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Artícle

Optimizing nitrogen ratios for blueberry cultivated under saline medium

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Abstract

Blueberry (*Vaccinium corymbosum* L.) production in Mexico is expanding rapidly, yet nitrogen management and salinity stress remain major challenges. This study evaluated the effects of ammonium (NH_4^+) and nitrate (NO_3^-) fertilization, with or without sodium chloride (NaCl, 30 mM), on growth, yield, and fruit quality of 'Biloxi' blueberry grown in coconut fiber substrate. A completely randomized 3x3 factorial design plus control was applied, varying nitrogen source, concentration (75% and 100%), and salinity. NH_4^+ significantly increased biomass (121.2%), flower production (316%), fruit number (231%) and yield (162.7%) compared with NO_3^-. A 100% N rate enhanced shoots (19.5%) and fruit count (43.4%) but reduced fruit size. Salinity reduced fruit number (-70.3%) and yield (-53.1%) without affecting vegetative growth. Significant interactions among nitrogen source, concentration and salinity influenced flowering, quality and agronomic traits. Results indicate NH_4^+ based fertilization improves blueberry productivity under saline conditions, supporting more efficient nitrogen management strategies.

Palabras clave:

Vaccinium corymbosum L., ammonium, nitrate, stress, quality, yield,

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Introduction

Over the past decade, Mexico has become one of the world's top five producers of blueberry (*Vaccinium corymbosum* L.), with annual growth in cultivated area and yield exceeding 15%, mainly concentrated in Jalisco, Michoacán, Sinaloa, Baja California, and Guanajuato (Trejo-Pech *et al*., 2024). This expansion is driven by increasing international demand and the recognized nutraceutical properties of the fruit (Krishna *et al*., 2023), encouraging cultivation in regions with specific edaphoclimatic conditions: altitudes between 1 500-4 700 m, temperatures ranging from 3-17 °C, acidic soils (pH 4-5) and electrical conductivity (EC) between 0.25 and 1.5 dS m^{-1} (Meléndez-Jácome *et al*., 2021).

As a calcifuge species, blueberry shows a preference for ammonium (NH_4^+); however, several studies report that nitrate (NO_3^-) can enhance nitrogen assimilation and improve physiological and agronomic traits (Alt *et al*., 2017; Anwar *et al*., 2024). While NH_4^+ can stimulate early growth, it also increases substrate EC, potentially inducing salt stress and limiting plant development (Machado *et al*., 2014; Hirzel *et al*., 2024).

In contrast, NO_3^- fertilization has been shown to promote photosynthesis, accumulation of antioxidant compounds, and stress tolerance, particularly under saline conditions (Leal-Ayala *et al*., 2021; Cárdenas-Navarro *et al*., 2024). Since salt stress disrupts key physiological processes but can also induce beneficial antioxidant synthesis (Krishna *et al*., 2023), it is crucial to evaluate nutritional strategies that mitigate its effects. Therefore, the objective of this study was to assess the impact of different NO_3^-/NH_4^+ ratios, combined with NaCl, on blueberry growth and yield, aiming to optimize nitrogen management under salinity stress.

Materials and methods

Experimental conditions and plant material

The experiment was conducted under low-tech greenhouse conditions in Ascensión, Aramberri, and Nuevo León, Mexico (24° 20' 14.96'' N, 99°56' 9.5'' W, 1961 masl, mean annual temperature 15.6 °C). Blueberry plants (*Vaccinium corymbosum* L.), variety Biloxi, propagated *in vitro* and acquired in 1 L pots, were transplanted on October 2, 2023, into 50 × 50 cm plastic bags filled with 20 L of prewashed, medium-texture coconut fiber. During acclimatization, plants received a standard nutrient solution (SN1) composed of K_2SO_4, KH_2PO_4, Ca $(NO_3)_2$ $4H_2O$, $MgSO_4$ $7H_2O$, $(NH_4)_2SO_4$ and micronutrients, following Frías-Ortega *et al*. (2020).

