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Response of *Agastache mexicana* subsp. *mexicana* to the nitrate: calcium relationship of the nutritive solution

Respuesta de *Agastache mexicana* subsp. *mexicana* a la relación nitrato: calcio de la solución nutritiva

López-Rodríguez, M.G.[1], *Villegas-Torres, O. G.*[1*], *Estrada-Soto, S. E.*[2], *Andrade-Rodríguez, M.* [1], *Perea-Arango, I.*[3], *Chávez-García, J. A.*[1], *Martínez-Fernández, E.*[4].

[1] *Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos Av. Universidad núm. 1001, Chamilpa, CP. 62209 Cuernavaca, Morelos, México.*
[2] *Facultad de Farmacia, Universidad Autónoma del Estado de Morelos Av. Universidad núm. 1001, Chamilpa, CP. 62209 Cuernavaca, Morelos, México.*
[3] *Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos Av. Universidad núm. 1001, Chamilpa, CP. 62209 Cuernavaca, Morelos, México.*
[4] *Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos Av. Universidad núm. 1001, Chamilpa, CP. 62209 Cuernavaca, Morelos, México.*

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ABSTRACT

Agastache mexicana subsp. *mexicana* is a medicinal plant used in the pharmaceutical and herbal industry; however, its nutritional requirements for its production are unknown. Therefore, the current investigation aimed to evaluate different NO_3^- : Ca^{2+} ratios of the nutrient solution in the vegetative stage of the *A. mexicana* to determine its effect on growth and dry matter production. Three concentrations of NO_3^- (10, 12, and 14 mEq L^{-1}) and Ca^{2+} (7, 9, and 11 mEq L^{-1}) were assessed, in a complete factorial arrangement of treatments. The Universal Nutrient Solution (Steiner, 1984) was the basis for the modifications of the NO_3^-: Ca^{2+} ratios, 10 repetitions per treatment and a completely random design were considered. The experimental unit was a black polyethylene container (15.14 L), with red tezontle gravel as substrate, with one plant per container. The results indicated that the solutions produced a statistically significant difference ($p \leq 0.05$) in the evaluated variables which were: root volume, main stem length, main stem diameter, leaf area, relative chlorophyll content, chlorophyll *a, b*, and total, root, stem and leaf dry matter. The NO_3^- : Ca^{2+} ratio of 10:9 mEq L^{-1} promoted the relative chlorophyll content, chlorophyll *a, b*, stem dry matter, total dry matter and optimized fertilization costs with respect to the 10:7 mEq L^{-1} ratio, while the 14:9 mEq L^{-1} ratio obtained the lowest values in the evaluated variables. The NO_3^- : Ca^{2+} ratio of 10:9 mEq L^{-1} of the nutrient solution allowed better assimilation of nitrogen and calcium, which led to favorable growth and therefore greater dry matter production.

KEY WORDS: *Medicinal plant, Mineral nutrition, Container cultivation, Semi-hydroponic cultivation, Dry matter.*

***Corresponding Author:**

Oscar Gabriel Villegas-Torres. Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos Av. Universidad núm.1001, Chamilpa, CP. 62209 Cuernavaca, Morelos, México. Teléfono: 52 777 180 6252. E-mail: oscar.villegas@uaem.mx

RESUMEN

El toronjil morado (*Agastache mexicana* subsp. *mexicana*) es una planta medicinal utilizada en la industria herbolaria y farmacéutica; sin embargo, se desconocen sus requerimientos nutrimentales para su producción. El objetivo de la investigación fue evaluar diferentes relaciones NO_3^- : Ca^{2+} de la solución nutritiva en etapa vegetativa del toronjil morado con la finalidad de determinar su efecto en el crecimiento y la producción de materia seca. Se evaluaron tres concentraciones de NO_3^- (10, 12 y 14 mEq L^{-1}) y de Ca^{2+} (7, 9 y 11 mEq L^{-1}), en arreglo factorial completo de tratamientos. La Solución Nutritiva Universal (Steiner, 1984) fue la base para realizar las modificaciones de las relaciones NO_3^- : Ca^{2+}, se consideraron 10 repeticiones por tratamiento y una distribución completamente al azar. La unidad experimental fue un contenedor de polietileno negro (15.14 L), con grava de tezontle rojo como sustrato, con una planta por contenedor. Los resultados indicaron que las soluciones produjeron una diferencia estadísticamente significativa ($p \leq 0.05$) en las variables evaluadas que fueron: volumen de raíz, longitud de tallo principal, diámetro de tallo principal, área foliar, contenido relativo de clorofila, clorofila *a*, *b*, total, materia seca de raíz, tallo y hojas. La relación NO_3^- : Ca^{2+} de 10:9 mEq L^{-1} benefició el contenido relativo de clorofila, clorofila *a*, *b*, materia seca de tallo, materia seca total y optimizó los costos de fertilización con respecto a la relación de 10:7 mEq L^{-1}, mientras que la relación 14:9 mEq L^{-1} no benefició al crecimiento de la planta. La relación NO_3^- : Ca^{2+} de 10:9 mEq L^{-1} de la solución nutritiva, permitió una mejor asimilación de nitrógeno y calcio, lo que condujo a un crecimiento favorable y por lo tanto mayor producción de materia seca.

