Emirates Journal of Food and Agriculture. 2021. 33(3): 237-244
doi: 10.9755/ejfa.2021.v33.i3.2666
http://www.ejfa.me/

RESEARCH ARTICLE

Effect of nutrient solution concentration on phenology, stevioside content, total phenolic compounds and total flavonoids in leaves of Stevia *rebaudiana* Bertoni

Karla Rubí Jiménez Sánchez[1], Adán Mercado Luna[1]*, Ana Angélica Feregrino Pérez[1], Juan Fernando García Trejo[1], Jorge Luis Chávez-Servín[2], Reynaldo Hernández Maldonado[3], Karla Nicol Hernández Puente[3], Carlos Alberto Murillo Cardenas[4].

[1]Departamento de Biosistemas, Facultad de Ingeniería, Centro de Investigación y Desarrollo Tecnológico en Materia Agrícola, Pecuaria, Acuícola y Forestal (CIDAF), Universidad Autónoma de Querétaro, Campus Amazcala. Carretera a Chichimequillas S/N, Amazcala, El Marqués, Querétaro 76130, Mexico, [2]Facultad de Ciencias Naturales, Campus Juriquilla, Universidad Autónoma de Querétaro. Av. de las Ciencias S/N, Juriquilla, Querétaro 76320, Mexico, [3]Facultad de Ingeniería, Campus Amealco, Carretera Amealco –Temascalcingo km 1 col. Amealco, Querétaro 76850, Mexico, [4]Campus Jalpan, Universidad Autónoma de Querétaro. Blvd. Policarpio Olvera s/n Frac. El Coco, Jalpan, Querétaro, 76340, Mexico

ABSTRACT

Among the most interesting areas of food technology research today is the search for natural alternatives to common sweeteners. One of these is *Stevia rebaudiana* Bertoni, which contains antioxidants and stevioside whose concentration can be affected by factors such as plant nutrition during its production. The objective of the study was to determine the effect of mineral nutrition levels (N, P, K Ca, Mg, S, Fe, Mn, B, Zn, Cu) in Steiner nutrient solution (SNS) on phenology and to quantify chlorophyll concentration (CC), stevioside content (SC), total phenolic compounds (TPC) and total flavonoids (TF) in the leaves of *Stevia rebaudiana* Bertoni. Five concentrations of SNS were evaluated: 0%, 25%, 50%, 75% and 100%. The experimental design was randomized with three replications. The experimental unit consisted of six plants per treatment (6 x 3 = 18 plants). The highest plant height (18.58 cm) was obtained with the 25% and 100% SNS concentrations; while the highest fresh weight and dry weight (21.70 ± 0.70 and 4.17 ± 0.12 g/ plant) were obtained with a 50% solution. The highest CC (41.31 ± 0.93 SPAD Units) and SC (8.88 ± 0.54 mg of stevioside / g dry matter (DM) were observed with 0% SNS. The highest contents of TF (16.42 ± 1.12 µg RE / g DM) and TPC (8.57 ± 1.12 µg GAE / g DM) were obtained with a 50% SNS solution. Finally, the compounds of interest were analyzed with respect to the total biomass obtained. The 75% SNS solution resulted in the highest amount of stevioside (17.03 mg / plant) and the second highest value for TPC (27.19 µg GAE / plant) and TF (64.57 µg RE / plant) content.

Keywords: Steiner nutrient solution; *Stevia rebaudiana*; stevioside; total flavonoids; total phenolic compounds

INTRODUCTION

Lifestyle changes in the general population have been accompanied by changes in diet in recent decades, including the consumption of more refined sugars and foods with high energy density, which have contributed to the increase in the incidence and prevalence of obesity, type II diabetes mellitus and its comorbidities (Aranda et al., 2014). In the soft drinks industry, a variety of synthetic substances are used to sweeten drinks while contributing fewer calories. Natural sources of sweeteners with very low caloric intake and high sweetening power are a subject of growing interest. The stevioside found in *Stevia rebaudiana* Bertoni has high sweetening power and low energy contribution compared to most sweeteners (Zhang et al., 2017; Soufi et al., 2019) so it may be an appropriate substitute for the sucrose that is used in the food industry (Rao et al., 2014).

Plant mineral nutrition has been found to play a definitive role in the synthesis of these sweetening molecules (Jarma et al., 2012). Most of the sulfur in plants is found in proteins, specifically in the amino acids cysteine and methionine. Other essential sulfur-containing compounds include the vitamins thiamine and biotin, as well as

***Corresponding author:**
Adán Mercado Luna, Departamento de Biosistemas, Facultad de Ingeniería, Centro de Investigación y Desarrollo Tecnológico en Materia Agrícola, Pecuaria, Acuícola y Forestal (CIDAF), Universidad Autónoma de Querétaro, Campus Amazcala. Carretera a Chichimequillas S/N, Amazcala, El Marqués, Querétaro 76130, Mexico. **E-mail:** adan.mercado@uaq.mx

Received: 14 December 2020; **Accepted:** 28 February 2021

coenzyme A, an essential compound for respiration, synthesis and degradation of fatty acids in the synthesis of diterpene glycosides. Sulfur deficiency has been reported to cause a decrease in stevioside content, in addition to other symptoms such as chlorosis and a reduction in the number of new leaves (Utumi et al., 1999).

