose Application (DA)			
Dose A	3.79 ± 0.03 a	12.86 ± 0.14 a	182.86 ± 7.54 a
Dose B	3.82 ± 0.01 a	12.64 ± 0.14 b	172.77 ± 9.01 b
Significance ^(x)			
B	***	***	***
MA	ns	ns	***
DA	ns	*	***
B*MA	ns	***	***
B*DA	ns	***	***
MA*DA	ns	ns	***
B*MA*DA	*	**	***
C.V (%)	3.11	4.20	6.63

^xEach value represents the mean of eight observations ± standard error (n = 8). ^{*}Significance level: ns = not significant, * p < 0.05, ** p < 0.01, *** p < 0.001. C.V. = coefficient of variation. Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05)

Table 10. Means and Tukey's comparison of blueberry fruit quality for the triple interaction of biostimulants, application method, and dose application

Treatment	Firmness (kg/cm ²)	TSS (°Brix)	Anthocyanins (mg 100g ⁻¹ DW)
C Foliar	3.55 b	11.80 h	123.34 i
C Drench	3.65 ab	11.85 h	124.20 i
MT 50µM Foliar	3.78 ab	12.90 bcdefgh	182.00 de
MT 100µM Foliar	3.90 a	12.00 fgh	159.72 efgh
MT 50µM Drench	3.85 ab	12.55 defgh	145.83 fghi
MT 100µM Drench	3.79 ab	13.25 bcdef	144.11 fghi
SA 2mM Foliar	3.85 ab	12.38 defgh	224.30 c
SA 4mM Foliar	3.83 ab	13.50 abcde	160.33 efg
SA 2mM Drench	3.55 b	13.25 bcdef	129.17 hi
SA 4mM Drench	3.90 a	14.00 ab	133.83 ghi
GA 500mg L ⁻¹ Foliar	3.89 a	12.10 fgh	126.16 i
GA 1000mg L ⁻¹ Foliar	3.78 ab	13.00 bcdefg	175.03 def
GA 500mg L ⁻¹ Drench	3.90 a	12.50 efgh	200.32 cd
GA 1000mg L ⁻¹ Drench	3.90 a	12.35 defgh	201.82 cd
SI 5g L ⁻¹ Foliar	3.75 ab	14.70 a	219.07 c
SI 10g L ⁻¹ Foliar	3.88 ab	13.70 abcd	218.51 c
SI 2g L ⁻¹ Drench	3.85 ab	14.15 ab	274.04 b
SI 4g L ⁻¹ Drench	3.90 a	12.00 fgh	134.57 ghi
YE 5g L ⁻¹ Foliar	3.90 a	13.90 abc	258.64 b
YE 10g L ⁻¹ Foliar	3.83 ab	12.25 efgh	348.92 a
YE 3g L ⁻¹ Drench	3.81 ab	12.18 efgh	187.30 de
YE 6g L ⁻¹ Drench	3.90 a	11.90 fgh	148.83 fghi

^aEach value represents the mean of eight measurements per treatment (n = 8). Means followed by the same letter within a column are not significantly different according to Tukey's test (p < 0.05). C= Control, MT= Melatonin, SA= Salicylic Acid, GA=Glutamic Acid, SI= Silicon and YE= Yeast Extract

Total soluble solids (°Brix) in factor B showed highly significant differences (p < 0.001), with the treatments containing SI and AS exhibiting the highest values. No significant differences were observed for the application method (AM), whereas the application dose (DA) had a significant effect (p < 0.05). Significant interactions were found for B*AM, B*DA, and B*AM*DA, the highest total soluble solids (TSS) content was observed in treatment 15, with an average of 14.70 (% °Brix) (Table 10).

Anthocyanin content showed highly significant differences (p < 0.001) among the biostimulant treatments; the control presented the lowest content, whereas YE led to the highest anthocyanin concentration. The application method (AM) had a significant effect (p < 0.001), with foliar application being superior. Regarding the application dose (DA), foliar application was the most effective. Significant interactions were also found for B*AM, B*DA, and B*AM*DA (Table 10), the treatment that most increased anthocyanin content was T20, with a mean value of 348.92 (mg 100g⁻¹ DW).