PALABRAS CLAVE: Planta medicinal, Nutrición mineral, Cultivo en contenedor, Cultivo semi hidropónico, Materia seca.

Introduction

Agastache mexicana subsp. *mexicana (Kunth) E.F. Linton & Epling* (toronjil) is an aromatic herbaceous plant native to Mexico, it grows wild in pine-oak forests and is primarily concentrated along the Neovolcanic Axis in central Mexico, particularly in the Popocatépetl volcano region, within the Ozumba municipality, State of Mexico, and in the Milpa Alta County, Mexico City. The species is distributed across the states of Guanajuato, Michoacán, Puebla, Querétaro, Hidalgo, Veracruz, Chihuahua, Morelos, and Tlaxcala (Martínez-Gordillo *et al.,* 2017; Palma-Tenango *et al.,* 2021). Its socioeconomic importance lies in its high demand as a medicinal product, mainly due to its biological activities, including anti-atherogenic, anti-inflammatory, antihypertensive, antidiabetic, and vasorelaxant effects (Carrillo-Galván *et al.,* 2020). Additionally, traditional medicine recommends it for rheumatic symptoms, asthma treatment, and gastrointestinal conditions such as stomach pain, among others. Ethnomedical records describe different consumption dosages, with dried plant material being the most commonly used form. The primary plant parts utilized are the leaves and flowers, while the roots are used less frequently. The preparation methods include decoctions, infusions, teas, and dried (powdered) form for oral administration. Common dosages include 6 g of plant material per liter of water or one tablespoon per day, boiled until the "water takes on color," either alone or mixed with other plants such as hinojo (*Foeniculum vulgare Mill*.), tila (*Ternstroemia pringlei* (Rose) *Standl*.), toronjil blanco (*Agastache mexicana* subsp. *xolocotziana*), Artemisa (*Artemisia absinthium L*.), among others (Archundia, 2005; Monroy-Ortiz & Castillo-España, 2007; Santillán-Ramírez *et al.,* 2008; CONAFOR, 2010). The first written mention of *A. mexicana* under its Nahuatl name tlalahuehuetl dates back to the 16th century (1552), when it was recorded in the *Libellus de medicinalibus indorum herbis* (Badianus Manuscript), one of the oldest manuscripts on Mexican medicinal plants, since then, its approaches of consumption have diversified significantly (Linares & Bye, 2013). Phytochemical and pharmacological studies have validated this traditional knowledge, demonstrating that the previously mentioned biological activities are primarily attributed to two major classes of bioactive compounds: flavonoids such as acacetin, tilianin, and hesperitin, and terpenoids including monoterpenes, sesquiterpenes, diterpenes, and triterpenes, such as ursolic, oleanolic, corosolic, and maslinic acids. Furthermore, no toxicological effects have been reported (Hernández-Abreu *et al.,* 2012; Estrada-Reyes *et al.,* 2014; Flores-Flores *et al.,* 2016; Carmona-Castro *et al.,* 2019; Cruz-Torres *et al.,* 2023).

Due to these potential applications in the pharmaceutical and medicinal industries, domestication of the species is necessary, as cultivated plant material enables greater control over the supply chain and reduces chemical variability in bioactive compounds (Najar *et al.,* 2019). However, research on this species has primarily focused on its phytochemical, pharmacological, ethnomedical, and ethnobotanical properties, while its nutritional requirements for cultivation remain unknown (Palma-Tenango *et al.,* 2021).