Research work on fertilization in S. *rebaudiana* and its possible effects on the accumulation of steviosides is still limited. Ramirez (2011) reported that the following formula can be used to fertilize S. *rebaudiana*: 180 kg of nitrogen (N) / ha, 60 kg of potassium (K) / ha and 92 kg of phosphorus (P) / ha. His study indicates that inadequate fertilization can lead to low dry leaf production yields. Also, nitrogen deficiencies in particular can cause a decrease in growth and development, mainly in leaves, which are the main focus of commercial interest. Phosphorus is an essential part of many glycophosphates such as uridine diphosphate glucose (UDP-glc), a glucose donor molecule in the synthesis of diterpene glycosides, among other metabolic processes. Potassium deficiencies can cause a reduction in the number of branches per plant (Jarma et al., 2010). Tavarini et al. (2015) demonstrated the effect of different doses of nitrogen (0, 50, 150 and 300 kg N / ha) on the production of glycosides, flavoniodes and antioxidants in S. *rebaudiana* plants. These authors reported that maximum stevioside content (6.47 mg / g) was obtained in plants without any nitrogen treatment, but the highest content of rebaudioside A was obtained in 7 plants fertilized with 150 and 300 kg N / ha (3.88 and 3.75 mg / g, respectively). Likewise, the flavonoid content was improved with doses of 150 kg N / ha, with values of 104.03 mg / g. These results suggest that nitrogen manipulation may be a way to increase secondary metabolites in S. *rebaudiana*.

Two different varieties of stevia (Morita and Eriete) were evaluated for the amount of glycosides (stevioside, rebau-dioside A, and rebaudioside C) they contained under organic and conventional fertilization. The results showed significant differences for total phenols in organic peppermint (62% higher content). Also, the DPPH test revealed differences for peppermint and stevia (572% and 16% greater in organic). Stevia and peppermint grown under organic conditions are shown to display higher levels of polyphenols and antioxidant activity; but additional research is necessary to confirm possible differences between conventional and organic stevia and peppermint in order to provide better guidance to farmers and consumers (Garcia-Mier et al., 2021).

More information is necessary on the management of this plant in order to maximize yield and production of compounds of interest to the food industry.

The objective of the study was to determine the effect of mineral nutrition levels (N, P, K Ca, Mg, S, Fe, Mn, B, Zn, Cu) supplied through application of SNS, on phenology and to quantify chlorophyll concentration (CC), stevioside content (SC), total phenolic compounds (TPC) and total flavonoids (TF) in leaves of *S. rebaudiana* Bertoni. This study will contribute information that can help in developing more efficient fertilization programs and improving the performance of sweeteners.

MATERIALS AND METHODS

Location and type of greenhouse

The study was developed at the Engineering Faculty of the Autonomous University of Querétaro, on the Amazcala campus, municipality of Marques, Mexico, located 20° 31'and 20° 58' N, 100° 09'and 100° 24' W, and altitude of 1921 m. The region has a semi-dry climate. A 528 m^2 (9.6x55 m) Gothic-style greenhouse was used, with an 800-gauge-thick polyethylene plastic roof covering, and a transparent polycarbonate plate covering on the walls. The greenhouse has overhead ventilation, thermal screening, temperature and relative humidity control and a drip irrigation system (Fig. 1).

Plant material, substrate and containers

The vegetable matter was obtained from cuttings of *S. rebaudiana*, Morita II variety, from an agro-industry

Fig 1. Greenhouse and block distribution

company (STEVIA MAYA) located in the state of Yucatán, Mexico. The substrate used was commercial organic coconut fiber (coco peat) from Colima, Mexico, with a pH of 5.5- 6.5; electrical conductivity <0.8 mS / cm; aeration percentage 10-40%; water retention capacity 25-50%; CEC (cation exchange capacity) 70-100 mEq / 100g; C/N carbon-nitrogen ratio 80: 1; and cellulose content 20-30%. The containers used were flexible black plastic pots 11 cm base x 15 cm high x 15 cm round at the top, with a fill volume of 1,240 mL.