Experimental design and treatments

A completely randomized design was used with nine treatments: eight from a 3 × 3 factorial combination of nitrogen source (NO_3^- or NH_4^+), concentration (75% or 100%) and salinity (0 or 30 mM NaCl) and 50/50 NO_3^-/NH_4^+ (control). Treatments were: T1: NH_4^+ 100%; T2: NH_4^+ 100% + NaCl; T3: NH_4^+ 75%; T4: NH_4^+ 75% + NaCl; T5: NO_3^- 100%: T6: NO_3^- 100% + NaCl; T7: NO_3^- 75%; T8: NO_3^- 75% + NaCl; T9: 50% NH_4^+ / 50% NO_3^-. Each treatment had 10 replicates (one plant per pot), arranged in a 48 m² area, with rows oriented north-south, spaced 0.3 m between pots and 0.8 m between rows. Anti-aphid mesh was used to prevent soil contact.

Treatment application and fertigation

Treatments began on April 1, 2024. Irrigation was manual and daily, using treatment-specific nutrient solutions (Table 1), with 6-20% drainage. Water characteristics: EC 0.51 dS m^{-1}, pH 6.95, containing Ca^{2+}, Mg^{2+}, Na^+, HCO_3^-, Cl^-, and SO_4^{2-}.

Fertilizers included were: A) K_2SO_4; B) Mg $(NO_3)_2$ $6H_2O$; C) $(NH_4)_2$ SO_4; D) KH_2PO_4; E) KNO_3; F) Ca $(NO_3)_2$ $4H_2O$; G) $CaSO_4$ $2H_2O$; H) $MgSO_4$ $7H_2O$; I) $(NH_4)_2$ SO_4 and J) micronutrients (Table 1). For salinity treatments (T2, T4, T6 and T8), irrigation once weekly included 30 mM NaCl only.

Revista Mexicana de Ciencias Agrícolas
REMEXCA
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Table 1. Fertilizers used in the formulation of nutrients solutions (g L^{-1}).

Treatments	A	B	C	D	E	F	G	H	I	J
					(g L^{-1})					
T1-T2	0.17		0.08				0.22	0.19	0.35	0.02
T3-T4	0.17		0.08			0.14	0.1	0.19	0.25	0.02
T5-T6	0.04	0.22		0.09	0.19	0.26	0.06			0.02
T7-T8	0.11	0.22		0.09	0.03	0.26			0.09	0.02
T9	0.13			0.09		0.26		0.19	0.16	0.02

Growth and yield measurements

Every 14 days for 182 days, two tagged stems per plant were measured for length (cm), diameter (mm), number of leaves, secondary shoots, and buds. Flower count included fully developed white corollas. Fruits >5 mm in diameter were counted. On March 4, 2025, shoot biomass was collected by cutting stems 30 cm above the crown, chopped into 3-5 cm pieces, weighed fresh, and dried at 28.3 °C until constant weight.

Fruit quality and yield parameters

Harvest began 180 days after treatment initiation. For each harvest, a random fruit was measured for polar and equatorial diameter (mm). All fruits with 8-13 mm diameter was considered for yield analysis, excluding outliers (Cortés-Rojas *et al*., 2016). Total fruit number and accumulated yield (g) per plant were recorded. Juice was extracted (0.5 ml), and soluble solids content (°Brix) and juice temperature were measured using a digital refractometer (HANNA HI96801).

Statistical analysis

Data was analyzed using IBM SPSS v25. Anova was applied to detect treatment effects, and Tukey's test (p< 0.05) was used for post hoc comparisons where significant differences were found.

Results

Agronomic characteristics

Plants fertilized with NH_4^+ exhibited significantly higher values across all measured variables compared to those treated with NO_3^-: stem length (18.1%), diameter (21.3%), number of leaves (41.7%), secondary shoots (70.6%), total shoots (42.5%), flowers (316%) and fruits (193.5%). Biomass also increased by 121.2% (209.4 g *vs* 94.7 g with NO_3^- (Table 2). These findings align with reports showing NH_4^+ promotes root development and nutrient absorption (Arias *et al*., 2024), although proper pH management is critical for effective assimilation (Jiang *et al*., 2019).

