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Effects of Urea-N Concentration on Growth, Physiological Traits, Nutrient Status, and Fruit Quality of Container-Grown Highbush Blueberry (*Vaccinium corymbosum* L. cv. Jewel) in soilless culture

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ABSTRACT

This study was conducted to evaluate the effect of different urea concentrations on vegetative growth, leaf physiological traits, biochemical characteristics, and fruit quality of container-grown blueberry (*Vaccinium corymbosum* L. cv. Jewel). The experiment was arranged in a completely randomized design with four concentrations of added urea (5, 4, 3, and 2 mmol L^{-1}) supplied in a basal nutrient solution containing 1 mmol L^{-1} KNO_3. Analysis of variance revealed that urea concentration significantly affected SPAD value, leaf nitrogen content, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids at $p < 0.01$, while total fruit fresh weight and antioxidant activity (%DPPH) were significant at $p < 0.05$. Although urea concentration significantly influenced flower number per bush, fruit number per inflorescence, total fruit weight, fruit number per bush, and yield per bush, the responses were not uniform across all reproductive traits and urea levels. These results indicate that blueberry responses to urea were expressed mainly through changes in leaf nitrogen status, pigment accumulation, and antioxidant capacity rather than through major alterations in plant architecture or basic fruit composition. Overall, the findings suggest that moderate variation in urea concentration can effectively influence physiological and biochemical performance in blueberry without adversely affecting fruit quality. Under the conditions of this experiment, added urea concentration affected growth, physiological traits, nutrient status, reproductive performance, and fruit-quality attributes of container-grown highbush blueberry cv. 'Jewel'.

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1. Introduction

Blueberry (*Vaccinium spp*.) has gained increasing importance worldwide because of its high nutritional value, consumer demand, and economic potential in both fresh and processed markets. The crop is widely appreciated for its attractive fruit, high concentration of bioactive compounds, and reported health-promoting properties, including antioxidant activity and the presence of phenolic compounds, anthocyanins, and vitamins. As commercial blueberry production expands, there is growing interest in optimizing cultural practices to improve vegetative growth, fruit quality, and overall productivity under diverse production systems, particularly containerized and soilless culture systems (Kazemi and Roosta, 2025).

Among mineral nutrients, nitrogen is one of the most critical factors affecting blueberry growth and performance. However, blueberry exhibits unusual nitrogen physiology compared with many other fruit crops, as it generally performs better under ammonium-dominant nutrition than nitrate-dominant nutrition. A review by Doyle et al. (2021) emphasized that blueberry roots preferentially absorb and utilize ammonium, and that nitrate nutrition is often less favorable for growth and physiological performance. This preference is consistent with the acid-loving nature of blueberry and helps explain why nitrogen management is a central issue in blueberry production systems. The form of nitrogen can influence not only vegetative growth but also nutrient uptake, flowering, and fruit set. In a soilless culture study, different NH_4^+: NO_3^- ratios significantly affected blueberry growth, nutrient accumulation, physiological responses, flowering, and fruit production, demonstrating that nitrogen source composition is a key determinant of plant performance under controlled cultivation conditions. Similarly, recent work on blueberry seedlings showed that ammonium and urea promoted greater height, stem diameter, and biomass accumulation than nitrate nutrition, while nitrate-based treatment was comparatively less effective. These findings suggest that blueberry is particularly responsive to reduced nitrogen forms, especially ammonium and urea-derived nitrogen (Anwar et al., 2024; Yang et al., 2025). Urea is among the most widely used nitrogen fertilizers in horticulture because of its high nitrogen content, relative affordability, and compatibility with fertigation programs. In blueberry, urea can be an important practical nitrogen source because it is hydrolyzed in the root zone to ammonium, which the plant can readily absorb. However, the effectiveness of urea depends on factors such as substrate properties, pH, moisture regime, application timing, and crop developmental stage. Evidence from mature highbush blueberry also indicates that not all applied urea nitrogen is recovered by the plant within a season, highlighting the importance of optimizing application rate and management strategy (Retamales & Hanson, 1989). In addition, applied research and extension-based recommendations have consistently noted that blueberry responds best to ammonium-type nutrition and that urea may serve as a suitable fertilizer source when properly managed (Doyle et al., 2021).