Crop management

The containers were filled with coconut fiber previously disinfected with quaternary ammonium salts in the form of soluble liquid (Anibac® plus, Mexico), with a percentage composition by weight: 45.02% quaternary ammonium salts (equivalent to 521.47 g of active ingredient / L at 20°C), copper sulfate pentahydrate 19.49%, metallic copper 4.96% (equivalent to 225.75 g of active ingredient / L at 20°C); diluent and adjuvant 35.49% (inert ingredients). The substrate was deposited in the black pots, then washed with acidified water (pH 5.5) using phosphoric acid (H_3PO_4) at a 75% concentration and a density of 1.573 g / cm^3 to remove the salts that the coconut fiber could contain.

Transplant to pots

The establishment of the plants was carried out when they had an average height of 15 cm and during the afternoon to avoid stress. The seedling was carefully removed from the germination tray and holes were made in the substrate of the pots. The plants were accommodated in these holes and watered with running water during their adaptation stage, which was two weeks (Fig. 2).

Irrigation of plants with Steiner nutrient solution (SNS)

The plants were watered manually applying 500 mL of nutrient solution per plant per day. The nutrient solution was applied for 90 days after transplantation in each of the treatments, and the volume was measured with a 1000 mL graduated cylinder. Irrigation with running water was carried out twice a week in the morning, to avoid damage from excess temperature in later hours. The application of the SNS was carried out in such a way that the solution did not touch the leaves of the plants (Fig. 3).

The preparation of the nutrient solution was based on that reported by Steiner (1984). This solution has been gradually evaluated in concentrations of 45, 90 and 100% in ornamental species (Evangelista-Lozano et al., 2015). Likewise, it has been evaluated in physallis peruviana in concentrations ranging from 25 to 100%, obtaining significant results on the height of the plants and the accumulation of biomass (Gastelum et al., 2013). There is insufficient information on the evaluation of these concentrations and their effect on S. *rebaudiana* Bertoni. Taking into consideration the contribution of nutrients from the water used in this study (Table 1). Preparing the 100% SNS, the corresponding dilutions were made for the other treatment concentrations (0%, 25%, 50%, 75%, and 100%

Table 1: Steiner Nutrient Solution Adjustment

Element	Steiner Nutrient Solution (mg L^{-1})	Water contribution (mg L^{-1})	Adjusted Steiner Nutrition Solution (mg L^{-1})
Nitrogen (N)	168	1.91	66.1
Phosphorus (P)	31	0	31
Potassium (K)	273	2.73	270.27
Calcium (Ca)	180	11.8	168.2
Magnesium (Mg)	48	0.24	47.62
Sulfur (S)	110	3	107
Iron (Fe)	3	0	3
Manganese (Mn)	1.97	0	1.97
Boron (B)	0.44	0	0.44
Zinc (Zn)	0.11	0	0.11
Copper (Cu)	0.02	0	0.02

Fig 2. Seedlings of S. rebaudiana

Fig 3. Application of SNS treatments

Table 2: Source of fertilizer and quantity to prepare the treatments

Fertilizer (g m^{-3})	Treatment 1 (0% SNS)	Treatment 2 (25% SNS)	Treatment 3 (50% SNS)	Treatment 4 (75% SNS)	Treatment 5 (100% SNS)
$Ca(NO_3)_2$	0	212.01	424.03	636.04	884.06
$MgSO_4^{2-} + 7H_2O$	0	107.54	215.08	322.62	430.16
KNO_3^-	0	70.29	140.58	210.62	281.16
$KH_2PO_4^-$	0	31.47	62.95	94.43	125.91
$K_2SO_4^{2-}$	0	138.71	277.43	416.15	554.87
Fe-EDTA 13.2%	0	5.68	11.36	17.04	22.72
Mn-EDTA 13%	0	3.78	7.57	11.36	15.15
Zn-EDTA 14%	0	0.19	0.39	0.58	0.78
Cu-EDTA 14%	0	0.03	0.07	0.10	0.14
H_3BO_3 17.5%	0	0.62	1.25	1.88	2.51
Electrical conductivity (dS m^{-1})	0	1.04	1.58	1.88	2.50
pH	0	5.98	5.83	6.06	5.94

SNS: Steiner nutrient solution; EDTA: Ethylenediaminetetraacetic acid

Table 3: Electrical conductivity and osmotic pressure of the Steiner nutrient solution

Treatment	Steiner Nutrient Solution (%)	Electrical conductivity (dS m^{-1})	Osmotic pressure (bar)
1	0	0.20	0.0720
2	25	1.04	0.3744
3	50	1.58	0.5688
4	75	1.88	0.6768
5	100	2.5	0.9000

SNS). A granatary scale (OHAUS brand) with a precision of 0.1 g was used to weigh each fertilizer (Table 2). The osmotic pressure (OP) of the nutrient solution was obtained based on the electrical conductivity (EC) with the following relationship: OP (bars) = 0.36 EC (EC in dS m^{-1}) (Alcántar et al., 2010) (Table 3).

