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Article

Organic amendments in the growth of *Stevia rebaudiana* in Tabasco

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Abstract

The crop of *Stevia rebaudiana* Bertoni is an alternative for the replacement of some sweeteners due to its yield and short growth periods. Nevertheless, before proposing this species in tropical areas, it is necessary to generate information on agronomic management under particular edaphoclimatic conditions. The objective was to evaluate the effect of organic amendments on the growth of *Stevia rebaudiana* in Tabasco, Mexico. The experiment was established in raised beds in the open field, under a system of tape irrigation and mulching in August 2019. The design was completely randomized with three repetitions, in which the following was evaluated, three organic materials as organic amendments: sheep manure, bovine manure and vermicompost, and a chemical fertilizer as a control. Survival at transplantation was determined, and growth variables were measured: survival percentage, plant height, number of shoots, root length, dry weight of leaves, stem and root, as well as foliar nutrient concentration. The results indicated that the plants grown with VC was the best treatment as it obtained the highest values in all the variables evaluated: 98.9% survival at transplantation, 40 cm plant height, 17.7 root length, 11.49 g total dry biomass. No statistical differences were found in foliar nutrient concentration for macroelements. According to the findings, it is concluded that the use of vermicompost as an organic amendment improves the growth of the stevia crop.

Keywords: fertilizers, manure, medicinal plant, vermicompost.

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Introduction

More than 100 species of the genus *Stevia*, distributed in America, belong to the family Asteraceae (Compositae) (Díaz-Gutiérrez *et al*., 2020 b), which includes *Stevia rebaudiana* Bertoni, a perennial plant native to the eastern region of Paraguay. Its cultivation has increased in countries such as China, Japan, the United States of America, Indonesia, Brazil and Canada, among others, it is valued for having 250 to 300 times more sweetener than sugarcane sucrose due to the content of steviol glycosides in its leaves (Rahayu *et al*., 2020).

In Mexico, it was introduced a decade ago and its cultivation is documented in research conducted by the National Institute of Forestry, Agricultural and Livestock Research (INIFAP, for its acronym in Spanish) in the states of Yucatan and Nayarit. However, to propose its cultivation in some states of the country, it is necessary to generate knowledge that allows understanding the adaptation and management of the crop in each particular area since the amount of sweetening glycosides varies according to the environmental conditions of the sowing region of the crop (Aghighi *et al*., 2018).

Stevia is a plant that requires temperatures of 18 to 30 ºC for its growth and rainfall of 1 000 to 1 400 mm per year, it can be grown in terrains with an approximate slope of 30%, clayey soils and at heights of 500 to 1 500 masl (Alva *et al*., 2017; Rojas *et al*., 2018). Therefore, Tabasco, Mexico, can be an option for its implementation, since it has a climate that is characterized by a rainfall of 1 926 to 3 437 mm per year and an average annual temperature of 22 ºC to 29 ºC (INEGI, 2018).

With respect to research carried out, different results of phenological variables, production and nutrient content of stevia have been found under different research conditions. For example, Cauich *et al*. (2018) found a root length and height of 11.14 and 42.86 cm, respectively, in stevia grown with different irrigation sheets with a mixture of black soil and henequen. For this last parameter, other authors reported a height of 94.3 cm in a loamy soil (Karimi *et al*., 2019), under salt stress a height of 48 cm (Sheikhalipour *et al*., 2021) and 43 cm (Aghighi *et al*., 2019) is mentioned, and in black soil combined with earthworm humus under greenhouse conditions, a height of 47 cm (González *et al*., 2019).

In addition, Díaz-Gutiérrez *et al*. (2020a) recommend the application of organomineral fertilizers to increase the productivity *of S. rebaudiana*, as an efficient sustainable crop management system that decreases dependence on mineral fertilizer, because its cost increases year after year, so an alternative that appears is the use of organic amendments. In relation to this, Velázquez *et al*. (2017) report that the application of 60 t ha^{-1} of bovine manure in the stevia crop increased the yield of dry biomass of stems and leaves; whereas Villalba *et al*. (2018) report an increase in the number of shoots (9) shoots and dry biomass (19 g) in the production of stevia with vermicompost, compared to the use of chicken manure.

Regarding the nutritional status of stevia, Aghighi *et al*. (2019) report a similar concentration of Na^+ in leaves and stems and differences in K^+ content; for their part, Yu-Ming *et al*. (2021) found higher concentrations of N relative to K content. In this sense, Aghighi *et al*. (2018) mention that, in the stevia crop, micronutrients play an important role in the development and growth of plants,

as well as the dose and time of application of fertilization since they affect the accumulation of the biomass of the stevia plant. The objective of this research was to assess the effect of organic amendments on the growth of *Stevia rebaudiana* in Tabasco, Mexico.