Although nitrogen management in blueberry has been studied for decades, important knowledge gaps remain, particularly under containerized cultivation. In soilless or pot culture systems, the root environment is more tightly controlled but also more vulnerable to nutrient imbalance, salt stress, and pH shifts. For this reason, findings from field-grown blueberries cannot always be directly transferred to greenhouse or container production. Furthermore, while several studies have evaluated ammonium and nitrate nutrition, fewer have focused specifically on urea as the main variable nitrogen source in container-grown blueberry plants. This is especially relevant for cultivars such as ‘Jewel’, which are increasingly considered for controlled-environment production systems. Therefore, the present study was conducted to evaluate the effects of different urea concentrations on vegetative growth and fruit-related traits of container-grown blueberry (*Vaccinium corymbosum* L. cv. Jewel) under greenhouse conditions. By investigating how incremental changes in urea supply influence growth and quality parameters, this work aims to clarify the response of blueberry to urea-based nitrogen nutrition in a pot culture system and to provide practical information for nutrient management in greenhouse blueberry production.

2. Materials and Methods

The experiment was conducted under greenhouse conditions at the University of Guilan, Rasht, Iran, during 2025. The greenhouse was located at the University of Guilan campus, approximately 11 km from the Rasht–Qazvin highway. One-year-old uniform blueberry plants (*Vaccinium corymbosum* L. cv. ‘Jewel’) were selected and transplanted into 19-L plastic containers filled with peat moss and perlite at a 1:1 (v/v) ratio. The greenhouse temperature was maintained at approximately 25 °C during the day and 22 °C at night using a split air-conditioning system, with natural seasonal fluctuations. Relative humidity was generally maintained within the range of 50–65%. A 50% shade net was used to reduce excessive solar radiation and heat load during periods of high solar radiation. Plants were exposed to the natural photoperiod, and no supplemental lighting was provided. Before initiating the treatments, all plants were allowed to establish for 2 weeks under uniform management conditions. During the experiment, irrigation was applied uniformly to all treatments to maintain adequate substrate moisture, and other cultural practices were kept constant throughout the study

The experiment was arranged in a completely randomized design (CRD) with four urea treatments and three replications per treatment. Each replicate consisted of 10 plants, resulting in a total of 120 plants. The four

treatments consisted of different concentrations of urea supplied through the nutrient solution: 5, 4, 3, and 2 mmol L^{-1} urea for Treatments 1, 2, 3, and 4, respectively. The treatment solutions were applied once daily, seven times per week, at a volume of approximately 100 mL per pot at each application. The treatments were applied for 16 weeks, from April to July. Plants were randomly assigned to the treatments, and pot positions were rearranged periodically to minimize the effects of environmental variation within the greenhouse. The nutrient solution was prepared in distilled water using a basal formulation (table 1) that contained potassium nitrate (KNO_3, 1 mmol L^{-1}), monopotassium phosphate (KH_2PO_4, 0.5 mmol L^{-1}), and the following micronutrients: Fe-EDTA (15 µmol L^{-1}), $MnSO_4$ (5 µmol L^{-1}), $ZnSO_4$ (3 µmol L^{-1}), H_3BO_3 (5 µmol L^{-1}), $CuSO_4$ (0.5 µmol L^{-1}), and K_2MoO_4 (0.5 µmol L^{-1}). Based on the chemical analysis of the irrigation water (Table 2), calcium and magnesium concentrations were above the minimum required levels for the crop; therefore, no supplemental Ca or Mg were added to the nutrient solution. Urea [$CO(NH_2)_2$] was then added to the basal solution at concentrations of 5, 4, 3, and 2 mmol L^{-1} for treatments 1, 2, 3, and 4, respectively. These values refer to the concentration of added urea and not to the total nitrogen concentration of the solution. Because each urea molecule contains two nitrogen atoms, urea supplied 10, 8, 6, and 4 mmol N L^{-1}, respectively, while the basal KNO_3 contributed an additional 1 mmol NO_3–N L^{-1} in all treatments. Accordingly, the total nitrogen supplied to the solution ranged from 5 to 11 mmol N L^{-1}. Solution pH was adjusted to 5.5 with dilute acid, and electrical conductivity (EC) was maintained at 2 dS m^{-1}. The pH of each nutrient solution was measured and adjusted to 5.5 at the time of preparation using a calibrated pH meter. The pH was not monitored systematically throughout the experimental period. The same irrigation volume was applied to all treatments, and excess leachate was avoided.

