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Nutritional requirements of macronutrients in stevia crop (*Stevia rebaudiana* Bertoni)

Requerimientos nutricionales de macronutrientes en el cultivo de estevia (*Stevia rebaudiana* Bertoni)

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ABSTRACT

The stevia crop is native to America and has gained much popularity among consumers in the last decades; however, optimal nutrition and fertilization are required. Therefore, the objective of the present experiment aimed to determine the effect of fertilization on nutrient extraction and yield. Stevia plants were selected and propagated for field experiments. The study was installed in a randomized complete block design (RCBD) with four treatments: T0 (Control), T1 (10N-10P2O5-35K2O), T2 (80N-15P2O5-70K2O), T3 (160N-20P2O5-140K2O); With three repetitions each. The evaluation parameters were height, stem diameter, yield, nutrient concentration, and extraction (N, P, and K). ANOVA was performed, and the comparison of means by the Scott-Knott test ($p \leq 0.05$). The results indicated that the treatment with higher fertilizers was the best, indicating that larger doses are needed to reach an optimal value. The most required nutrient for stevia cultivation is nitrogen, followed by potassium and phosphorus, respectively. The crop extraction per ton was approximately 30 kg N, 4.4 kg of P₂O₅, and 18 kg of K₂O for treatment with higher yield.

Keywords: concentration; fertilization; nutrient extraction; stevia; yield

RESUMEN

El cultivo de estevia es originario de América y popular entre los consumidores en las últimas décadas, sin embargo, se requiere una nutrición y fertilización óptimas, para ello, el experimento buscó determinar el efecto de la fertilización en la extracción y el rendimiento de nutrientes. Las plantas de estevia fueron seleccionadas y propagadas para el experimento de campo. El estudio se instaló en un diseño de bloques completamente aleatorios (DBCA); con cuatro tratamientos: T0 (control), T1 (10N-10P2O5-35K2O), T2 (80N-15P2O5-70K2O), T3 (160N-20P2O5-140K2O); con tres repeticiones cada una. Los parámetros de evaluación fueron altura, diámetro del tallo, rendimiento y concentración y extracción de nutrientes (N, P y K). Se realizó ANOVA y la comparación de medias mediante la prueba de Scott-Knott con una probabilidad estadística de 0,05. Los resultados indicaron que el tratamiento con dosis más altas de fertilizantes fue el mejor indicativo de que se necesitan dosis más grandes para alcanzar un valor óptimo. El nutriente más requerido para el cultivo de estevia es nitrógeno seguido de potasio y fósforo, respectivamente. La extracción del cultivo por tonelada fue de aproximadamente 30 kg de N, 4,4 kg de P₂O₅ y 18 kg de K₂O para el tratamiento con mayor rendimiento.

Palabras clave: concentración; estevia; extracción de nutrientes; fertilización; rendimiento

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1. INTRODUCTION

The Stevia crop (*Stevia rebaudiana*, Bertoni) is native to the southeast of Paraguay and has been known since the sixteenth century by Guaraní settlers. It has been introduced in many countries in Asia and South America, with China being the leading producer (75%) with a volume of 150 thousand tons (20,000 hectares planted), representing 75% of world production (Acosta, 2015). In Peru, exports reached US \$13,757 in 2017, with Japan, China, Malaysia, Israel, South Korea, and Brazil being the main commercial destinations (Koo, 2017). In recent years, interest in the consumption of stevia in its different presentations has grown by 400% compared to the last decade, decreasing the purchase of synthetic sweeteners by increasing the consumption trend of natural products (Brito Castrejón, 2018).