In the cultivation of medicinal plants, mineral nutrition plays a crucial role in achieving high productivity in terms of vegetative growth and dry matter accumulation. Nutrient deficiencies or excesses negatively affect processes such as photosynthesis, respiration, nutrient assimilation and transport, as well as anabolic and catabolic pathways of amino acids and proteins, ultimately impacting the chemical composition of bioactive molecules (Xie *et al.,* 2019; He-Chao *et al.,* 2020; de Oliveira *et al.,* 2021). Nitrogen fertilization during the vegetative growth phase is essential for plant nutrition, as nitrogen ($N\text{-}NO_3^-$; $N\text{-}NH_4^+$) is required for plant growth and development (Larios-González *et al.,* 2021). Nitrogen also increases leaf area and dry matter accumulation in leaves and contributes to the activation of enzymes involved in the biosynthesis of numerous essential oil compounds, thereby improving the yield of aromatic plants (Nurzyńska-Wierdak, 2013). The importance of N in plants lies in its role as a structural component of proteins, amino acids, nucleic acids, and chlorophyll, making it essential for photosynthesis (Ontiveros-Capurata *et al.,* 2022).

Regarding calcium (Ca^{2+}), its absorption can be substantially reduced by antagonistic interactions with K^+, Mg^{2+}, and NH_4^+, depending on their relative concentrations in the nutrient solution, although NO_3^- stimulates its uptake (Torres-Olivar *et al.,* 2015), besides, calcium is

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a structural component of cell walls and membranes, regulates osmotic pressure, selectively modifies cation transport across membranes, regulates hormone transport, and influences enzymatic activity (Baran, 2021). In this context, maintaining an optimal balance of macronutrient ratios is fundamental for improving nutrient uptake efficiency and, consequently, achieving better production outcomes. While higher nitrogen concentrations in the soil can promote greater plant growth, proper management of the balance between anions and cations in the nutrient solution is necessary to maximize plant response (Torres-Olivar *et al.,* 2018; Lara-Herrera *et al.,* 2023). Therefore, this research aimed to evaluate different NO_3^- : Ca^{2+} ratios in the nutrient solution during the vegetative stage of the *A. Mexicana* to determine their effects on growth and dry matter production.

Material and Methods

The experiment was conducted in a greenhouse covered with white polyethylene (30 % shade) at the experimental field of the Faculty of Agricultural Sciences (18° 58' 51" N, 99° 13' 57" W, at an altitude of 1,868 masl) at the Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico, from June to September 2022.

Plant material

A. mexicana seeds were collected from the orchard of the Directorate of Sustainable Development at the Universidad Autónoma del Estado de Morelos (18° 58' 53'' N, 99° 13' 58.4'' W). The seeds were sown in 50-cell polyethylene trays, and once the seedlings emerged, they were irrigated with nutrient solution Steiner (1984) nutrient solution at 40%. A total of 800 adult plants were cultivated, from which three outstanding individuals were visually selected based on height growth, stem diameter, secondary stem production capacity, and leaf area. The sample was identified and deposited by curator M.Sc. Gabriel Flores Franco in the herbarium of the Center for Research in Biodiversity and Conservation at the Universidad Autónoma del Estado de Morelos (HUMO) (voucher No. 39795). The three selected plants served as mother plants for the production of 12 cm-long cuttings used as plant material. The nursery phase had its own agronomic management and was not subject to evaluation. This section was conducted because, to date, no hybrid or improved seeds or seedlings of *A. mexicana* are reported to be commercially available.

Experimental unit

The experimental unit consisted of a black polyethylene container (15.14 L) filled with red tezontle (0.5–1.0 ±1 cm particle size) were used as the substrate, which is chemically inert (Ojodeagua *et al.,* 2008). with a previously rooted 12 cm-high *A. mexicana*. Transplanting was performed when the roots of the cutting completely filled the root ball in the tray cavity.

Treatments and experimental design

Nine treatments were established based on three levels of NO_3^- concentration (10,

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12, and 14 mEq L^{-1}) and three levels of Ca^{2+} concentration (7, 9, and 11 mEq L^{-1}). The total ionic concentration of the nutrient solution was maintained at 100 %. A completely randomized experimental design was used, with 10 replicates per treatment. The control treatment followed the Steiner (1984) formulation, which considers an NO_3^- concentration of 12 mEq L^{-1} and a Ca^{2+} concentration of 9 mEq L^{-1} (treatment 5), with a total anion and cation concentration of 20 mEq L^{-1}, respectively (Table 1). The experiment lasted 90 days, from transplanting until inflorescences appeared on 90 % of the stems.