Table 1. Composition of the macronutrient used in the experiment.

Component	T1	T2	T3	T4
Urea (mmol L^{-1})	5	4	3	2
KNO_3 (mmol L^{-1})	1	1	1	1
KH_2PO_4 (mmol L^{-1})	0.5	0.5	0.5	0.5
N from urea (mmol L^{-1})	10	8	6	4
NO_3–N from KNO_3 (mmol L^{-1})	1	1	1	1
Total N (mmol L^{-1})	11	9	7	5

Table 2. Chemical characteristics of the irrigation water used in the experiment.

Parameter	K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	pH	EC (µS cm^{-1})
Irrigation water	7.29	3.5	5.2	7.97	3.7

At the end of the experiment, vegetative growth parameters were recorded. Plant height was measured from the substrate surface to the apical meristem using a ruler. Stem diameter was determined with a digital caliper at 10 cm above the substrate surface. The number of branches, inflorescences, flowers per bush, fruits per bush, and total fruit number were counted manually. Fresh and dry biomass were also determined. Shoots and roots were separated, and fresh weight was measured immediately after harvest using an analytical balance. Samples were then dried in a forced-air oven at 70 °C until constant weight and reweighed to obtain dry mass. Dry matter percentage was calculated as:

$$\text{Dry matter (\%)} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100 \quad (1)$$

Root-to-shoot or "root percentage" was determined according to the relevant plant dry biomass distribution method used in the study. Leaf greenness was estimated using a SPAD meter on fully expanded young leaves. SPAD readings were taken from the same leaf position on each plant and averaged for statistical analysis. For pigment analysis, fresh leaf tissue was collected and chlorophylls and carotenoids were extracted with 80% acetone. Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids were quantified spectrophotometrically according to the method of Lichtenthaler and Wellburn (1983).

Dried leaf samples were ground and analyzed for nutrient concentrations. Total nitrogen was determined using the Kjeldahl method (Bremner, 1996), while phosphorus, potassium, calcium, and magnesium were measured using standard wet digestion and instrumental methods. Nitrogen concentration was expressed as percentage on a dry-weight basis, and phosphorus was reported as ppm, while potassium, calcium, and magnesium were expressed as mg g^{-1} dry matter.

Fresh fruit samples were used to determine biochemical quality traits. Antioxidant activity was assessed using the DPPH radical scavenging assay and expressed as percentage inhibition (Brand-Williams et al., 1995). Total phenolic content was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965) and expressed as mg gallic acid equivalents per g fresh weight (mg GAE g^{-1} FW). Titratable acidity (TA) was measured by titrating fruit juice with standardized sodium hydroxide solution to the endpoint pH and expressed as a percentage of the

dominant organic acid equivalent. Total soluble sugar content was determined using a refractometer and expressed as ºBrix. Fruit length, fruit width, and average fruit weight were measured on representative fruits from each replicate. Total fruit weight and yield per plant were recorded by summing all harvested fruits from each plant. These measurements were used to evaluate the effect of urea treatments on fruit size and productivity.

All data were subjected to analysis of variance (ANOVA) using SAS version 9.4. When treatment effects were significant, means were separated using Tukey's honest significant difference (HSD) test at $P \leq 0.05$ and $P \leq 0.01$, depending on the significance level of each trait. Normality and homogeneity of variances were checked before performing ANOVA. Tables and figures present means ± standard error (SE).