Correlation analysis

The correlation analysis between morphological and quality variables in blueberry fruits under the application of treatments showed positive and significant relationships. Fruit weight exhibited a strong positive correlation with fruit width and height (Figure 2), while width and height were also positively correlated with each other. The variables yield, plant height, stem diameter, and SPAD index showed relevant positive correlations among them, indicating that greater vegetative growth is associated with higher relative chlorophyll content and increased yield. Fruit width was positively and significantly correlated with fruit height; likewise, fruit weight showed a positive correlation with fruit width. Regarding color characteristics, a

strong correlation was observed between L* and C*, indicating that fruits with higher luminosity also present more intense chromaticity. Anthocyanins play an important role in fruit pigmentation, which explains their positive correlations with C*, L*, and h°.

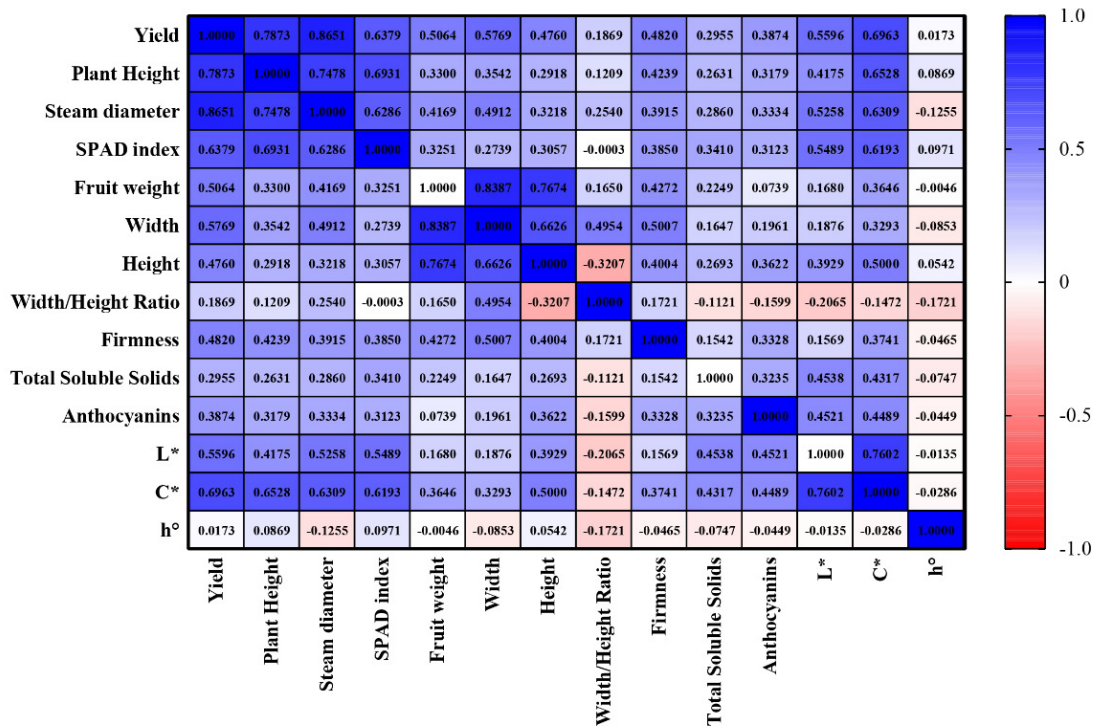


Figure 2. Heatmap of Pearson correlation coefficients among the variables analyzed in blueberry plants and fruits

Discussion

Biostimulants enhance yield, plant height, stem diameter, and SPAD index

Biostimulants significantly improved plant yield relative to the untreated control, consistent with evidence showing that melatonin, silicon, glutamic acid, and yeast extracts enhance yield across diverse crops (Sadak *et al.*, 2020; Fan *et al.*, 2022; Almutairi *et al.*, 2022; Parecido *et al.*, 2022; Ebaid *et al.*, 2022). Yield improvements associated with biostimulant application can be attributed to enhanced photosynthetic efficiency, metabolic activity, nutrient uptake, and redox homeostasis, which collectively promote abiotic stress defense mechanisms, resulting in higher productivity (Bulgari *et al.*, 2015; Rouphael and Colla, 2020). The greatest yield increase was recorded with 100 μ M melatonin applied via drench (T6) which exceeded the control by 41%, confirming the strong yield promoting capacity of melatonin observed previously in strawberry under non-stress conditions (Okatan *et al.*, 2022) and maize under stress (Ali *et al.*, 2024). Similarly, foliar application of 100 μ M melatonin in wheat, applied via seed coating produced comparable physiological results (Li *et al.*, 2025). According to the authors, melatonin enhanced antioxidant enzyme activity, soluble protein content, and chlorophyll concentration while reducing ROS accumulation. These physiological improvements supported sustained photosynthetic performance and greater seedling vigor under drought stress, ultimately promoting more efficient grain filling, which was reflected in the overall yield increase reported in the study. The yield increase associated with melatonin application can be attributed to its dual function as an indolamine