Table 1. Treatments for the mutual relationships between NO_3^-: Ca^{2+} of the Universal Nutrient Solution (Steiner, 1984) at 100 %, for the cultivation semi hydroponic of *Agastache mexicana* subsp. *mexicana* in a greenhouse.

Treatments	Relative ion concentration (mEq.L^{-1})					
	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	K^+	Ca^{2+}	Mg^{2+}
1	10.00	1.25	8.75	8.27	7.00	4.73
2	10.00	1.25	8.75	7.00	9.00	4.00
3	10.00	1.25	8.75	5.73	11.00	3.27
4	12.00	1.00	7.00	8.27	7.00	4.73
5 (T)	12.00	1.00	7.00	7.00	9.00	4.00
6	12.00	1.00	7.00	5.73	11.00	3.27
7	14.00	0.75	5.25	8.27	7.00	4.73
8	14.00	0.75	5.25	7.00	9.00	4.00
9	14.00	0.75	5.25	5.73	11.00	3.27

T= Control treatment

Preparation of nutrient solutions

Highly soluble fertilizers were used (potassium nitrate, calcium nitrate, potassium sulfate, monopotassium phosphate, and magnesium sulfate). In addition, each treatment included the following micronutrients: Fe, 5 mg L^{-1} (source: Fe-EDTA); H_3BO_3, 2.88 mg L^{-1}; Mn, 0.502 mg L^{-1} ($MnCl_2$); Zn, 0.05 mg L^{-1} ($ZnSO_4$); Cu, 0.045 mg L^{-1} ($CuSO_4$); Mo, 0.01 mg L^{-1} (H_2MoO_4). All materials, as well as the plant material, were disinfected with organic products. The nutrient solutions were prepared with tap water, and prior to their formulation, the chemical composition and hardness

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of the water were analyzed in a certified soil laboratory (ER-0223/2020, ISO 9001:2015). The pH was adjusted to 6 using H_2SO_4 (1M).

The nutrient solutions were supplied through an automated drip irrigation system equipped with Netafilm® self-compensating drippers with a flow rate of 8 L h^{-1}. Seven irrigation events were programmed with a timer, each lasting one minute during the first week after transplanting. Subsequently, irrigation time was increased to two minutes as the plant's water requirements for growth increased. The cost of the 10:9 and 14:7 solutions was calculated based on the price per bag of the following commercial fertilizers: Calcinit®, NKS®, SOP®, Sulmag®, and MAP®. The amount used for 450 liters was extrapolated to determine the cost per liter for 1,100 liters of solution.

Variables evaluated

The response variables included root volume, measured using the water displacement method which is described below: a 1000 mL graduated cylinder was filled with a known volume of water, and the entire root system was submerged; the difference between the final and initial volume was recorded as root volume in cubic centimeters. The main stem length was measured in centimeters using a tape measure (Pretul®), from the base of the main stem to the apex of the longest stem, the main stem diameter was recorded in millimeters, measured 2 cm above the substrate using a digital caliper (Stainless Hardened®), the leaf area, in square centimeters, was quantified with a leaf area integrator (LI-COR LI3100C), considering all leaves of each plant per treatment, the relative chlorophyll content was measured with a SPAD 502 Plus Spectrum portable chlorophyll meter (Minolta®), averaging three intermediate-aged leaves per plant, the chlorophyll *a*, *b*, and total chlorophyll were determined using the spectrophotometric method at wavelengths of 645 and 663 nm, using a GENESYS 10S UV spectrophotometer, following the methodology proposed by Rodés & Collazo (2006), a sample of four leaves per treatment was used to measure the relative chlorophyll content, subsequently, for each treatment, a 0.1 g sample of fresh leaf tissue was taken, 10 mL of 80 % cold acetone was added, and the mixture was macerated for 30 seconds at 14,000 rpm using an ULTRA TURRAX T8 (IKA®). The samples were filtered through filter paper and stored in amber vials until analysis. An 80 % acetone solution was used as a blank, and 10 mm rectangular quartz cuvettes (Thomas Scientific) were cleaned with distilled water between each measurement, the dry weight of roots, stems, and leaves was recorded in grams using an analytical balance after drying the samples in a mechanical convection oven at 70 °C for 72 hours.