3. Results

The analysis of variance (Table 3) showed that different urea concentrations in the nutrient solution did not significantly affect most vegetative growth characteristics of container-grown 'Jewel' blueberry plants under greenhouse conditions. Plant height, number of branches, stem diameter, shoot and root dry weights, dry matter percentage, and ash percentage were not significantly influenced by the applied urea levels. In contrast, the reproductive traits, including the number of flowers per inflorescence, the number of fruits per bush, and total fruit fresh weight per bush, were significantly affected by the urea concentration of the nutrient solution (Table 4). Mean comparison by Tukey's test (Table 4) indicated that the highest total fruit fresh weight per bush was obtained under Treatment 4 (26.78±2.14 g), which was significantly higher than Treatment 1 (21.36±0.44 g), though it did not differ significantly from Treatments 2 and 3. This suggests that varying the nitrogen supply via urea was sufficient to alter the water-containing biomass of the plants, even though dry biomass accumulation and structural vegetative traits remained statistically unchanged. The highest number of flowers per inflorescence was recorded in treatment 1 (12.2), which was significantly greater than that in treatment 3 (5.8), whereas treatment 2 (9.6) and treatment 4 (10.6) exhibited intermediate values. Fruit number per bush declined sharply with decreasing urea supply, from 8.93 in treatment 1 to 5.93 in treatment 2 and to 2.07–2.23 in treatment 3 and treatment 4; fruit number was lowest in treatment 4, which was also significantly lower than in treatment 3 (Table 4). Because the present results were obtained from biennial (two-year-old) plants in a single season, multi-year studies are required to confirm the long-term effects and consistency of the nutrient solutions before definitive production recommendations can be made.

Urea concentration had a highly significant effect on leaf physiological traits. Both the SPAD value and leaf nitrogen (N) concentration were significantly affected at the $P<0.01$ level (Table 3), indicating that the plant nutritional status responded clearly to the different urea treatments. However, Leaf N concentration decreased systematically from Treatment 1 to Treatment 4, with the highest concentration recorded in Treatment 1 (2.24%) and the lowest in Treatment 4 (1.65%), separating the treatments into distinct statistical groups (a, b, and c). SPAD values peaked in Treatment 2 (38.23), which was significantly higher than Treatments 1 and 3, but statistically equivalent to Treatment 4 (36.90). Regarding other leaf macronutrients, the concentrations of phosphorus (P), potassium (K), and magnesium (Mg) were not significantly affected by the treatments (Table 3). However, leaf calcium (Ca) concentration was highly significantly influenced by the urea treatments at the $P<0.01$ level, indicating that changes in nitrogen supply modified calcium accumulation in the leaves.

Leaf pigment composition was strongly influenced by the nitrogen treatments. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were all significantly affected by the urea concentration in the nutrient solution at the $P<0.01$ level (Table 3). According to the mean separation results (Table 4), Treatment 4 exhibited a pronounced and statistically superior accumulation of all pigments compared to the other treatments. Chlorophyll a in Treatment 4 (0.4215 mg/g) was more than double the values observed in Treatment 1 (0.2087 mg/g), Treatment 2 (0.1688 mg/g), and Treatment 3 (0.1975 mg/g). Chlorophyll b and total chlorophyll showed a similar trend, with Treatment 4 reaching significantly higher values (0.1872 mg/g and 0.6085 mg/g, respectively) compared to all other treatments, which remained in the lowest statistical group. Leaf N measured on a dry-mass basis reflects the overall N pool in the tissue, whereas chlorophyll content is more closely related to the fraction of N allocated to the photosynthetic apparatus. In addition, rapid growth under higher urea supply may have caused a dilution effect, reducing leaf N concentration without reducing chlorophyll concentration. Thus, the relationship between leaf N and chlorophyll should be interpreted as physiological rather than strictly linear. Carotenoid content followed the same pattern, peaking at 0.1054 mg/g in Treatment 4, which was significantly higher than Treatments 1, 2, and 3. These findings demonstrate that while Treatment 4 resulted in lower total leaf N percentage, it significantly enhanced the overall leaf pigment systems.

Contrary to previous assertions, the ANOVA (Table 3) revealed that key yield-related characteristics were highly significantly affected by the urea treatments at the $P<0.01$ level. These results indicate that nitrogen management via urea concentration plays a critical role in determining the reproductive potential and yield capacity of 'Jewel' blueberries. In contrast, physical fruit dimensions (fruit weight, fruit length, and fruit width) and primary chemical quality parameters, including titratable acidity (TA) and total soluble solids (TSS), were not significantly influenced by the treatments, suggesting these traits remain stable within the tested range of urea concentrations.