Stevia is a shrub and semi-perennial species that adapts to different tropical zones and climates. The importance of the crop lies in the leaves used in solids (dust) and liquid forms. They also have glycosides, such as stevioside, steviolbioside, and rebaudioside A, B, C, D, E, and F, which are responsible for the sweet taste, being 100 to 300 times greater than sucrose (EFSA, 2010). Due to its excellent benefits as a natural antioxidant, anti-fungal and antibacterial component, and low in calories, diseases such as diabetes, overweight, obesity, hypertension, and dental caries can be treated. It is also a nutritional supplement (Lemus-Mondaca et al., 2012). The percentage level of glycosides is very varied and depends on the genotype, agronomic practices, and climatic conditions (Salvador-Reyes et al., 2014).

One of the main limiting factors of production is the physicochemical attributes of the soil, such as salinity, pH, organic matter, and acidity, since they are directly involved in the absorption of essential nutrients for the growth and development of stevia (Alcaraz Ariza, 2012). Soil fertilization is an important agronomic practice in the management of stevia crops and involves the application of inputs of inorganic and organic origin. Using these inputs increases the availability of nutrients (mainly N, P, and K) for their absorption by plants, improving their nutrition and productivity (Rodríguez & Florez, 2004).

Few studies exist on the fertilization and nutrition of stevia under Peruvian conditions since there are no extensive areas of this crop in this country. However, areas have increased. In this matter, Zetina Lezama et al. (2014) mention that in the culture of Stevia, investigating the effects of nitrogen, phosphorus, and potassium sources, significant responses were obtained with high doses in the dry matter. Other studies conducted by Vera Peralta (2016) mention that a similar response obtained with the application of inorganic (nitrogenous) sources reported higher average dry leaf yields in stevia culture, indicating that fertilization promotes higher production in this crop.

In this way, stevia cultivation continues with the global consumption trends towards more natural products and conserving biodiversity, so research that improves its efficiency and crop management is a priority. To extend the areas and increase productivity, the present experiment aimed to determine the effect of fertilization on nutrient extraction and yield.

2. MATERIAL AND METHODS

Localization

The study was conducted in the Experimental Station "Juan Bernito" of the Instituto de Cultivos Tropicales - ICT, located in La Banda del Shilcayo District, Province and Department of San Martín - Peru, which is located at a South Latitude 06° 00 '28" and West Longitude 76° 00 '18", at an altitude of 315 m.a.s.l.

Plant propagation

For stevia propagation, cuttings of 15 cm in length from agricultural plots of selected plantations of stevia crops were collected; afterward, the basal part was applied with a rooting product (Stim-Root 3), to facilitate the development of adventitious roots. The cuttings were arranged in a substrate based on agricultural land and compost, and the nursery period was approximately two months until the seedlings were ready for field transplantation.

Field preparation and soil analysis

The plot was prepared using machinery with a tiller and plows to facilitate the soil's aeration, permeability, and homogeneous preparation. After crop rows were formed, the stratified blocks were left to facilitate sowing. Soil samples were taken randomly in a zigzag pattern at a depth of 20 cm, and 500 g of soil was collected. The sample was sent to the soil laboratory of the ICT for physicochemical analysis.

The results of the soil analysis were: Sand (67.52%), Lime (8%), Clay (24.48%), pH in water (4.58), EC (0.07 dS/m), Organic Matter (1.45 %), CEC (2.46), $Al^{+3}+H^{+}$ (0.61 cmolc⁺ kg⁻¹), N (0.07%), P (12.51 mg kg⁻¹), K (108 mg kg⁻¹), Ca (1.24 cmolc⁺ kg⁻¹), Mg (0.34 cmolc⁺ kg⁻¹), S (<10 mg kg⁻¹), Fe (55.40 mg kg⁻¹), Cu (0.60 mg kg⁻¹), Zn (0.06 mg kg⁻¹), Mn (2.70 mg kg⁻¹), B (<0.4 mg kg⁻¹).

Sowing and fertilizer application

The Stevia seedlings were manually installed in the experimental field at a distance of 0.20 m plants x 0.50 m rows. Thirty days after planting, chemical fertilizers (Urea, SPT, and KCl) were applied according to the treatments, three applications every two months. In the maintenance of the experimental area, agricultural work was carried out, such as weeding, plant thinning, hilling, fertilization, pests, and disease control.