At the end of the experiment, a nutrient analysis was performed on the roots and shoots of the NO_3^- 10 mEq $L^{-1}$and Ca^{2+} 9 mEq L^{-1}; NO_3^- 14 mEq L^{-1} and Ca^{2+} 9 mEq L^{-1} treatments in a certified soil laboratory (ER-0223/2020, ISO 9001:2015).

Statistical analysis

The data obtained were analyzed using analysis of variance in SAS software (version 9.0). For variables significantly affected by treatments, a multiple means comparison was performed using Tukey's test ($p \leq 0.05$).

Results and Discussion

The analysis of variance for vegetative growth variables indicated that at least one nutrient solution resulted in statistically significant differences ($p \leq 0.05$) in relative chlorophyll content, chlorophyll *a*, *b*, and total chlorophyll, root volume, stem length and diameter, and leaf area. Regarding relative chlorophyll content, the nutrient solution with a NO_3^- : Ca^{2+} ratio of 10:9 yielded the highest SPAD readings (51.62), whereas the 14:9 ratio yielded the lowest value (46.180). The relative chlorophyll content is an indicator of the relationship between the degree of supply and availability of nutrients and is related to the amount of N in the plant, which is modified according to the phenological phase of the crop (Juárez-Rosete *et al.,* 2007; Kalaji *et al.,* 2017). The response observed in *A. mexicana* was that by increasing NO_3^- to 14 mEq L^{-1} the SPAD readings decreased to 46.180 conflicting with what was reported by Ontiveros-Capurata *et al.* (2022) in the cultivation of Basil (*Ocimum basilicum*), whereby increasing the NO_3^- content to 12 mEq L^{-1}, the relative chlorophyll content increased to 52.70 and with 16 mEq L^{-1} to 56.80, which suggests that this species is assimilating nitrogen at higher concentrations for its growth; however, for *A. mexicana*, it was observed that with a nitrogen input of 10 mEq L^{-1}, the SPAD reading was 51.62, which could be sufficient to maintain its growth without requiring high concentrations of NO_3^-, this could be attributed to greater efficiency in the use of nitrogen due to its interaction with calcium. For chlorophyll *a*, the 10:9 ratio yielded a value of 16.87, representing 38.27 % more than the 14:9 ratio. Chlorophyll *b* also exhibited the highest concentration under the same treatment, as did total chlorophyll, which was 45.90 % higher than the 14:9 treatment (Table 2). The laboratory-determined chlorophyll and nitrogen concentrations correlated with SPAD readings, a result consistent with various reports on vegetables, ornamental plants, and medicinal plants, where a correlation between SPAD readings and N and chlorophyll content has been established (Basyouni *et al.,* 2015; Mendoza-Tafolla *et al.,* 2019).

For root volume, the nutrient solution with a 10:7 NO_3^-: Ca^{2+} ratio produced a root volume of 40 cm^3, while the 12:11 ratio resulted in a 103.75 % higher volume, reaching 81.50 cm^3. The treatments with NO_3^-: Ca^{2+} ratios of 10:9, 12:7, 12:9, and 14:7 mEq L^{-1} were statistically similar, in this case, the greater root volume can be attributed to the 11 mEq L^{-1} Ca^{2+} concentration, as it was the only factor that, when increased, produced a significant effect. Root growth depends on nutrient availability in the substrate, while shoot growth relies on nutrient transport from the roots. Roots require carbohydrates produced in the aerial part through photosynthesis, while shoots depend on water and nutrients absorbed by the roots, maintaining a balance between dry matter distribution in roots and shoots (Barrios *et al.,* 2014).

Greenhouse cultivation utilizing inert substrates, along with optimal water and nutrient supply, encourages plant growth while maintaining a reduced root system, as evidenced by a smaller fraction of the plant's total dry matter. For example, in cucumbers, the dry matter allocated to roots ranges from 8 % to 15 % during the early vegetative growth stage, in tomatoes, it varies between 17 % and 20 % in the initial stage and from 1 % to 10 % in the generative stage (Peil & Gálvez, 2005). These findings correspond with the results of this study, despite the 12:11 solution having the highest root volume, it did not produce the best vegetative growth. Conversely, the 10:9 and 14:7 solutions exhibited significant values in the assessed growth variables, despite root

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volumes of 54.70 and 62.50 cm^3, respectively (Table 2). The percentage of dry matter allocated to roots was 21.20 % and 22.80 %, while the dry matter fraction for stems was 48.16 % and 40.90 %, and for leaves, 30.8 % and 36.7 %, respectively, this suggests that, in the *A. mexicana* cultivated in an inert substrate, a higher root volume does not necessarily lead to greater aerial growth.

