Colegio de Postgraduados

Evaluation of Commercial Biostimulants at Three Planting Densities in Roselle (*Hibiscus sabdariffa* L.) Crop, Guerrero Variety

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ABSTRACT

Objective: The objective was to evaluate the physiological and productive response of roselle crop in the induction of three commercial biostimulants in three greenhouse planting densities.

Design/methodology/approach: The experiment consisted of three treatments and a control; T1 (high density) with Super Hormonal, T2 (low density) with Cito Xplosion, T3 (medium density) with Vitaminum Forte and Control (TES). The physiological variables evaluated were (plant height, stem diameter, number of branches, fresh and dry biomass), yield (fresh and dry weight of calyxes) and quality (titratable acidity, total soluble solids (°Brix), pH and electrical conductivity).

Results: plant heights of 168.3, 187.3, 219.5, 183.3 cm were obtained for T1, T2, T3 and control. For stem diameter, T2 was better with 2.9 cm, while T3 was better in number of branches. T2 presented a greater number of calyxes, however, the best yield was obtained in T1 with 25.9 t ha^{-1} of fresh calyxes and 3.86 t ha^{-1} of dry calyxes. The control was better with 10.1 in °Brix and 45.8 meq/100 g of titratable acidity, while the T1 was better in pH and electrical conductivity as quality parameters.

Limitations on study/implications: evaluate other biostimulants in other planting densities and modify the concentration of the nutrient solution to know the physiological and productive response of the plant.

Findings/conclusions: The use of biostimulants in combination with agronomic management and nutrition can substantially improve the yield and quality variables of dry calyxes.

Keywords: *Hibiscus sabdariffa* L., chalice quality, hydroponics, yield.

Citation: Mendoza-Pérez, C., Rubiños-Panta, J. E., Exebio-García, A. A., Delgadillo-Piñón, Ma. E., & Cazares-González, B. (2024). Evaluation of Commercial Biostimulants at Three Planting Densities in Roselle (*Hibiscus sabdariffa* L.) Crop, Guerrero Variety. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i11.2893

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: May 06, 2024.
Accepted: October 13, 2024.
Published on-line: December XX, 2024.

Agro Productividad, 17(11). November. 2024. pp: 165-174.

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an annual crop that belongs to the Malvaceae family, generally cultivated in tropical and subtropical regions (Rojas *et al.*, 2023). Although it is native to tropical Asia and Africa, it is now grown in tropical and subtropical regions due to its edible calyxes, which can be distinguished in four colors: green, pink, red, and dark red (Babalola *et al.*, 2001; Mariod *et al.*, 2017).

In 2019, 18,654 hectares of Roselle were cultivated in Mexico, distributed across 11 states, including Guerrero, Michoacán, and Oaxaca. In Michoacán, 1,780 hectares were cultivated with an average yield of 884.3 tons of dry calyxes, and a gross economic output of $69,510,950 Mexican pesos (SIAP-SADER, 2022).

Michoacán is the second-largest producer of both organic and conventional Roselle (Coria *et al.*, 2022). However, the yield per hectare obtained is 0.570 t ha^{-1}, which is lower than that of other states such as Oaxaca and Guerrero, which reached 1 t ha^{-1}. This is related to management practices, environmental conditions, phytosanitary problems, and seed selection (Coria *et al.*, 2022).

The low yield of Roselle is due to the lack of technical knowledge among producers and the insufficient transfer of the technological package for its production. However, improvements such as agronomic management, irrigation and balanced nutrition, seed selection, and the application of biostimulants can lead to higher yields of dry calyxes per hectare (Mofoke, 2006; Babatunde and Caro-Velarde *et al.*, 2012; El-Naim *et al.*, 2012; Bobadilla-Carrillo *et al.*, 2016).

The use of new production systems such as hydroponics, along with nutrient solutions, climate variable control, soil or substrate moisture balance, and balanced nutrition are other techniques that can be implemented to increase the yield of this crop (Vargas-Canales *et al.*, 2014).

Sánchez-Prado *et al.* (2019) mention that the nutrient solution in Roselle cultivation can improve the yield of dry calyxes and the accumulation of dry matter in plants. Another technology to enhance the quality of Roselle calyxes is the use of plant phytohormones to control physiological processes, growth timing, fruit maturation, and the crossing of plant species for the improvement of industrial products, which are naturally challenging processes to regulate in a conventional growing medium (Núñez-Ramírez *et al.*, 2023).