Table 2. Agronomic behavior of blueberry under different nitrogen sources in saline medium.

Factor	Level	SL (cm)	SD (mm)	NL (Nº)	SS (Nº)	TS (Nº)	Fo (Nº)	Fr (Nº)	DM (g)
(N)	NH_4^+	42.1a	4.2a	27.9a	2.1a	66.8a	100.4a	140.4a	209.4a
	NO_3^-	35.6b	3.4a	19.7b	1.2b	46.9c	24.2c	47.9c	94.6c
	Control	38.7ab	4a	25.2a	1.8ab	53.6b	41.7b	80.8b	168.8b
(C)	100	39a	3.8a	22.5a	1.7a	61.9a	67.9a	110.9a	155.3a
	75	38.7a	3.8a	25.1a	1.7a	51.8b	56.6b	77.4b	148.7a
	Control	38.7a	4a	25.2a	1.8a	53.6b	41.7a	80.8ab	168.8a
(S)	30	37.9a	3.6a	22.9a	1.4b	54.9a	51.8b	78.2b	165.3a

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Factor	Level	SL (cm)	SD (mm)	NL (Nº)	SS (Nº)	TS (Nº)	Fo (Nº)	Fr (Nº)	DM (g)
	0	39.9a	4a	24.7a	2a	58.8a	72.7a	110a	138.7b
	Control	38.7a	4a	25.2a	1.8ab	53.6a	41.7b	80.8ab	168.8a
Interaction									
N x C		0.01	0.05	0.01	0.11	0	0	0	0
N x S		0.74	0.34	0.8	0.64	0.81	0	0.93	0.69
C x S		0.17	0.59	0.18	0.11	0.53	0	0.94	0.13
N x C x S		0.13	0.05	0.02	0.04	0.44	0.01	0.12	0.58

SL= stem length (cm); SD= stem diameter (mm); NL= number of leaves; SS= secondary shoots; TS= total shoots; Fo= number of flowers; Fr= number of fruits; DM= dry matter; N type (N); concentration (C) and NaCl (S, mM). Different le tters in the means per column in each factor indicate significant differences (Tukey, *p*≤ 0.05).

In contrast, the lower efficiency of NO_3^- may stem from its higher energetic demands for reduction (Ali, 2020; Berger *et al*., 2020). Nonetheless, Alt *et al*. (2017) observed that blueberry plants can adapt to NO_3^- and activate nitrate reductase, highlighting their metabolic plasticity. Increasing nitrogen concentration to 100% further enhanced shoot number (19.5%), flowers (20.1%), and fruits (43.4%) compared to 75% N (Table 2). These results support the role of nitrogen in synthesizing amino acids, enzymes, and hormones essential for floral development (Santiago and Sharkey, 2019; Cárdenas-Navarro *et al*., 2024). Notably, fruit production rose by 43.3% in plants with 100% N *vs* 75% (Table 2) and 87.4% in harvested fruits (Table 3).

Table 3. Productive behavior of blueberry under different nitrogen sources in saline medium.