The nutrient solution with a 10:11 resulted in a 9.5 % greater stem length compared to the Steiner solution (12:9) (Table 2), it was observed that increasing NO_3^- concentrations from 10 to 14 mEq L^{-1} and Ca^{2+} concentrations from 7 to 11 mEq L^{-1} reduced stem length, indicating that higher NO_3^- and Ca_2^+ concentrations negatively affected plant growth. However, when NO_3^- was reduced while maintaining a high Ca^{2+} concentration at 11 mEq L^{-1}, plant height was favored, with the highest value recorded at 91.10 cm for the 10:11 ratio. This result contrasts with the findings of Torres (2019) for *A. mexicana* cultivated in 5 L polyethylene containers under plastic cover, where plants supplied with 24 mEq L^{-1} NO_3^- reached a height of 77.66 cm, showing no significant differences compared to treatments with 6, 12, and 18 mEq L^{-1} NO_3^-. These differences, despite involving the same species, may be attributed to agronomic management, substrate composition, and the mutual interaction between NO_3^- and Ca^{2+}.

Table 2. Growth of the *A. mexicana* in response to NO_3^-: Ca^{2+} relationship of the Steiner universal nutrient solution (1984).

NO_3^-: Ca^{2+}	RCC (SPAD)	chlorophyll a	chlorophyll b	Total chlorophyll	RV (cm^3)	SL (cm)	SD (mm)	LA (cm^2)
10:7	48.540 ^{ab}	16.75 ^{ab}	5.73 ^{ab}	23.33 ^{b}	40.0 ^{d}	78.30 ^{bcd}	4.66 ^{abc}	2352.3 ^{ab}
10:9	51.620 ^{a}	16.87 ^{a}	6.00 ^{a}	26.54 ^{a}	54.70 ^{c}	85.30 ^{ab}	4.92 ^{ab}	2555.3 ^{ab}
10:11	50.310 ^{ab}	14.45 ^{d}	5.55 ^{b}	20.16 ^{d}	78.90 ^{ab}	91.100 ^{a}	4.90 ^{ab}	2427.3 ^{ab}
12:7	49.650 ^{ab}	15.35 ^{c}	5.72 ^{ab}	21.05 ^{cd}	60.30 ^{c}	83.00 ^{abc}	4.70 abc	2411.2 ^{ab}
12:9 (T)	49.910 ^{ab}	15.13 ^{c}	5.73 ^{ab}	20.86 ^{d}	56.20 ^{c}	83.10 ^{abc}	4.36 ^{c}	2340.8 ^{ab}
12:11	49.660 ^{ab}	15.33 ^{c}	5.56 ^{b}	21.13 ^{cd}	81.50 ^{a}	81.00 ^{bcd}	4.92 ^{ab}	2358.2 ^{ab}
14:7	50.610 ^{ab}	14.53 ^{d}	5.54 ^{b}	20.06 ^{d}	62.50 ^{c}	82.30 ^{bcd}	5.03 ^{a}	2715.2 ^{a}
14:9	46.180 ^{b}	12.20 ^{e}	5.91 ^{ab}	18.19 ^{e}	67.00 ^{bc}	75.30 ^{cd}	4.26 ^{c}	2130.2 ^{ab}
14:11	48.570 ^{ab}	16.51 ^{b}	5.53 ^{b}	22.16 ^{bc}	67.50 ^{bc}	74.30 ^{d}	4.48 ^{bc}	1972.0 ^{b}
MSD	5.31	0.28	0.42	1.20	13.39	8.28	0.51	590.29
CV (%)	7.51	1.31	5.15	3.91	14.82	7.1	7.74	17.46

NO_3^-: Ca^{2+}= Nitrate: calcium relationship; RCC= Relative chlorophyll content in SPAD values; RV=Root volume; SL= Stem length; SD= Stem diameter; LA= Leaf area; T= Control treatment; MSD= Minimum significant difference; CV= Coefficient of variation; treatments with the same letter were statistically equal (Tukey, p >0.05).