The yield of Roselle flowers is influenced by planting density. The use of an appropriate greenhouse planting can optimize higher density on a smaller surface area. Additionally, the induction of biostimulants combined with planting density can help the plant to express its maximum yield potential. Therefore, it is important to evaluate planting density and biostimulants, as they can be key variables for maximizing the yield of Roselle flowers. The objective of this study was to evaluate the physiological and productive response of Roselle cultivation in the induction of three commercial biostimulants at three planting densities, grown hydroponically under greenhouse conditions.

MATERIALS AND METHODS

Study Site

The experiment was conducted at the Colegio de Posgraduados, Montecillo Campus, State of Mexico (19° 27' 58" North latitude and 98° 54' 58" West longitude and altitude of 2,431 m).

Selection and Growth of Seedlings

Guerrero variety seeds of Roselle were germinated in 200-cavity Styrofoam trays, sown on February 18. They were transplanted on April 22, and the harvest of flowers was completed on September 19, 2022. The material was grown in greenhouse conditions under hydroponic system using black polyethylene bags (8 L) with red tezontle as substrate. The planting was arranged in a staggered pattern at 40 cm apart from plants in twin lines (20 m long and 40 cm between rows).

Irrigation System and Nutrient Solution

The irrigation system consisted of a watering line (16 mm diameter) with self-compensating drippers (0.4 m apart), flow rate of 8 L h^{-1} and operating pressure of 68.64 kPa. The irrigation was applied with Steiner's nutrient solution (1984) maintaining an osmotic potential of −0.087 MPa and a pH of 6.5 throughout the entire crop cycle.

Description of Treatments

The treatments consisted of using three planting densities and applying three commercial biostimulants in each treatment (Agricultural Solutions Chemiorganic) (Table 1).

The experiment was arranged as a randomized complete block design.

Each experimental unit was 20 m^2 with 15 plants and four replications per treatment, resulting in a total area of 80 m^2 per treatment.

Data was subjected to analysis o varianza. To determine significant differences in the variables studied, they were subjected to a Tukey mean comparison test with $p \leq 0.05$, using the MINITAB® statistical package version 16.

Evaluation of Response Variables

Plant height was measured with a 5-meter Truper tape measure (model 14582). The stem diameter was measured with a Truper digital caliper every eight days. The number of branches was counted directly. Fresh and dry weight of calyxes was measured using a portable digital scale Ocony (model UWE HGM-20) with a capacity of 20,000 ± 1g.

Table 1. Description of treatments and applied biostimulants.

Treatments	Planting density (plants m^2)	Biostimulants
T1 (High density)	6	Súper Hormonal
T2 (Low density)	2	Cito Xplosión
T3 (Medium density)	3	Vitaminum Forte
Control	TES	No application

Table 2. Rates of Biostimulants.

Treatments Biostimulants	Biostimulants	Vegetative stage	Beginning of flowering	Beginning of calyx formation	Calyx formation (50%)
		Dose (mL L^{-1})			
T1 (High density)	Súper Hormonal	1	2	3	5
T2 (Low density)	Cito Xplosión	1	2	3	5
T3 (Medium density)	Vitaminum Forte	1	2	3	5
Control	No application	0	0	0	0

For Fresh Biomass

A destructive sampling of the plant was conducted at the end of the growing cycle, which involved extracting the plant from the pot and then separating each organ, such as leaves, stems, roots, and calyx. Subsequently, they were weighed fresh and placed in a forced air oven at 70 °C for 72 hours for dehydration, and finally weighed to obtain the dry weight.

Yield Variable

The number of harvested calyces per plant was counted to maintain a record in each treatment. Subsequently, the capsule or ovary was removed from the calyces for drying. For fresh and dry weight of calyx: The harvested calyces from each treatment had their capsules removed and were weighed on a digital scale for fresh weight. They were then stored in paper bags for dehydration in a forced air oven at 60 °C for 72 h to obtain dry weight.

Evaluation of Biochemical Components

Total Soluble Solids (TSS) were realized by selecting ten calyces per treatment, then halved, and ground to extract the sample for determining total soluble solids using a refractometer (HI96801 Hanna) reported in °Brix. The pH was measured directly in the calyx juice with a potentiometer (Corning 12 Scientific Instruments, USA). Electrical Conductivity (EC) was measured directly in the calyx juice using a Conductronic PC18 (Puebla, Mexico), values were expressed in dS m^{-1}.