Experimental design

The experimental design was completely randomized with three blocks and three replications. The experimental unit consisted of six plants per treatment (6 x 3 = 18 plants for each treatment). The treatments were the concentration levels of the SNS (0%, 25%, 50%, 75% and 100%).

Determination of phenological variables

Plant height (AP): This variable was measured in cm using a Truper FH-3m scaler measure (Truper, Taiwan), with a precision of 0.01 mm. The measurement was taken from the base of the stem to the highest leaf. All measurements were taken at 3:00 p.m (Fig. 4).

Fresh weight (FW): Three *S. rebaudiana* plants were taken at random for each repetition, resulting in a total of nine samples per treatment. All the leaves of the stems were cut and then weighed using a Precisa model XB 220A analytical balance, with a maximum capacity of 220 g and a precision of 0.001 g.

Fig 4. Plant height measurement

Dry weight (DW): Once the leaves were separated from the stems, they were placed in labeled paper bags and placed in a stove with forced air circulation, RIOSSA model HSF-41 (Metler Toledo, Mexico), for 24 h at 60°C until the samples reached a constant weight. Weight was determined using a Precisa model XB 220A analytical balance, with a maximum capacity of 220 g and 0.001 g of precision. This process was carried out on each sample. For laboratory analysis the samples were ground in a mill (Thomas Wiley Model 4 Scientific, USA) with a 0.5 mm-diameter sieve. The powdered samples were then stored in an ultra-freezer (REVCO last II, USA) to -80°C until analysis.

Chlorophyll content (CC): This was measured six times during the culture cycle at 12:00 noon. The determinations were made directly on the leaves, according to Krugh et al. (1994), using a SPAD 502 chlorophyll meter (Konica Minolta, USA), in dimensional values from 0 to 199 SPAD units.

Table 4: Plant height, leaf fresh weight, leaf dry weight and chlorophyll concentration

Treatment (%)	Plant height (cm)	Leaf fresh weight (g/plant)	Leaf dry weight (g/plant)	Chlorophyll concentration (SPAD units)
0	14.25 ± 1.14[a]	15.56 ± 0.93[a]	1.84 ± 0.08[a]	41.31 ± 0.50[a]
25	18.58 ± 0.63[b]	16.08 ± 0.54[a]	3.21 ± 0.15[b]	39.15 ± 0.75[a]
50	17.89 ± 0.43[b]	21.70 ± 0.70[b]	4.17 ± 0.12[c]	39.26 ± 0.60[a]
75	18.45 ± 0.78[b]	21.41 ± 0.65[b]	4.09 ± 0.06[c]	38.53 ± 0.60[a]
100	18.58 ± 0.41[b]	21.06 ± 0.96[b]	3.88 ± 0.21[c]	40.71 ± 0.52[a]

Results are shown as the mean ± standard error of the mean (Mean ± SEM). Different letters in the same column indicate significant difference according to ANOVA followed by a Tukey *post hoc* test (p <0.05)

Laboratory Determinations

Stevioside content (SC): This was quantified using the technique explained by Vázquez-Baxcajay et al. (2014), with modifications for its coupling to a microplate. Briefly, 100 mg of dry matter (DM) from each of the samples were crushed in a mortar with 10 mL of 96% ethanol. The samples in alcoholic solution were placed in beakers and kept at room temperature and stirred for 4 h. The samples were centrifuged for 5 min at 1400 rpm at 4°C. The supernatant was concentrated to 1 mL using nitrogen injection. The concentrate was filtered through a 0.45 µm millipore membrane. 250 µL were used for the analyses, in 96-well plates. Stevioside content was quantified by performing a calibration curve prepared with a standard solution (maximum concentration: 2 mg / mL) of stevioside (Sigma-Aldrich, Toluca, Mexico). The absorbance readings were made in a spectrophotometer (Multiskan Ascent version 1.00.40) with a scan from 610 to 660 nm, at intervals of 10 nm, and the maximum absorption of stevioside was observed at 630 nm. The results were expressed in (mg of stevioside / g of dry matter [DM]).

Total Flavonoid (TF) content: A methanolic extraction was performed according to a previously reported method (Cardador-Martínez et al., 2002). The determination of total flavonoids was carried out with a method adapted to a microplate (Oomah et al., 2005), which consists of a colorimetric reaction that is quantifiable at an absorbance of 404 nm. The results were expressed in µg rutin equivalents (RE) / g DM.

Total Phenolic Compounds (TPC): These compounds were quantified using the method described by Dewanto et al. (2002) adapted to a microplate. The colorimetric reaction is generated by the Folin Ciocalteu reagent, which reacts with phenolic compounds from the sample in an alkaline medium. After a period in darkness, this reaction is quantifiable at an absorbance of 750 nm. The results were expressed in µg gallic acid equivalents (GAE) / g DM.