and a regulator with auxin-like activity (Zhang *et al.*, 2022), which promotes cell expansion, vegetative vigor, and ultimately enhances flower formation and fruit set. In addition, exogenous melatonin modulates several key hormonal pathways, particularly ABA, JA, SA, and ethylene (Bychkov *et al.*, 2025), that coordinate physiological processes involved in flowering, fruit development, and stress resilience. Melatonin also plays a central role in maintaining chloroplast integrity, stabilizing thylakoid membranes, and stimulating chlorophyll synthesis and photosynthetic efficiency. Collectively, these mechanisms enhance carbon assimilation and optimize the allocation of photoassimilates to developing sink tissues such as fruits. The resulting improvement in source-sink dynamics support greater berry enlargement and contributes to the overall increase in yield observed in multiple fruit crops (Nawaz *et al.*, 2016; Wang *et al.*, 2022). From a horticultural perspective, these results indicate that melatonin at 100 μM delivered via drench represents the most productive strategy, especially in systems where root zone uptake is a priority, like fertigated crops in organic growing media or sandy soils.

The treatments also promoted substantial improvements in vegetative growth, as evidenced by notable increases in both plant height and stem diameter. Comparable responses to biostimulants have been documented in multiple crops, including melatonin induced growth in rice (Yan *et al.*, 2024), salicylic acid in cowpea (El-Taher *et al.*, 2021), glutamic acid in tomato (Alfosea-Simón *et al.*, 2021), silicon in soybean (Hussain *et al.*, 2021), and yeast extract in garlic (Abdelaal *et al.*, 2021). These effects are consistent with the recognized capacity of biostimulants to modulate core physiological pathways, particularly those regulating plant growth and development. For instance, several biostimulants stimulate the biosynthesis or signalling of growth-related hormones such as auxins and gibberellins (Rupawalla *et al.*, 2022), while others enhance the uptake and assimilation of essential nutrients including phosphorus, potassium, and nitrogen, contributing to greater biomass accumulation (Baltazar *et al.*, 2021). Notably, melatonin application produced method dependent effect. Foliar melatonin was more effective in increasing plant height, whereas drench application promoted greater stem diameter. These contrasting responses can be attributed to the distinct physiological routes activated by each application method. Foliar melatonin acts directly on photosynthetically active tissues, where it can rapidly modulate energy-metabolism processes, enhance chlorophyll stability and protect the photosynthetic apparatus against the oxidative stress (Ahmad *et al.*, 2020). Such localized improvements likely contribute to structural reinforcement, reflected in increased stem diameter. In contrast, melatonin applied via drench is absorbed through the root system, where it activates auxin-analogous signalling pathways that promotes root development (Korkmaz *et al.*, 2021), that in turn, improves water and nutrient acquisition, thereby stimulating shoot elongation. Collectively, these results indicate that the physiological impact of melatonin is strongly conditioned by the application method. Foliar melatonin preferentially enhances localized structural traits such as stem thickness, while drench application promotes stem elongation.

Relative chlorophyll content (SPAD index) increased significantly in response to biostimulant application, with glutamic acid and silicon treatments producing the greatest enhancements. Comparable findings have been reported in cucumber (Lozano-González *et al.*, 2021), sugarcane (Verma *et al.*, 2021), and pumpkin (Salim *et al.*, 2021), confirming the broad efficacy of these compounds in enhancing photosynthetic performance across crops. Silicon is well known for its capacity to stabilize thylakoid membranes, reduce membrane lipid degradation, and mitigate chloroplast oxidative stress, thereby enhancing photosynthetic efficiency under both optimal and stress conditions (Rastogi *et al.*, 2021; Kumar *et al.*, 2025). In contrast, the highest SPAD values were recorded when glutamic acid at 1000 mg L^{-1} was applied via drench. This response may be attributed to the central role of glutamic acid as a central precursor in nitrogen metabolism and its function as a key signaling molecule that regulates nitrogen assimilation pathways (Alfosea-Simón *et al.*, 2021). Exogenous glutamate enhances the activity of key enzymes such as glutamine synthetase and glutamate synthase, improving the assimilation of reduced nitrogen necessary for the formation of the tetrapyrrole ring and consequently, chlorophyll formation (Forde and Lea, 2007). Additionally, glutamate acts as a metabolic hub connecting carbon and nitrogen metabolism, optimizing the availability of carbon skeletons required for

chloroplast biogenesis (Quan *et al.*, 2022). Simultaneously, glutamate-mediated signalling contributes to nutritional status, redox homeostasis and stabilization of photosynthetic pigments which collectively explain the elevated SPAD values observed in drench-applied treatments.