Materials and methods

Experiment location

The work was developed in the productive sector of the Zona Olmeca Campus belonging to the National Technological Institute of Mexico located in Villa Ocuiltzapotlán, Centro, Tabasco, at coordinates 18 07' 7.75" and 18º 09' 39" north latitude and 92º 52' 24" and 92º 50' 55" west longitude. The study area presents a warm humid climate with abundant rainfall in summer, average annual temperature of 27 ºC and precipitation of 1 926 mm per year (INEGI, 2018).

Crop management

Cuttings of *Stevia rebaudiana* variety Morita II rooted in commercial peat for 30 days were used, the cuttings were obtained in Tekax, Yucatán, Mexico. The variety Morita II is a Japanese variety, has higher dry leaf yields and product quality than other varieties including the landrace variety (Herrera *et al*., 2012), also has a lower content of steviosides that cause the bitter taste of the leaf and has a higher amount of rebaudioside A, a product demanded by the industry (Ramírez *et al*., 2011).

The crop was established in the autumn-winter period (August 2020), in cultivation beds with the following dimensions: 0.7 m wide, 25 cm long and 0.3 m high. The soil used was in alluvial plain (Palma-López *et al*., 2007), the soil composition is shown in Table 1. The cultivation beds were covered with silver plastic mulch.

Table 1. Chemical analysis of the soil in the productive sector of the Zona Olmeca *Campus* of the Technological Institute of Mexico, in Villa Ocuiltzapotlán, Centro, Tabasco.

Texture class	FC	PWP	pH	EC (dS m^{-1})	OM (%)	P-Bray	Ca	Mg	K
	(%)					(ppm)			
Clay	26.7	15.9	7.95	0.47	2.37	18.9	4 864	378	246

The irrigation was by drip, an irrigation of 0.5 L day^{-1} was provided at the time of transplantation and supplemental irrigation was eventually given according to the requirement of the crop, since most of the water requirements of the plant were covered with precipitation. The sowing was carried out with a staggered arrangement with a distance of 30 cm between plants and 20 cm between rows, and at the time of transplantation a tipping was carried out to homogenize the plants.

Treatments

The treatments used were: bovine manure (BM), sheep manure (SM), vermicompost (VC) and chemical fertilizer (CF: control) with urea: 46-0-0, the nutrient analysis of the treatments is reported on a dry matter basis (Table 2). The manure was collected in the corrals of the region and exposed to the sun to standardize the moisture, the vermicompost was obtained from the microenterprise 'Rancho Alegre' of the municipality of Comalcalco, Tabasco.

Table 2. Chemical nutrient analysis of organic amendments used in the growth of *S. rebaudiana.*

Determination	Method	Organic amendments		
		Bovine	Sheep	Vermicompost
pH	NMX-FF-109-SCFI-2007	7.41	5.5	6.43
EC (dS m^{-1})	NMX-FF-109-SCFI-2007	3.5	17.07	1.14
TN (%)	Dumas	1.89	4.13	1.25
P (%)	Microwave digestion/ICP	0.24	2.44	3.76
K (%)	Microwave digestion/ICP	1.16	4.16	0.31
Ca (%)	Microwave digestion/ICP	2.79	4.69	0.77
Mg (%)	Microwave digestion/ICP	0.53	1.18	0.41
Na (%)	Microwave digestion/ICP	0.1	1.97	0.06
S (%)	Micro digestion/Turbidimetry	0.33	2.45	-
Fe (ppm)	Microwave digestion/ICP	27 250	9 393	8 080
Cu (ppm)	Microwave digestion/ICP	47.83	66.94	26.27
Mn (ppm)	Microwave digestion/ICP	593	518	5.7
Zn (ppm)	Microwave digestion/ICP	298	498	137.1

Soil analysis was considered to calculate the dose of organic amendments. Table 1, and the amount of manure used from the dose of 180 kg ha^{-1} of N (Casaccia and Álvarez, 2006) was determined by the equation: $DF= \frac{ND-NSS}{\% NC}$. Where: DF= dose of fertilizer; ND= nutrient demand; NSS= nutrient supply by soil; % NC= percentage of nutrient in the compost; with which the following was obtained: BM= 857 g m^{-2}, SM= 392 g m^{-2} and VC= 1 280 g m^{-2}.