Statistical analysis

The experimental data for scale variables were submitted to analysis of variance (ANOVA) followed by a *post hoc* Tukey test for significant differences between treatment concentrations (0%, 25%, 50% and 100% SNS). A confidence interval of 95% and a level of significance (p <0.05) was used. The analyses were performed using Origin Pro version 8. The results are shown as the means of the variables ± standard error of the mean.

Table 5: Stevioside content (SC), total phenolic compounds (TPC) and total flavonoids (TF)

Treatment (%)	Stevioside content (mg stevioside/g DM)	Total phenolic compounds (µg GAE/g DM)	Total flavonoids (µg RE/g DM)
0	8.88 ± 0.54[a]	1.80 ± 0.45[a]	10.06 ± 1.61[a]
25	2.96 ± 0.46[b]	7.49 ± 1.18[b]	16.36 ± 0.20[b]
50	2.79 ± 0.47[b]	8.57 ± 1.12[b]	16.42 ± 1.12[b]
75	4.16 ± 0.65[b]	6.64 ± 0.36[bc]	15.77 ± 0.54[b]
100	3.45 ± 0.39[b]	5.20 ± 0.80[c]	15.68 ± 0.33[b]

µg GAE / g DM: µg Gallic acid equivalents / g dry matter, µg RE / g DM: µg Rutin equivalents / g dry matter. Results are shown as the mean ± standard error of the mean (Mean ± SEM). Different letters in the same column indicate significant difference according to ANOVA followed by a Tukey *post hoc* test (p <0.05)

Table 6: Total stevioside content (SC), total phenolic compounds (TPC) and total flavonoids (TF) per plant

Treatment (%)	Stevioside content (mg stevioside/ plant)	Total phenolic compounds (µg GAE/plant)	Total flavonoids (µg RE/plant)
0	16.34 ± 0.99[a]	3.31 ± 0.14[a]	18.50 ± 0.83[a]
25	9.49 ± 0.45[c]	24.01 ± 1.15[c]	52.45 ± 2.51[b]
50	11.65 ± 0.33[bc]	35.77 ± 1.02[d]	68.54 ± 1.97[c]
75	17.03 ± 0.27[a]	27.19 ± 0.44[c]	64.57 ± 1.04[c]
100	13.39 ± 0.72[b]	20.18 ± 1.09[b]	60.84 ± 3.29[bc]

µg GAE: µg Gallic acid equivalents, µg RE: µg Rutin equivalents. Results are shown as the mean ± standard error of the mean (Mean ± SEM). Different letters in the same column indicate significant difference according to ANOVA followed by a Tukey *post hoc* test (p <0.05)

RESULTS

Phenological variables

Plant height (AP): For this variable, the statistical analysis showed no significant differences (p> 0.05) between results for the concentrations of 25%, 50%, 75% and 100% of SNS, except for the 0% treatment, which obtained the lowest value. The concentrations that obtained the highest values were 25% and 100% of SNS (Table 4). Fresh weight (FW) and dry weight (DW): Fresh weight was 139% greater

with application of the 50% SNS than with the 0% solution; and dry leaf weight was 226% higher using SNS at 50% than at 0%. Subsequent increases in SNS concentration did not result in further increases in either FW or DW, however ($p > 0.05$). Chlorophyll content (CC): For this variable, there were no significant differences ($p > 0.05$) for any of the levels (0%, 25%, 50%, 75% or 100%) (Table 4).

Laboratory analysis

Stevioside content (SC): In the measurement of stevioside content in stevia leaves, there were no significant differences ($p > 0.05$) between the different concentrations of SNS (25%, 50%, 75% and 100%). Contrary to expectations, the control group (0% SNS) obtained the highest concentration of stevioside (8.88 mg stevioside / g DM), between double and triple the content of stevioside than plants treated with SNS (Table 5). However, when we adjust the values to the total stevioside content produced per plant (mg stevioside / g DM x total dry matter from the leaves in each of the plants), it can be seen that the highest stevioside content (17 mg stevioside/plant) is observed with the 75% SNS concentration (Table 6).

Total phenolic compounds (TPC): The content of total phenolic compounds was significantly higher ($p < 0.05$) for all SNS treatments than for the control group. There were no significant differences between the 25%, 50%, and 75% SNS treatments. The mean value (50%) generated the highest values (8.6 μg GAE / g DM). The 0% treatment obtained the lowest value ($p < 0.05$) of total phenolic compounds (1.80 μg GAE / g DM) (Table 5). Likewise, the highest CFT content per plant was observed in the 50% SNS treatment group, with values of 35.77 μg GAE / plant (Table 6).