Factor	Level	8	9	10	11	12	13	TF	CY
				Size					
(N)	NH_4^+	8.9a	22.2a	39.9a	31.6a	22.5a	10.7a	142.4a	71.7a
	NO_3^-	2.3b	5.3b	10.1b	9.8b	8.5b	4.4b	43b	27.3b
	Control	0.8b	3.3b	12.6ab	14.3b	13.9b	8.1ab	57.5b	42.6b
(C)	100	9.4a	21.6a	36a	23.5a	17.9a	7.5a	120.9a	57.9a
	75	1.9b	5.9b	14a	17.9a	12.75a	7.6a	64.6b	41.1a
	Control	0.8b	3.3b	12.6a	14.3a	13.9a	8.1a	57.5b	42.6a
(S)	30	3.7ab	11.4ab	17a	16.9a	10.95b	5.4a	68.6b	39.1b
	0	7.6a	16.13a	33a	24.5a	19.7a	9.7a	116.8a	59.9a
	Control	0.8b	3.3b	12.6a	14.3a	13.9ab	8.1a	57.5ab	42.6a
Interaction									
N x C		0.02	0	0.03	0.05	0.03	0.08	0.01	0.03
N x S		0.02	0.5	0.23	0.6	0.58	0.75	0.27	0.79
C x S		0.09	0.25	0.11	0.91	0.95	0.45	0.3	0.95
N x C x S		0.12	0.95	0.4	0.87	0.5	0.55	0.76	0.62

Factors 8 to 13 correspond to the number of fruits per size, total fruits harvested (TF, quantity), cumulative yield (CY, g), N type (N), concentration (C) and NaCl (S, mM) Different letters in each column for each factor indicate significant differences (Tukey, *p*≤ 0.05).

Salinity at 30 mM NaCl reduced secondary shoots (-30.4%), flowers (-28.8%) and fruits (-28.9%), while vegetative growth (SL, SD, NL, TS) remained unaffected (Table 2). These effects are attributed to Na^+ displacing K^+, which inhibits flowering-related enzymes (Wu, 2018; Atta *et al*., 2023). The findings align with Molnar *et al*. (2024), who documented shoot growth suppression under salt stress *in vitro*. Significant interactions were observed between N source × concentration (N × C) for most agronomic traits (SL, SD, NL, TS, Fo, Fr, DM (Table 2). NH_4^+ at 100% showed the strongest response. At 75%, differences between N sources diminished, but NH_4^+ still outperformed NO_3^- in terms of shoots, flowers, and dry matter. This suggests a concentration-dependent ionic modulation (Cárdenas-Navarro *et al*., 2024).

Revista Mexicana de Ciencias Agrícolas
REMEXCA
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Under salinity, NH_4^+ fertilization further reduced flowering, while NO_3^- maintained production, likely due to NH_4^+ induced apoplastic acidification that exacerbates Na^+ influx and limits K^+ and Ca^{2+} uptake (Shilpha *et al*., 2023). Hence, nitrogen form and concentration must be jointly optimized under saline conditions.

Productive behavior

NH_4^+ significantly outperformed NO_3^- in total fruit number (231%) and cumulative yield (162.7%) compared to NO_3^- and 147.8% and 68% *versus* control, respectively (Table 3). Fruit sizes 9-12 predominated in both NH_4^+ and NO_3^- treatments, although NH_4^+ slightly increased the share (81.4% *vs* 78.5%). At 100% N, fruit count per plant increased by 87.2% vs 75% and 110.2% vs. control. Cumulative yield rose by 35.7% over control and 40.8% over 75% N (Table 3). Fruit sizes 9-12 also predominated at higher N. These results highlight the importance of N in chlorophyll production and photosynthesis, improving energy availability for reproductive development (Zhang *et al*., 2023; Yang *et al*., 2023). A saturation points around 206-222 kg N ha^{-1} has been reported (Fang *et al*., 2020a), validating 100% as optimal. Doyle *et al*. (2021) emphasized NH_4^+ efficiency in translocating carbohydrates without causing osmotic stress.

Salinity (30 mM NaCl) reduced total fruit production by 70.3% compared to non-saline conditions (116.9 *vs* 68.6 fruits $plant^{-1}$) and yield dropped 53.1% (Table 3). Fruit sizes decreased, especially size 10 (-94.3%), indicating a restriction in parenchyma expansion due to osmotic stress (Denaxa *et al*., 2022). The N × C interaction was significant for fruit sizes 8-12, total fruit and yield. NH_4^+ at 100% achieved the highest values, while NO_3^- performed better at 75%, confirming the importance of optimizing both source and concentration. Neither C × S nor N × C × S interactions showed significant differences, except for size 8 (Table 3), indicating that salinity effects were largely independent of N source.