Organic amendments were applied 100% at the time of transplantation, while for chemical fertilization 50% was applied at transplantation and the remaining 50% 30 days after transplantation (dat). Seven days after planting, a formation pruning was carried out by removing the apical bud and 20 dat the formation pruning was carried out.

Growth variables

The percentage of survival at transplantation was determined four days after transplantation, in which the edge effect was considered. The growth variables evaluated were plant height (cm), number of shoots at 30 and 45 dat. At 60 dat, root length (cm) was measured, and dry biomass of leaves, stems and roots was weighed on a digital scale (Ohaus, Pioneer) in grams. For the determination of dry biomass, leaves, stems and roots of plants previously washed with distilled water were separated, dried at 60 ºC in a forced-air oven (Lumistell, HTP-42 series) until constant weight.

Foliar nutrient concentration

The sampling for the foliar chemical analysis was performed at 60 dat. TN was determined by the modified micro-Kjeldahl method to include nitrates, P by colorimetry with molybdate and ammonium vanadate, K and Na by atomic emission and Mg, Na, Fe, Mn, Cu and Zn by atomic absorption with a Perkin Elmer Analyst 100® atomic absorption equipment.

Experimental design and statistical analysis

The experimental design was completely randomized, with three repetitions, four plants were used as an experimental unit. The data in percentages were normalized with the square root of the arcsine and the data obtained were analyzed using an Anova, the variables with significance were subjected to a comparison of means with the Tukey test ($p \leq 0.05$) using the statistical program Statical Analysis Software version 9.1 (SAS, 2003).

Results and discussion

Survival percentage

The percentage of survival of stevia was greater than 81% in all treatments; however, vermicompost (VC) presented the maximum survival with 98.9%, while sheep manure (SM) obtained the lowest percentage with 81.5% (Figure 1), which means that the treatments presented significant differences ($p \leq 0.05$), while the chemical fertilizer treatment (CF; control) was statistically equal to the treatments VC and BM (Figure 1).

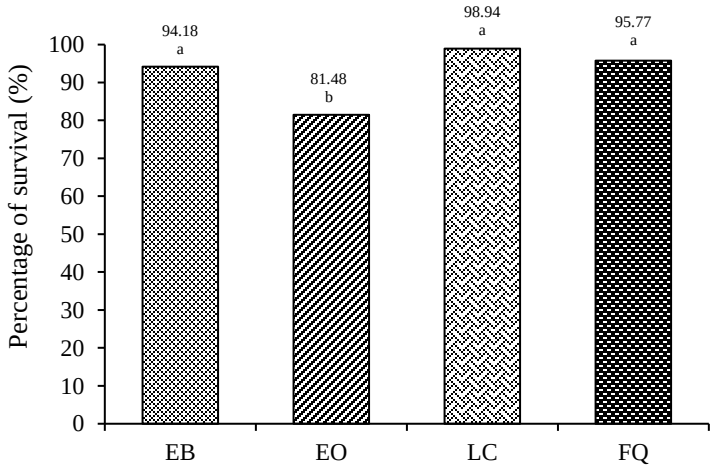

Figure 1. Percentage of survival at transplantation of *Stevia rebaudiana* Bertoni var. Morita II, established with different organic amendments. EB= bovine manure (BM), EO= sheep manure (SM); LC= vermicompost (VC); **FQ= chemical fertilizer (CF).** Means with equal letters are not statistically different (Tukey, $p \leq 0.05$).

The differences in the percentage of survival of plants established with vermicompost were favored because VC reduces the C:N ratio, the phytotoxicity of organic residues and has low electrical conductivity; this can be observed with the chemical analysis of each treatment (Table 2); likewise, Guerrero *et al*. (2012) mention that VC is a stable product that avoids risks in the soil-plant system associated with soil oxidation.

On the other hand, sheep manure used without prior decomposition process possibly affected the survival of plants by raising the microbial activity of the soil and facilitating the splitting of nitrogenous organic substances that generate ammoniacal nitrogen, which can cause root burning. It is important to mention that weather conditions could affect the survival of plants, because rainfall was higher than 300 mm per month and temperatures above 26 ºC (CONAGUA, 2020), environmental factors that contribute to the development and growth of stevia.

Plant height

Differences ($p \leq 0.05$) in plant height were found between treatments at 30, 45 and 60 dat (Figure 2). The plants grown with VC presented the highest growth in the three dates evaluated with 14.75, 23.767 and 40 cm respectively, and it exceeded the control (CF) by 34.1, 57.8 and 116.2%, which presented a height of 11, 15 and 18.5 cm, respectively.