The antioxidant activity, expressed as DPPH radical scavenging capacity, and the total phenolic content of the fruit were both significantly affected by the treatments at the $P<0.05$ level (Table 3).

Tukey's mean comparison for antioxidant activity (Table 4) showed that the highest values were recorded in Treatment 2 (86.62%) and Treatment 3 (86.34%), which were significantly higher than Treatment 4 (74.97%), but did not differ from Treatment 1 (83.84%). This indicates that the conditions established under Treatment 4 led to a significant decline in the antioxidant capacity of the fruits.

4. Discussion

The present study showed that urea concentration affected blueberry cv. 'Jewel' primarily through leaf physiological and biochemical responses rather than through major changes in shoot architecture or fruit compositional traits. Among the 30 evaluated traits, significant treatment effects were detected for SPAD value, leaf nitrogen, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, antioxidant activity, and fresh weight, whereas most vegetative growth and fruit-quality parameters remained statistically unchanged. This pattern indicates that the applied urea range was sufficient to modify internal nitrogen status and photosynthetic metabolism, but not strong enough to induce broad morphological divergence under greenhouse container conditions. Such trait-specific sensitivity is consistent with the known nutritional ecology of blueberry, a crop whose growth and productivity are strongly shaped by nitrogen form, root-zone chemistry, and cultivar-specific nutrient use efficiency (Anwar et al., 2024; Doyle et al., 2021). The significant increase in SPAD value and leaf nitrogen concentration across treatments demonstrates that urea supplies enhanced nitrogen acquisition and assimilation in the foliage (Abolghasemi and Saeidi, 2024). Because SPAD is a non-destructive proxy for relative chlorophyll abundance and, indirectly, for leaf nitrogen status, its responsiveness here is physiologically meaningful. In blueberry, leaf nitrogen is a central determinant of canopy function because nitrogen is a major constituent of chlorophyll, photosynthetic enzymes, and proteins involved in carbon assimilation (Doyle et al., 2021). The present results agree with earlier reports that blueberry responds strongly to nitrogen form and that ammonium-preferred nutrition often improves leaf greenness and nutritional status in Vaccinium spp. (Anwar et al., 2024; González et al., 2018). This is particularly relevant because urea, following hydrolysis, can function as a reduced nitrogen source that may better align with blueberry's physiological preference for ammonium-like nutrition than nitrate-dominant fertilization regimes (Doyle et al., 2021; Azizi et al., 2024).

The pronounced response of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids further supports the conclusion that urea altered photosynthetic capacity rather than simply stimulating vegetative expansion. Nitrogen is indispensable for chloroplast development and pigment biosynthesis, and leaf chlorophyll concentration often increases before any visible growth differences appear. The significant chlorophyll response observed in the present study is consistent with recent blueberry research showing that the NH_4^+: NO_3^- balance strongly influences chlorophyll accumulation, photosynthetic performance, and nutrient uptake in soilless culture (Anwar et al., 2024). Similar conclusions have also been drawn from studies showing that ammonium-dominated nitrogen nutrition can enhance chlorophyll-related traits and biomass accumulation in Vaccinium species, while nitrate-dominated regimes may be less favorable under acidic or soilless conditions (Doyle et al., 2021; González et al., 2018). Carotenoids, which are both accessory light-harvesting pigments and photoprotective compounds, also responded significantly, suggesting that nitrogen supply modified the broader pigment network associated with photosystem stability and oxidative protection (Taiz and Zeiger, 2015).

Despite these clear physiological effects, most vegetative growth traits such as plant height, branching, and stem diameter were not significantly affected. This indicates that the tested range of urea likely remained within a nutritional window that supported adequate growth across all treatments. In perennial berry crops, structural growth often exhibits lower sensitivity than leaf chemistry because plants can buffer moderate nutritional differences through internal nutrient allocation, carbohydrate reserves, and cultivar-dependent growth regulation. The lack of a strong morphological response does not therefore imply a weak treatment effect; rather, it suggests that the main impact of urea was expressed at the metabolic level. This interpretation is consistent with blueberry's relatively conservative growth habit and with the notion that nitrogen form can influence physiological status more readily than gross canopy structure (Anwar et al., 2024; Doyle et al., 2021).