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For stem diameter, the 14:7 ratio was 15.36 % superior to the 12:9 ratio. Regarding leaf area, the 14:7 ratio once again yielded the best results, with a total leaf area of 2,715.2 cm^2 (Table 2). On the other hand, the 10:9 nutrient solution consistently produced high leaf area values. This treatment was also optimal for relative chlorophyll content, chlorophyll *a*, *b*, and total chlorophyll, considering that chlorophyll concentration is related to leaf biomass. This effect could be attributed to the increase in NO_3^- concentration from 10 to 14 mEq L^{-1}, as stem diameter also increased.

Regarding dry matter accumulation, the 12:11 solution resulted in 14.85 g of root dry matter, while the 10:09 solution produced 23.02 g of stem dry matter, and the 14:07 solution yielded 18.51 g of leaf dry matter. The highest total dry matter accumulation was recorded for the 10:9 treatment, with 47.74 g, which was statistically similar to the 14:07 treatment, with 49.72 g (Table 3). Dry matter is considered a key parameter for quantifying growth, as it is directly related to photosynthetic efficiency and nutrient availability and utilization (Azcón-Bieto & Talón, 2008; Villegas-Torres *et al.,* 2012). In the *A. mexicana*, stems exhibited the highest nutrient demand due to their vegetative growth phase. Under the 10:9 nutrient solution, 21.20 % of the dry matter was allocated to roots, 48.16 % to stems, and 30.8 % to leaves. The 14:07 ratio treatment allocated 22.80 % to roots, 40.90 % to stems, and 36.7 % to leaves.

Table 3. Dry matter production in semi-hydroponic cultivation of *Agastache mexicana* subsp. mexicana in response to the mutual NO_3^-: Ca^{2+} ratios of the universal nutrient solution.

NO_3^-: Ca^{2+}	SDM (g)	RDM (g)	LDM (g)	TDM (g)	RDMP (%)	SDMP (%)	LDMP (%)
10:7	12.59 ^{d}	8.37 ^{c}	12.59 ^{ab}	33.56 ^{d}	24.80 ^{c}	37.44 ^{bc}	37.9 ^{a}
10:9	23.02 ^{a}	9.63 ^{bc}	15.08 ^{ab}	47.74 ^{a}	21.20 ^{c}	48.16 ^{a}	30.8 ^{a}
10:11	19.05 ^{abc}	11.85 ^{ab}	16.30 ^{ab}	47.20 ^{ab}	25.80 ^{bc}	40.17 ^{bc}	34.0 ^{a}
12:7	18.09 ^{abcd}	10.81 ^{bc}	15.67 ^{ab}	44.57 ^{abcd}	24.00 ^{c}	40.20 ^{bc}	35.6 ^{a}
12:09 (T)	18.41 ^{abcd}	10.71 ^{bc}	13.10 ^{ab}	42.24 ^{abcd}	25.30 ^{bc}	43.43 ^{ab}	31.1 ^{a}
12:11	15.23 ^{bcd}	14.85 ^{a}	15.42 ^{ab}	45.51 ^{abc}	33.10 ^{a}	33.34 ^{c}	33.5 ^{a}
14:7	20.15 ^{ab}	10.94 ^{bc}	18.51 ^{a}	49.72 ^{a}	22.80 ^{c}	40.90 ^{abc}	36.7 ^{a}
14:9	12.92 ^{d}	11.88 ^{ab}	10.66 ^{b}	35.46 ^{cd}	33.86 ^{a}	35.85 ^{bc}	30.3 ^{a}
14:11	13.88 ^{cd}	11.35 ^{bc}	10.61 ^{b}	35.84 ^{bcd}	31.80 ^{ab}	38.42 ^{bc}	29.9 ^{a}
MSD	6.12	3.33	6.15	11.54	6.98	7.79	9.11
CV (%)	25.14	20.89	30.28	19.4	18.19	13.69	19.1

NO_3^-: Ca^{2+}= Nitrate: calcium relationship; SDM= Stem dry matter; RDM= Root dry matter; LDM= Leaf dry matter; TDM= Total dry matter; RDMP= Root dry matter partition; SDMP= Stem dry matter partition; LDMP= Leaf dry matter partition; T= Control treatment; CV= Coefficient of variation; MSD= Minimum significant difference; treatments with the same letter were statistically equal (Tukey, $p > 0.05$).