Experimental design and variable parameters

The study was installed in a randomized complete block design (RCBD) with four treatments: T0 (Control), T1 (10N-10P₂O₅-35K₂O), T2 (80N-15P₂O₅-70K₂O), T3 (160N-20P₂O₅-140K₂O); with three replications each. The plot dimensions were 1.80 m long x 1.50 m wide, and one meter between streets. The experimental area was 48.6 m², with 336 plants in total.

The evaluation considered height, stem diameter, yield, and nutrient extraction (N, P, and K).

Statistical analysis

All statistical analyses were performed in the statistical package R, version 2017, where Variance Analysis (ANOVA) was performed, the data were adjusted to a normal distribution using the "Box-Cox method when necessary, and the comparison of means by the Scott-Knott test ($p \leq 0.05$).

3. RESULTS

Biometric and productive parameters

Table 1 shows the effects of different doses of inorganic fertilizers (N. P. K.) on height, stem diameters, and yield values. Overall, no significant differences were observed ($P > 0.05$), except for yield ($P < 0.05$).

The results showed that the height parameter values were variable as the doses of the fertilizers were increased. The lowest values were recorded with T1 (38.7 ± 1.06 cm), and the highest value was with the highest dose of T3 (47.5 ± 5.94 cm) (Table 1).

The values of the diameter parameter are statistically equal. However, there are minimal variations in the treatments. The lowest results were recorded with T1 (4.17 ± 1.18), and the maximum values were with the highest dose of T3 (4.43 ± 1.23) (Table 1).

For yield, values fluctuated between 802.8 ± 137.1 kg ha⁻¹ (T1) and 1536.4 ± 411.6 kg ha⁻¹ (T3). Treatment 3 presented the best results compared to the control. This treatment has the highest dose of inorganic fertilizers (urea, triple superphosphate, and potassium chloride) (Table 1).

Table 1.

Effect of treatments in height, stem diameter, and yield average of stevia

Treatments	Height (cm)	Steam diameter (mm)	Yield (kg ha ⁻¹)
T0	33.1 ± 5.03	4.09 ± 0.93	802.8 ± 137.1 c
T1	38.7 ± 1.06	4.17 ± 1.18	989.2 ± 236.2 b
T2	42.7 ± 5.92	4.37 ± 0.96	1101.7 ± 10.0 b
T3	47.5 ± 5.94	4.43 ± 1.23	1536.4 ± 411.6 a
P value*	>0.05	>0.05	<0.01

*Significant differences when P value <0.05

Concentration of macronutrients in Stevia

Table 2 presents the effects of applying the different doses of inorganic fertilizers (N, P, K) on shoot concentrations. In general, no significative differences were observed for the different treatments ($P > 0.05$)

Table 2.

The concentration of macronutrients in shoots of stevia crop, with the application of different doses of NPK

Treatments	Shoot Concentration		
	N (%)	P (%)	K (%)
T0	1.78 ± 0.11	0.14 ± 0.01	0.78 ± 0.01
T1	1.80 ± 0.15	0.14 ± 0.02	0.79 ± 0.01
T2	1.86 ± 1.01	0.16 ± 0.10	0.94 ± 0.57
T3	1.88 ± 0.16	0.17 ± 0.02	0.97 ± 0.46
P value*	0.88	0.99	0.12

*Significant differences when P value <0.05

The content of the macronutrients varied according to the treatments; the highest accumulated percentage corresponded to the T3 treatment with the maximum average of $1.88 \pm 0.16\%$ of nitrogen due to the high dose of nitrogen fertilizers, and a similar response occurred with phosphorus and potassium. The nitrogen absorbed was higher than the other elements, ranging from $1.78 \pm 0.11\%$ to $1.88 \pm 0.16\%$ respectively.