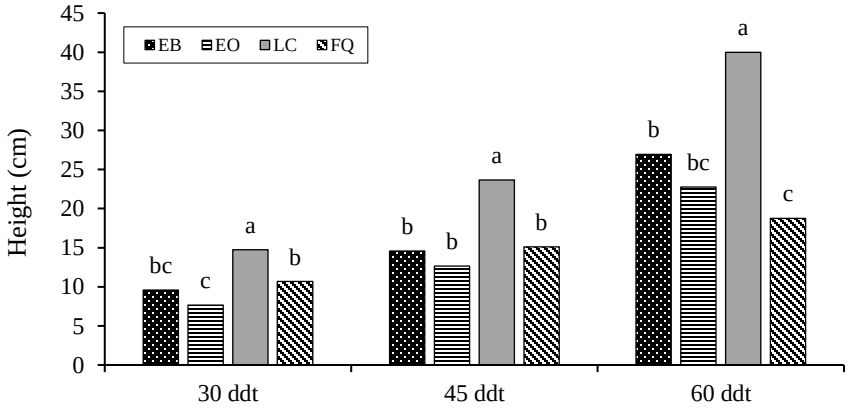

Figure 2. Plant height of *Stevia rebaudiana* Bertoni var. Morita with different organic amendments, at 30, 45 and 60 days after transplantation. EB= bovine manure (BM); EO= sheep manure (SM); LC= vermicompost (VC); **FQ= chemical fertilizer (CF).** Means with equal letters are not statistically different (Tukey, $p \leq 0.05$).

Our height results were lower than those reported by González *et al*. (2019); Karimi *et al*. (2019); Sheikhalipour *et al*. (2021) in other cultivation systems. However, the results of this experiment coincide with what was reported by Aghighi *et al*. (2019) and with Romero *et al*. (2017), who mention that at a higher proportion of vermicompost in plants of *Stevia pilosa* Lag. and *Stevia tomentosa* H. B. K., plant growth is improved by increasing nutrient availability.

In this regard, Domínguez *et al*. (2010) mention that the increase in the growth and productivity of horticultural crops fertilized with vermicompost is due to the fact that this material improves the physical and chemical structure of the soil and favors other biological mechanisms that stimulate plant growth, such as the production of humic acids, free enzymes and plant growth-regulating substances.

Number of shoots

In number of shoots there were no differences ($p \leq 0.05$) between treatments at 30 dat, all treatments produced on average 5.1 shoots per plant (Table 3). At 45 days of cultivation, the VC treatment presented the highest number of shoots per plant (23.1), surpassing the rest of the treatments. These results are similar to those reported by Villalba *et al*. (2018), who point out that vermicompost creates better conditions for the growth of stevia, producing three times more shoots than chicken manure; on the other hand, Avilés *et al*. (2019) mention that *Leucaena leucocephala* plants grown with vermicompost presented 18.3% more shoots than the control (without vermicompost). In addition, González *et al*. (2019) found a higher number of branches (33.8) per plant, at the age of 60 days in greenhouse conditions, grown in black soil with vermicompost.

Table 3. Number of shoots and root length of *Stevia rebaudiana* Bertoni var. Morita grown with different organic amendments.

Treatments	No. of shoots		Root length
	15 (dat)	45 (dat)	60 (dat)
BM	5 ab	11.8 b	15 b
SM	5.2 ab	10.6 b	14.6 b
VC	6 a	23.1 a	17.7 a
CF	4.4 ab	12.4 b	14.5 b
CV	40.73	43.18	18.51

BM= bovine manure; SM= sheep manure; VC= vermicompost; CF= chemical fertilizer; CV= coefficient of variation. Means with equal letters are not statistically different (Tukey, $p \leq 0.05$).

Root length

The use of vermicompost favored the growth of the roots of the plant (60 dat) as they presented the longest length with 17.7 cm, compared to the treatments BM, SM and CF (control) with a shorter length of 15, 14.6 and 14.5 cm respectively (Table 3). In relation to this, Salazar *et al*. (2018) report that vermicompost modifies the physical, chemical and microbiological properties of the soil, which positively affects the agronomic variables of the *Stevia rebaudiana* crop, these results coincide with Liu *et al*. (2011), who report that, from 60 dat, there is a greater vigor of *Stevia rebaudiana* roots in plants grown with organic residues compared to chemically fertilized plants, Campos *et al*. (2020) also report that the use of organic amendments to the soil favors root growth of plants, by improving soil structure, increasing microbial activity, nutrient absorption and moisture retention.