Biostimulant application enhances fruit size and weight

Fruit dimensions increased significantly following biostimulant treatments. Notably, glutamic acid increased fruit weight by 37% relative to the control. Foliar application of glutamic acid promotes cell expansion in the mesocarp by enhancing amino acid and protein synthesis, regulating the integration of carbon and nitrogen metabolism, and activating redox and nutritional signalling pathways, which improves the mobilization of photoassimilates to the fruit and increases both fruit weight and diameter (Asgher *et al.*, 2022; Almutairi *et al.*, 2022; Lee *et al.*, 2021). Pérez-León *et al.* (2023) similarly reported increases in fruit weight and both polar and equatorial diameters with glutamic acid at 500 mg L⁻¹. Yeast treatments induced the greatest polar diameter, likely due to the production of auxins, gibberellins, and other growth regulators that stimulate cell expansion (Ramos-Garza *et al.*, 2023), consistent with observations in banana (El-Gioushy *et al.*, 2022) and peach (Abdel-Mohsen and Kamel, 2015). The width-to-height ratio, an indicator of fruit morphological uniformity, was highest with melatonin treatment, producing fruits with greater consumer appeal (Canales *et al.*, 2024).

Effect of biostimulants on fruit color

Blueberry fruit color is a key indicator of maturity and quality (Krishna *et al.*, 2023). Luminosity (L*) increased significantly with biostimulant application, with silicon-treated fruits showing the highest values. Silicon reinforces cell walls by depositing as silica, increasing their rigidity and mechanical strength (Souri *et al.*, 2021). This produces a more turgid and uniform epidermis, reducing surface irregularities and enhancing light reflection. In addition, silicon can promote a more uniform epicuticular wax layer (Osei *et al.*, 2023), further increasing fruit brightness. Together, these structural effects improve epidermal integrity and turgor, which translates into a higher L* and a glossier fruit appearance. Chroma (C*), representing color intensity, was elevated in fruits treated with silicon and yeast, possibly reflecting increased anthocyanin accumulation responsible for the characteristic blue-violet hue of blueberries (Chung *et al.*, 2016). Silicon may enhance anthocyanin accumulation by reinforcing cell walls and improving epidermal turgor, which supports better pigment deposition and uniform light absorption (Afifi *et al.*, 2023). Yeast extracts, rich in amino acids, vitamins, and elicitor molecules, can stimulate phenylpropanoid metabolism, upregulating key enzymes in the anthocyanin biosynthetic pathway (Pastore *et al.*, 2020). Together, these effects increase pigment concentration and uniformity in the fruit skin, resulting in higher chroma values and a more intense blue-violet color. No significant differences were observed for hue angle (h°), indicating the overall tone remained consistent as typical blue-violet. This contrasts with Gonçalves *et al.* (2020), who reported hue changes in strawberry following glycine-betaine and *Ascophyllum nodosum* applications.

Biostimulants improve blueberry fruit quality

Fruit firmness was significantly enhanced by biostimulant treatments through multiple physiological mechanisms, similar firmness increases have been documented in previous studies (Tarantino *et al.*, 2018; Lobo *et al.*, 2019; Rodrigues *et al.*, 2020). Silicon and yeast reinforced cell walls by promoting silica deposition and stimulating lignin, cellulose, and hemicellulose synthesis, which increase mechanical strength and maintain cell-to-cell adhesion (Karagiannis *et al.*, 2021). This structural reinforcement preserves epidermal integrity and turgor pressure, key factors for resisting deformation (Wang *et al.*, 2024). Melatonin and salicylic acid applied via drench at 4 mM increased firmness, while their effect declined at 2 mM. The reduced response at 2 mM salicylic acid may be due to insufficient elicitation of phenylpropanoid metabolism and lignification at the

lower concentration, limiting the synthesis of secondary metabolites required for cell wall strengthening (Wani *et al.*, 2017). Glutamic acid and yeast also enhanced firmness by improving cell wall composition. Additionally, biostimulants enhance antioxidant defenses, reducing ROS-mediated cell wall degradation and maintaining tissue elasticity (Bulgari *et al.*, 2015).