Total flavonoids (TF): Flavonoid content was higher ($p < 0.05$) for all SNS treatment groups than for the control group (10.06 μg RE / g DM). There were no significant differences between the 25%, 50%, 75% and 100% SNS treatments. The highest concentration of flavonoids was observed with SNS at 50% (16.42 μg RE / g DM) (Table 5). Similarly, when calculating yield, the highest concentration of total flavonoids per plant (69 μg RE / plant) was observed with the 50% SNS treatment (Table 6).

DISCUSSION

Regarding the phenological variables, from the point of view of fertilizer optimization, for a better plant height the 25% SNS solution is best, but this results in a lower amount of fresh and dry leaf weight, which are important because they contain more antioxidants. For this reason, a 50% SNS concentration for this variable is suggested--sufficient to produce both fresh and dry matter. Romero-Figueroa et al. (2017) evaluated the growth dynamics of two Stevia species grown on organic substrates in greenhouses. They reported that as the crop cycle progressed, the values of specific leaf area, leaf area ratio, and leaf weight ratio for the two species decreased. This was due to the fact that in the early stages of growth, most of the photoassimilates are used to establish the foliar apparatus, an amount that gradually decreases as the plant accumulates more carbohydrates in other organs, showing that the availability of nutrients and ontogenetic development influence the allocation of biomass to the different organs of the plant.

Bonilla et al. (2007) evaluated the rooting process and growth of Stevia *rebaudiana* cuttings from basal and apical position of the stem grown in three rooting substrates (charcoal-sand, charcoal-compost and sand-compost) and with three sources of nitrogen (urea 46% N, compost 1.5% N and chicken manure 1.5% N). They reported that no significant differences were observed between the three nitrogen sources in the accumulation of leaf dry matter in two harvest periods. These results were similar to our own, as we observed that there was no increase in fresh weight or dry weight with increases in the SNS concentration to more than 50% (Table 4).

Clementelli et al. (2009) found that a concentration of 9 meq/ L of NO3- obtained the best results in Stevia *rebaudiana* Bertoni for the variables of plant height, number of stems, fresh weight and dry weight. In this research, the 50% SNS concentration is equivalent to 6 meq/ L of NO3-, which yielded the best results for fresh weight and dry weight. The 100% SNS solution, equivalent to 12 meq / L, resulted in the greatest height, but the difference was not significant ($p > 0.05$) compared to the 25%, 50%, and 75% SNS treatments. This shows that an acceptable plant height can be obtained from any of the nutrition levels.

Evangelista-Lozano et al. (2015) observed that ornamental plants (lily plant) treated with Steiner solution at concentrations of 75% and 100% grew higher, to 37.50 and 39.63 cm respectively, compared to the control group (28.87 cm). The plants treated with 75% and 100% SNS, also presented a larger leaf area of 50.34 and 47.82 cm^2 respectively, compared with the control group (39.85 cm^2). The results obtained in this investigation show a trend very similar to that reported by these authors, although leaf area was not measured. This behavior was observed in the plants treated with a concentration of 50% for the variables of fresh and dry weight of the leaf (Table 4). The results obtained in this research and compared with other studies in which different concentrations of SNS were used, show that the assimilation of nutrients depends on the type of plant.

On the other hand, regarding stevioside content, Jarma et al. (2012) reported that nutritional deficiency does not influence the amount of glycosides, but that the rebaudioside concentration is significantly reduced in the presence of P, S, K and Cu deficiencies. In our investigation, the content of stevioside was determined and it was observed that with the different levels (25, 50, 75 and 100%) of SNS, this glycoside decreased considerably compared to the control group, which lacked nutrient solution. Plants treated with no nutrient solution had the highest stevioside content, more than double that of all plants treated with different concentrations of nutrient solution (Table 5).

Utumi et al. (1999) induced Ca2+ deficiencies in their research, and reported symptoms of apical necrosis in leaf primordia, which ended in descending death and decrease in stevioside content. The conclusion was that because biomass was lower (new branches were brittle and roots were shorter and thinner), there is less stevioside content in the plant. For this reason, a yield analysis was carried out, calculating the content of compounds of interest (stevioside, total phenolic compounds and total flavonoids), in addition to per gram of dry matter, per plant. In this way it could be observed that although treatment with 0% SNS resulted in a higher content of stevioside in the leaves, it also resulted in the lowest biomass. The 75% SNS treatment resulted in the highest amount of stevioside per plant, while CFT and FT content were similar to those obtained from the SNS 50% treatment, which had the highest values in these variables. Soufi et al. (2019) evaluated the effect of different concentrations of saline water on growth, protein content, photochemical efficiency and nutrient content in Stevia *rebaudiana* Bert. The plants were watered with fresh water and with saline concentrations of 1.22, 3.40, 4.64 and 7.72 dSm-1. The results showed that the root / sprout relationship, the number of leaves and the foliar area decreased with saline water at 7.72 dSm-1, showing a decrease in protein content, in photochemical efficiency and an excessive accumulation of Na in the stem, reducing the presence of potassium in the cell due to the effect of saline stress.