On the other hand, Gayosso *et al*. (2021) mention that the roots of the plants in containers are distributed according to the physical characteristics of the substrate, such as porosity, and consequently the distribution of water. It is possible that the use of vermicompost as an organic amendment has improved the physical properties, as well as the water availability and biological activity of the soil (Cauich *et al*., 2018; Salazar *et al*., 2018), which was reflected in an increase in the root system.

Dry biomass

At 60 dat, the production of dry leaf biomass in treatments with organic amendments showed no significant differences ($p \leq 0.05$) between them, compared to the control (Table 4). Nevertheless, the total dry biomass of plants with vermicompost, bovine manure and sheep manure were superior to the biomass of plants grown with chemical fertilizers (CF). The production of dry biomass with organic amendments coincides with the results obtained by Liu *et al*. (2011) at 80 dat, where the dry biomass per plant in stevia was higher with organic fertilizers than with chemical fertilizers; on the other hand, Velázquez *et al*. (2017) mention that the application of bovine manure in stevia was significantly higher than the control in production of dry leaf biomass per plant.

Table 4. Dry biomass of leaf, stem and root of *Stevia rebaudiana* Bertoni var. Morita II at 60 days after transplantation grown with different organic amendments.

Treatments	Leaf	Stem	Root	Total biomass
BM	5 a	3 a	1 a	9 ab
SM	4 ab	2 a	1.5 a	7.5 ab
VC	6.16 a	3.33 a	2 a	11.49 a
CF	2 b	1.33 a	0.83 a	4.16 b
CV	24.62	36.45	47.18	30.01

BM= bovine manure; SM= sheep manure; VC= vermicompos; CF= chemical fertilizer; CV= coefficient of variation. Means with equal letters are not statistically different (Tukey, $p \leq 0.05$).

Foliar nutrient concentration

For N, P, K, Ca, Mg, Na and Mn, no significant differences ($p \leq 0.05$) were observed in foliar nutrient concentration analyzed at 60 days after transplantation (Table 5). With respect to N, it is due to the fact that the same amount was applied in all treatments; however, there were statistical differences in the content of Cu, Fe and Zn (Table 2); nevertheless, these results were lower than those obtained by Yu-Ming *et al*. (2021), the differences may be due to the experiment being conducted under other growing conditions.

Table 5. Foliar nutrient concentration in *Stevia rebaudiana* Bertoni var. Morita II at 60 days after transplantation grown with different organic amendments.

Nutrient	BM	SM	VC	CF	CV
N (%)	3.133 a	2.906 a	2.37 a	3.106 a	16.49
P (g kg^{-1})	3.853 a	4.58 a	3.523 a	2.74 a	20.69
K (g kg^{-1})	8.285 a	8.754 a	8.306 a	8.561 a	10.39
Ca (g kg^{-1})	0.675 a	0.589 a	0.615 a	0.817 a	13.34
Mg (g kg^{-1})	0.22 a	0.205a	0.22 a	0.24 a	16.44
Na (g kg^{-1})	0.075 a	0.072 a	0.095 a	0.075 a	41.16
Cu (mg kg^{-1})	10.696 a	9.123 a	5.15 b	6.433 b	35.38

Nutrient	BM	SM	VC	CF	CV
Fe (mg kg^{-1})	713.97 b	786.63 b	1205.35 a	523.2 b	33.62
Mn (mg kg^{-1})	83.68 a	72.38 a	74.82 a	84.09 a	18.04
Zn (mg kg^{-1})	41.55 a	36.93 a	32.943 a	4.34 b	37.77

BM= bovine manure; SM= sheep manure; VC= vermicompost; CF= chemical fertilizer; CV= coefficient of variation. Means with equal letters are not statistically different (Tukey, $p \leq 0.05$).

The concentrations of macronutrients in dry biomass in some cases are not related to the amounts present in organic amendments. For example, for Cu in the treatments BM and SM, there were no significant differences between them, despite the fact that SM contains 28.5% more Cu than BM. With respect to Fe, bovine manure contains the highest concentration, and in the nutrient analysis of the plant, VC reflected the highest concentrations. For Zn, sheep manure showed the highest concentration; however, in the nutrient analysis there were no statistically significant differences with the other fertilizers, although CF was statistically different, with less of this element.

Conclusions

The establishment of *Stevia rebaudiana* Bertoni in the field grown with the vermicompost amendment favored the survival of the plant, it showed greater height, number of shoots and root growth, which meant an increase in total dry biomass, surpassing the yield of conventional fertilization and other amendments evaluated. This indicates that the use of organic fertilizers improves the development and growth of *Stevia rebaudiana* Bertoni Variety Morita II, in the conditions of the humid tropics.

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