Regarding phosphorus, the highest cumulative percentage corresponds to the T3 treatment, with the maximum average of $0.17 \pm 0.02\%$, due to the high dose of phosphate fertilizers applied to the soil. The phosphorus concentration was lower concerning nitrogen and potassium, ranging from $0.14 \pm 0.01\%$ to $0.17 \pm 0.02\%$.

Potassium is also a significant macronutrient for plants; it is observed that the concentration in stevia leaves fluctuated in a range of $0.78 \pm 0.01\%$ - $0.97 \pm 0.46\%$, the maximum percentages reached were with T3, due to at a high dose of inorganic sources.

Nutrient extraction of stevia

Table 3 presents the extraction of nitrogen, phosphorus, and potassium by applying different doses of inorganic fertilizers (N, P, K). Significant differences were observed for all macronutrients studied ($P < 0.05$).

Table 3.

Nutrient extraction (N, P, K) in 1000 kg of stevia crop with different doses of inorganic fertilizers

Treatments	Nutrient Extraction (1000 kg)		
	N	P	K
T0	15.31±2.12d	1.2±0.19d	6.71±0.62d
T1	19.53±1.99c	1.52±0.16c	8.57±1.18c
T2	21.01±2.47b	1.81±1.09b	10.62±6.22b
T3	29.36±1.64a	1.92±0.12a	15.15±2.05a
P value	<0.05	<0.05	<0.05

The content of macronutrients differs according to the treatments studied; the highest cumulative percentage corresponds to T3 treatment due to a high dose of inorganic fertilizers. It was observed that the values of essential nutrients such as nitrogen ranged from $15.31 \pm 2.12 \text{ kg ha}^{-1}$ to $29.36 \pm 1.64 \text{ kg ha}^{-1}$, showing differences in the extraction rate concerning phosphorus and potassium. The proportion of nutrients required for stevia crops is $N > K > P$, which is common in tropical crops with very few exceptions.

Table 3 shows that the phosphorus extraction was much lower concerning nitrogen and potassium, fluctuating in a range of $1.2 \pm 0.19 \text{ kg ha}^{-1}$ to $1.92 \pm 0.12 \text{ kg ha}^{-1}$; Treatment 3 was statistically different with higher values compared to the control.

Potassium was the second most absorbed macronutrient, ranging from $6.71 \pm 0.62 \text{ kg ha}^{-1}$ to $15.15 \pm 2.05 \text{ kg ha}^{-1}$, with Treatment 3 having higher averages than the control.

4. DISCUSSION

Biometric and productive parameters

Chamba Caillagua & Merino Hidalgo (2018) experimented in the province of Loja - Ecuador, to improve yields in the cultivation of Stevia; where inorganic and organic fertilizers with different doses were applied, the yields achieved were lower than in the present study. Zetina Lezama et al. (2014), in Soledad de, folded - Mexico, applied nitrogen, potassium, and phosphate sources to the soil; the performance was not significant, being slightly lower than in this study. Villanueva Avellaneda (2009), experimented with Longar-Amazonas, using inorganic and organic sources in stevia culture, and the results showed a superior performance to that obtained in the present study.

Fertilization generally modifies the chemical properties of soils, increasing the availability of nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium, which are easily absorbed by plants (Ramírez et al., 2016). All essential elements are part of the physiological process of plants, mainly metabolizing protein synthesis, nutrient assimilation, translocation, photosynthesis, respiration, and other functions (Liu et al., 2011). The inorganic and organic sources also affect the physical and biological characteristics of the soils; that is to say, they alter the pH, the CEC, organic matter, and the population of the soil microfauna.

Concentration of macronutrients in Stevia

Katayama et al. (1976) conducted an experiment in Japan on stevia culture. The results showed that the nitrogen concentration was much lower than the study's. Lima Filho et al. (1997), experimented on the town of Piracicaba - Brazil, to evaluate the concentration of nitrogen in seven periods in stevia culture; the results, on average, were lower than those obtained concerning the present study.