Total soluble solids (TSS) content plays a fundamental role in blueberry flavour, contributing to sensory quality (Mennella *et al.*, 2017). TSS increased significantly with biostimulant treatments, particularly with salicylic acid and silicon, consistent with findings in strawberry (Munaretto *et al.*, 2018), tomato (Javaheri *et al.*, 2012), and litchi (Kumari *et al.*, 2023). Biostimulants enhance carbohydrate metabolism and photoassimilate transport: salicylic acid functions as a hormonal signal, stimulating photosynthesis and upregulating sugar transport genes (Shahrajabian and Sun, 2024), while silicon improves photosynthetic efficiency, stabilizes chloroplast structure, and enhances water-use efficiency, which collectively increase carbohydrate production and allocation to fruits (Savvas and Ntatsi, 2015). Furthermore, silicon strengthens vascular tissues and cell walls, facilitating more efficient phloem loading and translocation of sugars to developing berries (Abbai *et al.*, 2019). This structural and physiological enhancement likely explains why silicon was the most effective treatment in increasing TSS, regardless of foliar or drench application, as it simultaneously optimizes photosynthetic capacity, assimilate transport, and fruit sink strength.

Anthocyanin content was significantly increased in fruits treated with biostimulants, with yeast treatments showing the greatest enhancement. These results are consistent with Pastore *et al.* (2020), who reported elevated anthocyanin levels in Sangiovese grapes following application of a *Saccharomyces cerevisiae*-derived product, associated with upregulation of key biosynthetic genes. Biostimulants stimulate the phenylpropanoid pathway (Ahmad *et al.*, 2022), enhancing the activity of enzymes such as chalcone synthase and UDP-glucose: flavonoid glucosyltransferase, which drive flavonoid and anthocyanin biosynthesis (Yao *et al.*, 2023). In addition, they modulate hormonal signalling, including abscisic acid and jasmonic acid pathways, thereby activating transcription factors that regulate pigment production (Shi *et al.*, 2023). At the cellular level, improved turgor and membrane stability, promoted by silicon or osmoprotective metabolites present in biostimulants, facilitate efficient sequestration of anthocyanins into the vacuole, ensuring uniform epidermal pigmentation. Furthermore, enhanced antioxidant capacity reduces reactive oxygen species (ROS)-mediated pigment degradation (Cerqueira *et al.*, 2023), preserving both nutritional quality and stress resilience (Naing *et al.*, 2021). Collectively, these mechanisms optimize anthocyanin synthesis, stability, and distribution, resulting in fruits with higher pigment concentration and more intense coloration.

Relationship between growth, yield, and fruit quality under biostimulant application

The correlation analysis revealed clear relationships among some parameters in blueberry plants and fruits treated with biostimulants. Fruit weight was strongly associated with both width and height, indicating that larger fruits contribute more to overall yield, consistent with previous observations in highbush blueberries (Milić *et al.*, 2018). Likewise, yield, plant height, stem diameter, and SPAD index were positively correlated, suggesting that more vigorous plants with higher chlorophyll content tend to produce higher yields (Lenart *et al.*, 2024). Regarding color traits, L* and C* showed a strong positive relationship, meaning that brighter fruits also exhibit more intense coloration (Afonso *et al.*, 2024). Anthocyanin content was positively correlated with L*, C*, and hue angle, highlighting the role of pigment accumulation in determining fruit colour (Di Vaio *et al.*, 2021).

Conclusions

The preharvest application of biostimulants has a significant effect on yield, growth, and fruit quality in blueberry. Melatonin (MT) promoted higher yield and more vigorous vegetative growth, while silicon (SI) and glutamic acid (GA) increased SPAD index. GA and MT significantly enhanced fruit weight and size. SI and yeast extract (YE) enhanced fruit brightness and color intensity. Salicylic acid (SA) and SI increased total soluble solids content, and all evaluated biostimulants improved fruit firmness, whereas YE treatment favored anthocyanin accumulation. The presented results indicate that biostimulants represent a sustainable alternative to improve these parameters during preharvest. However, further studies are needed starting from earlier phenological stages, incorporating other factors, biostimulants, and application rates.

Authors' Contributions

Conceptualization, JAG-F; methodology, JYE-G and CEC-C; investigation, JYE-G; data curation, JYE-G and SG-M; software and formal analysis, LAV-A; validation and visualization, AB-M; resources and supervision, JCT-A and CEC-C; writing - original draft preparation, JYE-G; writing- review and editing JYE-G.

All authors read and approved the final manuscript

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Conflict of Interests

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

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