Titratable Acidity: Three grams of fresh calyces were placed in 50 m L^{-1} of deionized water and boiled for 3 minutes. The extracted solution was titrated with 0.08 N NaOH. Three drops of phenolphthalein were added as an indicator and results were reported in Meq/100 g of fresh sample, with a specific pH reading of 8.3 titratable acidity was calculated based on (Equation 1).

$$TA = \frac{(V_{\text{NaOH}})(N_{\text{NaOH}})}{P} \times 100$$

Where: TA=Titratable Acidity (meq/100 g of fresh sample). V_{NaOH}=Normality of $_{\text{NaOH}}$ (0.08 meq mL^{-1}). P=Weight of the sample (g).

RESULTS AND DISCUSSION

Evaluation of Physiological Variables

Plant Height

Plants that achieve greater height showed the ability to capture a larger amount of photosynthetically active radiation which produced the organic matter necessary for their growth and development. In this research, it was found that the growth in height of Roselle began slowly from transplanting until 22 days after transplanting (DAT). Subsequently, at 43 DAT, there was a noticeable increase in development across all treatments, reaching heights of 52, 55, 61, and 51 cm for T1, T2, T3, and the control treatment, respectively. It is noteworthy that at 50 DAT, the application of commercial bio-stimulants began for each treatment. The physiological response was most pronounced at 85 DAT, with greater vegetative development in the plants treated with bio-stimulants compared to the control treatment. The final heights were 168.3, 187.3, 219.5, and 183.3 cm for T1, T2, T3, and CT (Figure 1). This variable showed statistically significant differences ($p \leq 0.05$) compared to the other treatments.

Muñoz-Flores *et al.* (2022) mention that the difference in plant height is primarily due to competition for solar radiation, which directly impacts their growth, development, and production, as it is a photoperiodic plant. Additionally, the closer the distance between plants, the more they compete for water, nutrients, and radiation which is reflected in the increased height of the plants.

Stem Diameter

The results showed that plants on T2 exhibited a larger stem diameter after 43 days post-transplanting. In this treatment, the planting density allowed the plants to avoid competition for water, nutrients, and radiation. Therefore, due to the effect of the phytohormones, greater and better plant growth was achieved, as they utilized nutrient and water absorption more efficiently (Figure 2).

Number of Branches

In relation to the variable of number of branches, the following were obtained 30, 35, 37, and 34 branches were obtained for T1, T2, T3, and control, respectively. González and Chamorro (2017) reported a lower number of branches per plant, ranging from 25 to 30, with 215.7 calyxes per plant in Reina Salvadoreña variety at different plant densities.

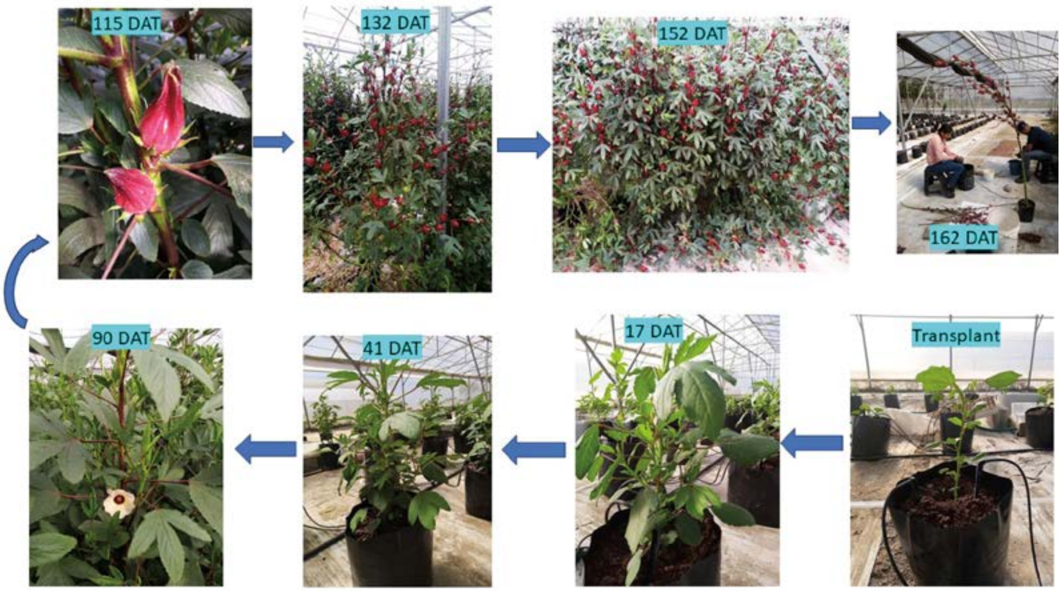

Figure 1. Growth development of roselle Crop.