Villalba Martínez & Oroa Pfefferkorn (2018) experimented in Paraguay, to determine the concentration of N.P.K in three varieties of stevia, the results were high concerning nitrogen compared to the study. The concentration of N varies depending on the physiological stages of the plants, soil management, organic matter, and other factors; specifically, the element interferes with plant tissue growth protein formation and intervenes in the cell wall and performance (Patil, 2010).

Villalba Martínez & Oroa Pfefferkorn (2018), experimented with Paraguay, to determine the concentration of NPK in three varieties of stevia; the results were higher compared to phosphorus compared to the present study. Katayama et al. (1976), conducted an experiment in Japan on stevia culture. The results showed that the Phosphorus concentration was higher than that obtained in the study.

Phosphorus is an indispensable element for plants; the requirement of anion is low compared to nitrogen and potassium. The specific functions of phosphorus are energy transfer root growth, they are involved in the synthesis of proteins, nucleic acids, and enzymes, and they also participate in the physiological processes of flowering and fruit formation (Harman, 2017). Taiz & Zeiger (2010), mention that the cation activates the enzymes of photosynthesis - respiration, intervenes in the osmotic potential of cells, formation of sugars in fruits, and acts as an inducer of disease resistance (Gómez-Merino & Trejo-Téllez, 2015).

Macronutrients such as Nitrogen, phosphorus, and potassium are indispensable in the development of stevia and mainly affect yield. Ion deficiency causes an alteration in the physiology of the plant, causing chlorosis in the leaves, inhibition of growth, decrease in biomass, and chemically affecting the synthesis of the glycosides responsible for the sweetness in the leaves (Lian et al., 2005).

Nutrient extraction of stevia

Pérez Chamorro & Calvache (2018), mention that the extraction of nutrients in the stevia culture over 120 days in the town of Santo Domingo - Ecuador, obtained lower results than the study.

Casaccia & Álvarez (2006), in Paraguay, mention that the nitrogen extraction in their study was greater concerning the results obtained in the present experiment. Jarma-Orozco et al. (2010), conducted an experiment in Colombia, to determine the extraction of nutrients in dried stevia leaves; the results were superior to those obtained in the present study. Nitrogen extraction was superior to phosphorus and potassium due to a maximum requirement by stevia plants; it is mainly absorbed to fulfill essential physiological functions and intervene in development and performance.

Jarma-Orozco et al. (2010), Casaccia & Álvarez (2006), Pérez Chamorro & Calvache (2018) mention that the extraction of nutrients in the stevia culture was superior to the present study. The low phosphorus extraction possibly corresponds to the fact that stevia plants absorbed small amounts of the ion due to their slow mobility in the soil; their adsorption is generally poor and easily lost due to leaching factors (Liao et al., 2001).

Jarma-Orozco et al. (2010), Pérez Chamorro and Calvache (2014), and Casaccia & Álvarez (2006) mention that the extraction of potassium by stevia plants was superior concerning the results obtained with the maximum doses in the present study. The application of potassium chloride to the soil increases the availability of absorption in plants, being essential in the activation of enzymes essential for photosynthesis and respiration, influences the activation of enzymes for the formation of starch and proteins, and interferes in the stomatic mechanism (Taiz & Zeiger, 2010). Variations in the values obtained are possibly due to the time of evaluation and phenological stages of the crop, soil nutrition, variety, and dry matter, among other factors.

CONCLUSIONS

Stevia (*Stevia rebaudiana*, Bertoni) is a crop with a growing demand. It requires good nutrition for its growth and development, so soils with good characteristics and fertilization are essential for reasonable productivity. A fertilizer experiment with different doses was carried out, considering initial soil analysis to assess their productivity and extraction. The results indicated that the treatment with higher fertilizers was the best, indicating that larger doses are needed to reach an optimal value. The most required nutrient for stevia cultivation is nitrogen, followed by potassium and phosphorus, respectively. The crop extraction per ton was approximately 30 kg N, 4.4 kg of P₂O₅, and 18 kg of K₂O for treatment with a higher yield.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTION

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