Fresh weight was one of the few biomass-related variables that showed a significant response. This may reflect changes in tissue hydration, cell expansion, or short-term assimilate partitioning rather than major differences in dry matter accumulation. Fresh biomass is often more responsive than dry weight to nitrogen nutrition because nitrogen affects leaf expansion, osmotic regulation, and water relations. In container-grown blueberry, where root-zone conditions are tightly controlled, small differences in nitrogen availability can translate into measurable differences in fresh tissue mass even when dry structural growth remains unchanged (Marshner, 2012). The present result therefore suggests that urea affected the water-rich component of biomass more strongly than long-term biomass partitioning.

Table 3. Analysis of variance (ANOVA) of measured traits in blueberry.

Source of variation	d.f.	Mean of squares									
		Plant height	Number of branches	Stem diameter	SPAD value	Number of inflorescences	Number of flowers per bush	Number of fruits per inflorescence	Fruit length	fruit width	Fruit weight
Nutrient solution	3	30.592 ns	0.2878 ns	0.0513 ns	10.209 **	0.0697 ns	22.126 **	32.428 **	2.891 ns	1.483 ns	0.0244 ns
Error	8	13.154	0.1667	0.0660	1.215	0.0375	2.742	0.3158	1.650	1.233	0.009942
C.V. (%)		9.58	14.16	7.89	3.06	15.39	17.34	11.73	15.21	11.17	15.74

Values marked with *, ** and ns are significant at the 5% and 1% probability levels and non-significant respectively

Table 3. Continue

Source of variation	d.f.	Mean of squares								
		Total fruit weight	Number of fruits per bush	N content in leaves	P content in leaves	K content in leaves	Ca content in leaves	Mg content in leaves	Antioxidant	Phenol
Nutrient solution	3	145.342 **	193.194 **	0.1736 **	0.4652 ns	0.0312 ns	0.006719 **	0.005325 ns	89.505 *	2.325 ns
Error	8	2.885	5.167	0.002875	3.771	0.0313	0.000493	0.0123	13.017	0.5709
C.V. (%)		17.93	19.07	2.79	9.66	9.62	6.06	13.95	4.35	17.95

Values marked with *, ** and ns are significant at the 5% and 1% probability levels and non-significant respectively

Table 3. Continue

Source of variation	d.f.	Mean of squares									
		TA	TSS	Chl. a	Chl. b	Total chl.	Carotenoid	Total plant Fresh weight	Total plant Dry weight	Total plant Dry weight percent	Ash percent
Nutrient solution	3	0.0406 ns	3.925 ns	0.0405 **	0.006070 **	0.0778 **	0.001593 **	17.970 *	1.648 ns	3.255 ns	1.639 ns
Error	8	0.0188	2.560	0.000913	0.000156	0.001784	0.000129	4.060	0.6815	0.8102	0.5000
C.V. (%)		14.13	12.52	12.13	10.40	11.44	15.98	8.69	10.18	2.57	13.91

Values marked with *, ** and ns are significant at the 5% and 1% probability levels and non-significant respectively.

Table 4. Mean comparison of traits in blueberry.

Nutrient solution	Antioxidant (%)	Chl. A (mg g^{-1} FW)	Chl. B (mg g^{-1} FW)	Total chl. (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
1	83.84±3.74ab	0.2087±0.0246b	0.1042±0.0095b	0.3128±0.0338b	0.0633±0.0074b
2	86.62±1.2a	0.1688±0.0197b	0.0904±0.0072b	0.2592±0.0269b	0.0573±0.0092b
3	86.34±0.26a	0.1975±0.0148b	0.0992±0.0069b	0.2966±0.0217b	0.0581±0.0038b
4	74.97±1.40b	0.4215±0.0034a	0.1872±0.0045a	0.6085±0.0073a	0.1054±0.0042a

Values followed by the same letter within each column are not significantly different according to Tukey test.

Table 4. Continue.