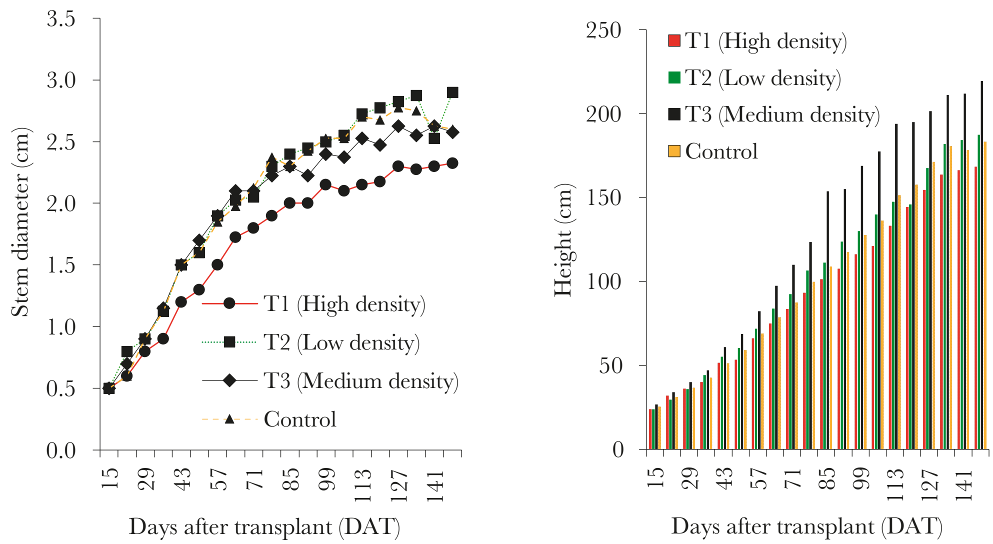

Figure 2. Stem diameter (left) and height (right) of the plant in the treatments.

On the other hand, Ovando *et al.* (2018) evaluated ten varieties of roselle and found 12 branches with 110 calyxes per plant; these values are lower than those found in the present study. González and Chamorro (2017) reported similar values for plant height and stem diameter in Reina Salvadoreña variety at different plant densities. Additionally, these authors reported between 25 and 30 branches with 215.7 calyxes per plant.

Fresh matter of organs

Figure 3 shows the accumulated fresh biomass in each organ for the evaluated treatments, it can be observed that control presented the highest accumulation in the leaves, flowers, and calyx, while T1 showed the greatest accumulation in the stem and seeds. Sánchez-Prado *et al.* (2019) reported 387.07, 1062, 1372.6, and 3656.3 g of dry biomass for the

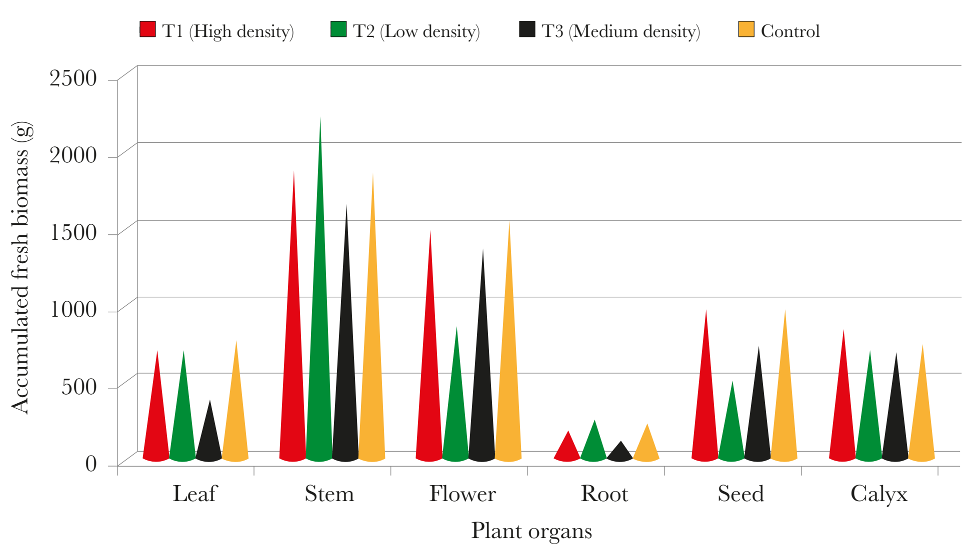

Figure 3. Biomass accumulation in plant organs across treatments.

roots, leaves, stems, and fruits plus calyx respectively in treatments with nutrient solution and osmotic potential of −0.072 MPa.