The influence of nitrogen on dry matter accumulation has been well-documented across various crops, including vegetables, medicinal plants, and ornamentals, for example, in chilhuacle pepper (*Capsicum annuum L.*), supplying 10 and 14 mEq L^{-1} NO_3^- increased dry matter accumulation in plant organs (Fajardo-Rebollar *et al.,* 2022), similarly, in strawberry cultivation, a concentration of 14 mEq L^{-1} NO_3^- (Mixquititla-Casbis *et al.,* 2020), as was also reported for habanero pepper (*Capsicum Chinense Jacq*), where 14 mEq L^{-1} NO_3^- benefited dry matter accumulation (López-Gómez *et al.,* 2017). The positive effects of NO_3^- have also been observed in aromatic plants, not only in dry matter accumulation but also in the enhancement of their bioactive compounds, for instance, research on rosemary (*Salvia rosmarinus*) has shown that essential oil yield significantly depends on both the absolute and relative concentrations of NO_3^- and K (Puttanna *et al.,* 2010). Similar findings have been reported for basil (*Ocimum basilicum*) (Taie *et al.,* 2010) and lemon balm (*Melissa officinalis*) (Abbaszadeh *et al.,* 2009), among others. However, this effect depends on agronomic management and the interactions between nutrients in the nutrient solution. In the present study, the interaction between NO_3^- and Ca^{2+} optimized nutrient assimilation for plant growth. This contrasts with the findings of Torres (2019), who reported no significant response in dry matter accumulation resulting from the gradual increase of nitrogen in fertilization.

Regarding nutrient concentration and uptake, the 10:9 ratio resulted in higher extraction and concentration of N, P, K, Ca, and Mg in both roots and stems of the *A. mexicana* compared to the 14:9 solution (Table 4). This aligns with the findings of Torres-Olivar *et al.* (2018) in poinsettia (*Euphorbia pulcherrima* (Willd. ex Klotzsch), where the NO_3^- and Ca^{2+} ratio influenced nutrient uptake efficiency.

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Table 4. Concentration and extraction of total macroelements in root, stem and leaf, used in the universal nutrient solution Steiner (1984) for the cultivation of *A. mexicana* in containers under plastic cover.

NO_3^-: Ca^2	Macroelement	Dry matter	Nutrient concentration	Nutrient Extraction
10:9	N	47.74	7.58	1.234
14:9		35.46	6.73	0.766
10:9	P	47.74	0.78	0.121
14:9		35.46	0.56	0.065
10:9	K	47.74	7.51	1.282
14:9		35.46	6.29	0.738
10:9	Ca	47.74	3.02	0.462
14:9		35.46	2.91	0.334
10:9	Mg	47.74	1.68	0.202
14:9		35.46	1.53	0.177
10:9	Na	47.74	0.10	0.010
14:9		35.46	0.06	0.007
10:9	S	47.74	0.82	0.110
14:9		35.46	1.07	0.124

grams per plant

The nutrient solutions with ratios of 10:9 and 14:7 showed the highest values in terms of growth and dry matter accumulation, therefore, to determine the most suitable solution for the *A. mexicana* production, the cost of preparing these nutrient solutions was estimated for a volume of 1,100 L. The cost for the 10:9 NO_3^- : Ca^{2+} solution was $154.12, while the 14:7 solution amounted to $176.82. These results confirm the importance of balanced anion-cation relationships in nutrient solutions, during the vegetative growth stage, applying high concentrations of NO_3^- and Ca^{2+} can hinder plant growth, reduce dry matter accumulation, and lead to excessive fertilization costs.

Conclusions

Agastache mexicana subsp. *mexicana* production in containers under plastic cover, maintaining a balanced NO_3^- : Ca^{2+} ratio based on the universal Steiner nutrient solution, specifically a 10:9 mEq L^{-1} ratio, promotes vegetative growth and dry matter accumulation while optimizing fertilization costs.

Author Contributions

Work conceptualization: LRMG, VTOG, and ESSE; methodology development: LRMG, VTOG; software management: LRMG, CHGJA; experimental validation: ESSE, ARM, PAI, and MFE; analysis of results: LRMG, VTOG, and CHGJA; data management: LRMG, VTOG, and MFE; manuscript writing and preparation: LRMG, VTOG, and ARM; writing, review, and editing: CHGJA, ARM, and PAI; project manager: VTOG; funding acquisition: LRMG, VTOG.

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Conflicts of Interest

The authors declare no conflicts of interest.

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