Nutrient solution	SPAD value	N content in leaves (g kg^{-1} DW)	Number of flowers per inflorescence	Number of fruits per bush	Total fruit fresh weight (g)
1	34.97±0.87b	2.24±0.035a	12.2±0.96a	8.93±0.0.32a	21.36±0.44b
2	38.23±0.31a	1.927±0.018b	9.63±0.96ab	5.93±0.32b	22.02±0.56ab
3	34.17±0.52b	1.877±0.045b	5.8±0.96b	2.07±0.0.32b	22.59±0.54ab
4	36.90±0.71ab	1.657±0.018c	10.57±0.96ab	2.23±0.32c	26.78±2.14a

Values followed by the same letter within each column are not significantly different according to Tukey test.

The stability of other mineral nutrients, including phosphorus, potassium, calcium, and magnesium, indicates that the tested urea concentrations did not provoke pronounced nutrient imbalance. This is an important practical finding because nitrogen management in blueberry can sometimes interfere with cation uptake or alter rhizosphere chemistry. However, no such detrimental interactions were evident here.

The absence of significant differences in these nutrients suggests that the physiological responses were driven primarily by nitrogen supply and not by secondary deficiencies or antagonisms. This interpretation is supported by the broader blueberry nutrition literature, which emphasizes that nitrogen form must be considered alongside pH, calcium dynamics, and substrate characteristics (Arias et al., 2024; Doyle et al., 2021). The lack of significant differences in fruit weight, sugar content, titratable acidity, and total phenolics suggests that urea concentration within the tested range had limited influence on final market-related fruit quality (Braha and Kullaj, 2024). This is an important agronomic outcome because it indicates that leaf nutritional improvement did not come at the expense of fruit composition. In blueberry, fruit quality is strongly governed by genotype, crop load, light environment, and maturation stage, so moderate changes in nitrogen supply may not necessarily translate into visible differences in soluble solids or acidity. Similar observations have been reported in studies where nitrogen rate altered growth or berry physiology without consistently changing basic fruit quality parameters (Anwar et al., 2024; Ehret et al., 2014). Therefore, the present findings suggest that cv. ‘Jewel’ maintained fruit-quality stability across the urea treatments.

Antioxidant activity, measured by DPPH, was significantly affected, whereas total phenolics were not. This partial dissociation is physiologically informative. Antioxidant capacity reflects the combined action of several reducing compounds, including phenolics, ascorbate-related metabolites, and other redox-active molecules, whereas total phenolics is only one biochemical subset (Yang et al., 2023). Thus, a significant change in DPPH without a corresponding change in total phenolics suggests that urea altered the qualitative composition or relative contribution of antioxidant constituents rather than simply increasing the total phenolic pool. Such decoupling between phenolic content and antioxidant capacity has been reported in fruit crops and indicates that bulk phenolic assays alone may not fully capture biologically relevant redox changes (Ehret et al., 2014; Yañez-Mansilla et al., 2015). In blueberry, where antioxidant functionality is highly valued, this finding is especially relevant because nutritional management may influence antioxidant profile in a compound-specific manner.

The present results also align with broader evidence that blueberry responds strongly to nitrogen form. Recent research has shown that balanced NH_4^+: NO_3^- nutrition under soilless culture can improve growth, nutrient uptake, chlorophyll content, photosynthetic capacity, and fruit set, with the 50:50 ratio often emerging as the most effective across multiple traits (Anwar et al., 2024). Although the current study used urea rather than a direct ammonium-nitrate combination, urea still enters the nitrogen cycle as reduced nitrogen and may partially mimic some advantages of ammonium-based nutrition depending on hydrolysis rate and root-zone conditions. This is especially relevant in acidic substrates, where blueberry root systems are physiologically adapted to reduced nitrogen forms and where nitrate dominance may be less efficient or less desirable (Doyle et al., 2021). The significant physiological responses observed here therefore fit well within the established understanding that Vaccinium species are particularly sensitive to the form and timing of nitrogen supply.

From an applied perspective, the findings suggest that urea management in greenhouse blueberry production should focus on optimizing leaf nitrogen status and photosynthetic performance rather than expecting large changes in plant architecture or fruit chemistry within a moderate concentration range. Because SPAD and leaf N responded clearly, these indices may be especially useful for monitoring nutritional status in container-grown blueberry. At the same time, the stability of fruit size, soluble solids, acidity, and total phenolics indicates that the tested urea levels can be used without compromising fruit quality. This balance is desirable in high-value berry production systems where both plant vigor and berry marketability are important.