Pal et al. (2015) commented that plant nutrition, strongly determined by soil characteristics and climatic conditions, plays an extremely important role in the growth and production of secondary metabolites in Stevia *rebaudiana* plants. These authors reported that the different applications of N, P2O5 y K2O influenced morphology, accumulation of secondary metabolites, and stevioside content in leaves. Likewise, they reported that the application of different SNS concentrations influenced the morphology and the content of secondary metabolites, but not of stevioside, in the leaves.

This suggests that stevioside production can be increased with the minimum use of mineral fertilization. Since these nutrients are not available in sufficient quantities at the rhizosphere level, some nutritional stress is probably generated by the competition of these minerals, inducing a increase in the formation of stevioside. However, care must be taken not to sacrifice biomass yield. Finally, Arias-Zabala et al. (2009), describe that as the level of nutrients available to plants increases, these are probably diverted to the formation of some secondary metabolites, a trend that was observed in this research in the case of total phenolic compounds and total flavonoid content. These results coincide with that reported by Tavarini et al. (2015) who used different concentrations of nitrogen (0, 50, 150 and 300 kg N / ha) in S. *rebaudiana* plants and obtained a higher amount of steviosides in plants that were not treated with nitrogen supplementation.

CONCLUSIONS

Plant phenology was influenced by the concentration of Steiner nutrient solution used to treat them. Stevioside content in *Stevia rebaudiana* leaves did not increase with changes in SNS concentration; in fact, it decreased. Stevioside levels were the highest when mineral nutrition was not applied at all, but the least amount of biomass yield was observed. An SNS concentration of 50% favored the production of secondary metabolites (total phenolic compounds and total flavonoids). The results of this study suggest using a 75% solution of SNS, because this results in increased fresh weight and the dry weight in the plant leaves, and biomass is also higher, which results in the highest stevioside content per plant, with a high content of bioactive compounds in terms of yield. *S. Rebaudiana* Bertoni leaves could be used not only as a source of non-energy sweetener, but also as a source of bioactive compounds of natural origin. More studies are required to evaluate the results of using SNS concentrations between 50% and 75%. It is also necessary to evaluate the specific content of secondary metabolites.

ACKNOWLEDGEMENTS

This study was supported by the Fund for Strengthening Research at the Autonomous University of Queretaro [grant number FOFIUAQ: FIN201607].

Authors´ contributions

KRJS: Conceptualization, data curation and methodology. AML: Conceptualization, data curation, methodology, writing - original draft and writing - review & editing. AAFP: Methodology and writing - review & editing. JFGT:

Data curation and writing - review & editing. JLCS: Data curation, writing - original draft and writing - review & editing. RMH: Writing - review & editing. KNHP: Writing - review & editing. CAMC: Writing - review & editing.

REFERENCES

Alcántar-González, G. and L. I. Trejo-Téllez. 2010. Nutrición de Cultivos. Colegio de Posgraduados and Mundi-Prensa. Mexico, DF, pp. 454.

Aranda-González, I., M. Segura-Campos, Y. Moguel-Ordoñez, D. Betancur-Ancona. 2014. *Stevia rebaudiana* Bertoni. Un potencial adyuvante en el tratamiento de la diabetes mellitus. J. Food. 12: 218-226.

Arias-Zabala, M., M. J. Angarita-Velásquez, A. M. Aguille-Cardona, M. Restrepo and C. Montoya-Vallejos. 2009. Strategies for the improvement of secondary metabolites production in plan cell cultures. Rev. Fac. Nal. Agr. Medellín. 62: 4881-4895.

Bonilla-Correa, C. R., M. S. Sánchez and D. F. Perlaza. 2007. Evaluation of propagation methods, nitrogen fertilization and phenology in stevia in the Cauca Valley, Colombia. J. Acta Agron. 56: 131-134.

Cardador-Martínez, A., G. Loarca-Piña and B. D. Oomah. 2002. Antioxidant activity of common beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 50: 6975-6980.

Clementelli, A. and R. Zevallos. 2009. Fertilización nitrogenada mineral y orgánica en el cultivo de stevia (*Stevia rebaudiana* Bertoni). J. Univ. Cienc. Soc. 1: 47-50.

Dewanto, V., X. Wu, K. K. Adom and R. H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50: 3010-3014.

Evangelista-Lozano, S., M. Estrada-García, S. L. Escobar-Arellano, M. L. Arenas-Ocampo, B. H. Camacho-Díaz and A. R. Jiménez-Aparicio. 2015. *Eucharis grandiflora* Planch. and Linden development fertirrigated with different osmotic potentials. J. Plant Nutr. 38: 551-567.