Evaluation of the productive variables

Fresh Calyx Weight

Statistical differences were found between treatments for fresh calyx weight. The yield of fresh calyx was 25.9, 7.6, 6.8, and 6.6 t ha^{-1} for T1, T2, T3, and control, respectively. The results obtained in this study were higher than those reported by Ramos-Gutiérrez *et al.* (2020) who found yields of 1.7, 1.3, and 1.5 t ha^{-1} of fresh calyx in UAN8, UAN6, and Porcelana varieties (Table 3).

Roselle Flower Yield

The yield obtained in the evaluated treatments were 3.86, 1.25, 1.09, and 0.95 t ha^{-1} of dry calyx for T1, T2, T3, and Control, respectively. Statistically significant differences were found between treatments for this variable. The nutrients Fe, Cu, Mn, B, and Zn present in the biostimulants Súper Hormonal, Cito Xplosion, and Vitaminum Forte influenced the leaves of the plants to have a greater amount of photosynthates. Additionally, hormones such as cytokinins, auxins, gibberellins, and vitamins induced a higher number of branches in the treatments, resulting in a greater number of calyces per plant.

Biostimulants increased tolerance to abiotic stress and enhanced yields in other crops, such as potatoes (Wadas and Dziugiel, 2020) and the number of pods per plant in beans (Kocira *et al.*, 2018).

The results found in this study are similar to those reported by Ramos-Gutiérrez *et al.* (2020), who obtained yield of 1.4, 0.9, and 1.1 t ha^{-1} of dry calyx in the UAN8, UAN6, and China varieties with a plant density of 10,000 plants per hectare. However, these results were higher than those reported by Muñoz-Flores *et al.* (2022), who recorded yield of 0.57 t ha^{-1} at a density of 13,888 plants ha^{-1} and 0.35 t ha^{-1} at a density of 8,264 plants ha^{-1} (Table 3).

The management of nutrient solution concentration is key to improve the productivity of agricultural crops. Sánchez-Prado *et al.* (2019) found a direct relationship between yield increases and different concentrations of the Steiner nutrient solution. They reported that the management of the osmotic potential of −0.018 MPa yielded 0.88 t ha^{-1}, −0.036 MPa yielded 1.1 t ha^{-1}, −0.054 MPa yielded 1.74 t ha^{-1}, −0.072 MPa yielded 1.98 t ha^{-1}, and −0.090 MPa yielded 1.56 t ha^{-1}. The treatment with the highest yield

Table 3. Results for number of branches, calyces, seeds, and fresh weight of calyces.

Treatments	Number of Branches	Calyx number	Number of seeds	Fresh weight of calyx t ha^{-1}
T1 (High density)	30 b	276 b	29 a	25.9 a
T2 (Low density)	35 a	315 a	30 a	7.6 b
T3 (Medium density)	37 a	151 d	25 b	6.8 b
Control	34 a	256 c	29 a	6.6 b

Columns with different letters are statistically different. Tukey mean separation test ($P<0.05$).

of dry calyx was 1.98 t ha^{-1}, which corresponded to a nutrient solution with an osmotic potential of −0.072 MPa. The results presented in this research clearly demonstrate that the use of innovative technologies, such as bio-stimulants, in combination with agronomic management such as planting density and nutrient solution, can substantially improve the yield of dry calyxes in this crop.

Evaluation of quality variables

For the variable of total soluble solids (°Brix), statistically significant differences were found among treatments due to application of bio-stimulants. The values obtained were 7.2, 9.2, 8.6, and 10.1 °Brix for T1, T2, T3, and control, respectively. It was observed that in control treatment, the highest °Brix value was recorded. The results found are similar to the values reported by Ramos-Gutiérrez *et al.* (2020), which were 7.38, 7.39, and 11.52 °Brix for the UAN8, UAN6, and China varieties (Table 4). It is important to consider that variations in total soluble solids are due to the fact that mature calyces concentrate a higher amount of °Brix. This formation is attributed to plants having a larger leaf area or greater planting density. These plants have the capacity to capture a greater amount of photosynthetically active radiation, CO_2 from the air, water, and nutrients. According to the results obtained in this research, the soluble solids in the calyces do not contain high concentrations of °Brix. It is recommended for the production of wine or for use in refreshing beverages, as the flower is rich in antioxidants.