Quality behavior

NH_4^+ and NO_3^- treatments showed no significant differences in fruit diameter, weight, firmness or soluble solids (Table 4), consistent with previous studies (Petridis *et al*., 2018; Anwar *et al*., 2024). These traits are primarily genetically controlled and linked to source-sink dynamics (Ferrão *et al*., 2018).

Table 4. Quality behavior of blueberry under different nitrogen sources in saline medium.

Factor	Levels	DE	DP	P	SST	T
(N)	NH_4^+	11.87 b	8.92 a	0.78 a	14.51 a	20.61 a
	NO_3^-	11.8 b	8.91 a	0.75 a	13.42 ab	17.49 b
	Control	12.25 a	9.12 a	0.81 a	13.35 b	17.11 a
(C)	100	11.58 b	8.75 b	0.72 b	14.41 a	19.19 a
	75	11.86 b	8.96 a	0.77 a	13.51 a	18.91 a
	Control	12.47 a	9.26 a	0.86 a	13.35 a	17.11 b
(S)	30	11.47 c	8.7 b	0.7 b	14.4 a	19.51 a
	0	11.98 b	9.01 b	0.79 a	13.52 a	18.59 b
	Control	12.47 a	9.26 a	0.86 a	13.35 a	17.11 c
Interaction						
N x C		0.78	0.29	0.4	0.79	0
N x S		0	0	0.05	0.01	0
C x S		0.39	0.05	0.09	0.15	0
N x C x S		0.85	0.73	0.92	0.46	0

Equatorial diameter (SD, mm), polar diameter (PD, mm), weight (g), total soluble solids (TSS, % Brix), and fruit juice temperature (T, °C). Different letters per column for each factor indicate significant differences (Tukey’s test, $p\leq 0.05$).

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However, NH_4^+ increased juice temperature by 17.9%, likely due to enhanced respiration and sugar accumulation (Shilpha *et al*., 2023; Duan *et al*., 2023). Soluble sugars rose by 8.7% with NH_4^+ *vs* 0.5% with NO_3^-. At 100% N, fruit size and weight decreased (-7.1% ED, -5.5% PD, -16.3% weight), likely due to resource dilution among more fruits (Jorquera-Fontena *et al*., 2018; Doyle *et al*., 2021). Soluble solids remained stable, indicating homeostatic sugar transport (Sellami *et al*., 2019). Juice temperature increased by 12.2% (100%) and 10.6% (75%).

Salinity (30 mM NaCl) caused a significant reduction in ED, PD, and weight (-8%, -6%, -18.6%(Table 4). Soluble solids increased slightly (7.9%) as a stress response, and juice temperature rose by 14%. The N × S interaction was significant for ED, PD, firmness, and TSS. The combined N × C × S interaction only affected juice temperature, indicating that thermal accumulation is particularly sensitive to nutrient-salinity interactions (Table 4). Moderate N supplies enhance NO_3^- transport, GS/GOGAT activity and osmoprotection (Nazir *et al*., 2023; Farvardin *et al*., 2020). Additionally, apoplastic redox signaling explains reduced flowering under high NH_4^+ and salinity (Kesawat *et al*., 2023).

Conclusions

Nitrogen source and concentration, in interaction with salinity, significantly influenced the vegetative and reproductive performance of blueberry plants. Ammonium fertilization consistently promoted greater shoot vigor, flowering, and fruit set compared to nitrate, particularly at higher nitrogen concentrations. In contrast, saline conditions markedly reduced reproductive development, while exerting minimal effects on vegetative growth. These findings highlight the critical importance of optimizing nitrogen form and dosage to enhance productivity and mitigate the adverse effects of salinity. The significant interactions among nitrogen source, concentration, and salinity stress reinforce the need for integrated nutrient management strategies tailored to saline environments in blueberry cultivation.

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