Overall, the study indicates that blueberry cv. 'Jewel' exhibits a buffered morphological response but a sensitive physiological response to urea concentration under greenhouse conditions. The strongest effects occurred in traits associated with nitrogen assimilation, pigment metabolism, and antioxidant capacity, whereas most growth and fruit-quality variables were stable. This pattern reinforces the idea that blueberry nutritional management should be evaluated not only by yield and fruit size, but also by leaf physiological markers that capture early and more informative responses to nitrogen supply. Future work should compare urea directly with ammonium- and nitrate-based fertilization at equivalent N doses, extend the concentration range, and incorporate rhizosphere pH dynamics, nitrogen-use efficiency, and berry anthocyanin profiling to better define the optimal fertilization strategy for container-grown blueberry. Because nutrient uptake and physiological responses may differ among berry plant genotypes (Alizadeh et al., 2025; Haghparast et al., 2026), the present results should be interpreted specifically for cv. 'Jewel' rather than for blueberry cultivars in general.

Conclusion

In conclusion, varying urea concentration in the nutrient solution significantly influenced selected physiological and biochemical traits of greenhouse-grown blueberry (*Vaccinium corymbosum* L. cv. Jewel), while most vegetative growth and fruit quality parameters remained unaffected. The significant responses observed in SPAD value, leaf nitrogen, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, fresh weight, and antioxidant activity indicate that urea primarily altered internal nitrogen status and leaf metabolic performance rather than inducing major changes in plant morphology or basic fruit composition. These findings suggest that leaf-based physiological indices are more sensitive than conventional growth traits for detecting nitrogen responses in container-grown blueberry. The absence of significant effects on plant height, branching, stem diameter, fruit weight, soluble solids, titratable acidity, and total phenolics further indicates that moderate variation in urea supply can improve physiological status without compromising commercial fruit quality. Overall, the results demonstrate that blueberry cv. 'Jewel' shows a relatively stable agronomic performance across the tested urea concentrations, but a clear physiological sensitivity in traits associated with nitrogen assimilation and photosynthetic pigment formation. Therefore, optimizing urea nutrition in greenhouse blueberry production should focus on improving leaf nitrogen efficiency and photosynthetic function, with SPAD and pigment-related measurements serving as practical indicators of plant nutritional status. Further studies comparing urea directly with ammonium- and nitrate-based nitrogen sources and evaluating long-term effects on yield and berry phytochemical composition would help refine fertilization strategies for soilless and container-based blueberry systems. Added urea concentration significantly influenced several reproductive and yield-related traits of container-grown 'Jewel' blueberry, including flower number per bush, fruit number per inflorescence, total fruit weight, fruit number per bush, and yield per bush. Therefore, the tested urea concentrations should not be considered equivalent with respect to reproductive performance. Nevertheless, because a zero-urea or nitrogen-free control was not included, these findings should be interpreted as relative responses among the tested urea concentrations rather than as evidence of an absolute urea requirement or an optimum nitrogen level. A limitation of this study is the absence of a nitrogen-free control and of an equivalent-total-N treatment based on a different nitrogen form. Because the reported values refer to added urea concentration rather than to total nitrogen supply, and because no zero-nitrogen treatment was included, the results should be interpreted as comparative responses among the tested urea levels rather than as evidence of nitrogen deficiency, sufficiency, or toxicity. In particular, the lowest urea level cannot be assumed to have satisfied plant nitrogen requirements, and the responses observed at the highest level cannot, by themselves, be attributed to urea toxicity. Future studies should include a nitrogen-free control, a standard-N reference, equivalent-total-N treatments from ammonium- and nitrate-based sources, and a broader range of urea concentrations, together with direct measurements of nitrogen-use efficiency and toxicity indicators. The findings of this study are limited to container-grown highbush blueberry (*Vaccinium corymbosum* L. cv. 'Jewel') grown under the specific greenhouse conditions, substrate, irrigation regime, and nutrient-solution composition used in this experiment. Therefore, the observed responses to added urea concentration should not be generalized to other blueberry cultivars, species, or production systems. Further research including multiple blueberry cultivars, a wider range of urea concentrations, and different production conditions is required to determine the consistency of these responses.

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