Gastelum-Osorio, D. A., M. Sandoval-Villa, C. Trejo-López and R. Trejo-López. 2013. Fuerza iónica de la solución nutritiva y densidad de plantación sobre la producción y calidad de frutos de *Physalis peruviana* L. Revista Chapingo. Serie Hortic. 19: 197-210.

Jarma, A., M. Espitia and G. Fischer. 2010. Síntesis de esteviósidos enestevia (*Stevia rebaudiana* Bert.). Acta Biol. Colomb. 15: 263-268.

Jarma-Orozco, A., E. M. Combatt and J. Polo. 2012. Glycoside contents depending on the nutrient deficiencies in *Stevia rebaudiana* Bert. Rev. U.D.C.A Actual. Divulg. Cient. 15: 107-116.

Krugh, B., L. Bickam and D. Miles. 1994. The solid-state chlorophyll meter, a novel instrument for rapidly and accurately determining the chlorophyll concentrations in seedling leaves. J. Maize Genet. Coop. Newsl. 68: 25-27.

Garcia-Mier, L. A. E., S. N. Meneses-Reyes, A. M. Jimenez-Garcia, J. F. G. Luna, L. M. Trejo, A. A. Contreras-Medina and Feregrino-Perez. 2021. Polyphenol content and antioxidant activity of stevia y menta como resultado de la fertilización orgánica y convencional. J. Food Qual. 2021: 6620446.

Rao, G. N., P. R. Pamidighantam, K. Balaswamy and S. Akula. 2014. Antioxidant activity of stevia (*Stevia rebeudiana* L.) leaf powder and a commercial stevioside powder. J. Food Pharm. Sci. 2: 32-38.

Oomah, B. D., A. Cardador-Martínez and G. Loarca-Piña. 2005. Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L). J. Sci. Food Agric. 85: 935-942.

Pal, P. K., R. Kumar, V. Guleria, M. Mahajan, R. Prasad, V. Pathania, B. S. Gill, D. Singh, G. Chand, B. Singh, R. D. Singh and P. S. Ahuja. 2015. Crop-ecology and nutritional variability influence growth and secondary metabolites of *Stevia rebaudiana* Bertoni. J. BMC Plant Biol. 15: 1-16.

Ramirez, G. 2011. Paquete tecnológico estevia (*Stevia rebaudiana*) establecimiento y mantenimiento. In: Programa Estratégico Para el Desarrollo Rural Sutentable de la Región Sur-Sureste de México: Tropico Húmedo, pp. 1-15.

Romero-Figueroa, J. C., M. C. Gutiérrez-Castorena, C. B. Peña-Valdivia, M. N. Rodríguez-Mendoza, J. A. Escalante-Estrada, J. A. Cueto-Wong and E. Burguete-Hernández. 2017. Growth dynamics of *Stevia cultivated* in organic substrates in greenhouse. J. Fitotec. Mex. 40: 341-350.

Soufi, S., S. Aabaoui, S. Jelleli, R. Salah and T. Bettaieb. 2019. High Photochemical Efficiency and Nutrient Homeostasis in Stevia (*Stevia rebaudiana*) plant leaf increases tolerance to saline irrigation water. J. Agric. Biol. 22: 1059-1064.

Steiner, A. 1984. The Universal Nutrient Solution, Proceedings of IWOSC 1984 6th International Congress on Soilless Culture, Wageningen, The Netherlands, pp. 633-650.

Tavarini, S., C. Sgherri, A. M. Ranieri and L. G. Angelini. 2015. Effect of nitrogen fertilization and harvest time on steviol glycosides, flavonoid composition, and antioxidant properties in *Stevia rebaudiana* Bertoni. J. Agric. Food Chem. 63: 7041-7050.

Utumi, M., P. Monnerat, P. Gomes-Pereira, P. Rezende-Fontes and V. Campos-Godinho. 1999. Deficiência de macronutrientes em estévia: Sintomas visuais e efeitos no crescimento, composição química e produção de esteviosídeo. J. Pesq. Agropec. Bras. 34: 1039-1043.

Vázquez-Baxcajay, L., A. Robledo-Paz, A. Muratalla-Lúa and V. Conde-Martínez. 2014. Micropropagación de *Stevia rebaudiana* Bertoni y detección de steviósidos. J. Bioagro. 26: 49-56.

Zhang, Q., H. Yang, L. Yongning, H. Liu and X. Jia. 2017. Toxicological evaluation of ethanolic extract from *Stevia rebaudiana* Bertoni leaves: Genotoxicity and subchronic oral toxicity. J. Sci. Direct. 86: 253-259.