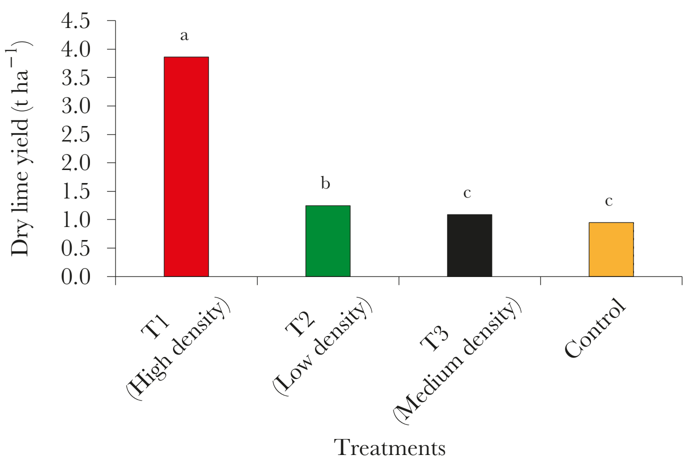

Figure 4. Yields obtained in the treatments.
Bars with different letters are statistically different. Tukey mean separation test (P<0.05).

Table 4. Quality variables of Roselle calyces in the three treatments and the control.

Treatments	Total soluble solids	Titratable acidity (meq $100g^{-1}$)	pH	Electrical conductivity (dS m^{-1})
T1 (High density)	7.2 d	45.6 b	1.91 a	6.50 a
T2 (Low density)	9.2 b	40.9 c	1.75 c	6.00 b
T3 (Medium density)	8.6 c	40.3 d	1.77 b	6.00 b
Control	10.1 a	45.8 a	1.90 a	5.80 c

The columns with different letters are statistically different. Tukey mean separation test (P<0.05).

Regarding the variable of titratable acidity in roselle calyces, values of 45.6, 40.9, 40.3, and 45.8 meq/100 g of citric acid were found for T1, T2, T3, and control, accordingly. Ramos-Gutiérrez *et al.* (2020) reported values of 40.7, 36.84, and 36.08 meq/100 g for the UAN8, UAN6, and China varieties (Table 4). Salinas-Moreno *et al.* (2012) explain that the dominant acids are oxalic and succinic, and relatively young roselle flowers exhibit higher acidity values of approximately 18.6% citric acid. Furthermore, Ariza-Flores *et al.* (2014) mention that the acidity of the extracts is related to the quantity of acids present, and in roselle, the following acids have been found: roselle acid, citric acid, ascorbic acid, stearic acid, benzoic acid, chlorogenic acid, 4-hydroxybenzoic acid, salicylic acid, vanillic acid, and protocatechuic acid, among others.

The pH values found were 1.91, 1.75, 1.77, and 1.90 for T1, T2, T3, and control (Table 4). Ramos-Gutiérrez *et al.* (2020) reported values of 2.72, 2.55, and 2.70 for the UAN8, UAN6, and China varieties. These values were higher than those found in the present study. Statistically significant differences were found among the treatments for the pH variable. Although higher pH values (3.3 to 3.4) of aqueous extracts of Roselle have been reported (González-Palomares *et al.*, 2009), Salinas-Moreno *et al.* (2012) recommend that values be less than 3.0. For the electrical conductivity variable, the results obtained were 6.50, 6.0, 6.0, and 5.8 Ds m^{-1} for T1, T2, T3, and control, respectively (Table 4).

CONCLUSIONS

The combination of plant density and biostimulants was crucial for increasing the yield per plant of calyces in both fresh and dry weight.

At a density of 6 p m^{-2}, the highest yield of 3.86 t ha^{-1} of dry calyces was obtained, compared to the control, which yielded 0.95 t ha^{-1}.

It was found that application of the biostimulant Súper Hormonal promoted more uniform plant growth on T1, as these products have the ability to control metabolic activities to ensure the intracellular and extracellular homeostasis of the plants. The use of innovative technologies such as biostimulants, combined with agronomic management (planting density) and plant nutrition, can substantially improve the yield and quality variables of the dry calyces of this crop.

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