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**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**
Volume II



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Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (<https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf>), published in 2023 by Leandris Argente-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of them contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argente-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscato, members of the Consolidated Academic Group ITVAYA-CA-3.

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Initial Agronomic Adaptation of *Stevia rebaudiana* Bertoni in the High Mountain Region of Veracruz and Sustainable Management of *Planococcus citri* Using a Neem-Based Bioinsecticide

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ABSTRACT

Assessment of seedling growth provides valuable information for understanding the early development and adaptation of plant species under specific cultivation conditions. In this study, *Stevia rebaudiana* Bertoni was evaluated as a plant model to analyze its early establishment in black soil under the environmental conditions of the High Mountain Region of Veracruz, Mexico. Additionally, the effectiveness of a potassium soap bioinsecticide enriched with *Azadirachta indica* (neem) extract, with potassium hydroxide as the active component, was evaluated for the control of the mealybug *Planococcus citri*. The study was conducted using a replicated 2² factorial experimental design, and the data were analyzed by ANOVA using Minitab 2023 software. The results showed that relative humidity had a positive and significant effect on stem growth, with a coefficient of determination of $R^2 = 94.51\%$. Under direct planting conditions, 30 seedlings showed better establishment and greater vigor, with an approximate average of 378 leaves per plant. In the phytosanitary trial, seven infested seedlings were sprayed every four days. Notably, in seedling 21, the visible insect population decreased from 53 to 7 individuals over a 13-day period, representing an 86.8% reduction. These results indicate that relative humidity is a determining variable for promoting the initial vegetative development of the soil-grown crop, whereas the evaluated bioinsecticide represents a sustainable alternative for the early management of this pest. Overall, this study provides an experimental basis for strengthening agronomic adaptation strategies and sustainable phytosanitary management for crops of regional interest.

Keywords: 2² factorial design, relative humidity, neem-based bioinsecticide, *Planococcus citri*, agronomic adaptation.

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[†] In memoriam

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INTRODUCTION

The growing interest in natural sweeteners has positioned *Stevia rebaudiana* Bertoni as a species of agronomic and industrial importance because of its content of steviol glycosides, mainly stevioside and rebaudioside A (Castillo-Vargas et al., 2025), compounds with high sweetening power and potential application in low-calorie products (Amaro-Arcos et al., 2025). The botanical nomenclature of this species was corroborated using the taxonomic record available in Plants of the World Online, Royal Botanic Gardens, Kew (n.d.).

The agronomic relevance of this crop is also associated with the need to strengthen leaf production systems, seedling management, and the generation of technical information useful for researchers, producers, and agricultural advisors, particularly in contexts where profitable and sustainable crops are sought under specific local conditions (Carneiro et al., 2024).

Although stevia is commonly associated with favorable development conditions at relatively low altitudes, edaphoclimatic gradients and soil properties can directly influence the physiological and metabolic responses of plants, thereby affecting their growth, adaptation, and stress tolerance (Ixmatlahua-Rodríguez et al., 2026). Therefore, its establishment under altitudinal conditions higher than those reported as optimal represents an aspect of agronomic interest, especially in regions where thermal variability and relative humidity may modify crop vegetative performance.

The early development of this species may also be affected by phytosanitary factors, including the presence of the mealybug *Planococcus citri*, which can reduce vegetative vigor and affect leaf formation. Mealybugs are pests of agricultural importance because they cause mechanical damage during feeding, reduce crop quality and productivity, and may act as vectors of plant viruses. Within this group, species of the genus *Planococcus* have been reported as relevant vectors; therefore, their monitoring and management are necessary within sustainable production schemes (Ahmed et al., 2023). In this context, the use of low-environmental-impact bioinputs, such as potassium soap and neem extract, represents an alternative compatible with sustainable agronomic management (Tamez et al., 2020).

The objective of this study was to evaluate, as a pilot trial, the initial establishment of *Stevia rebaudiana* Bertoni seedlings in black soil under the environmental conditions of Orizaba, Veracruz, as well as to assess the effectiveness of a neem-based bioinsecticide for the early management of this pest. This approach integrated the analysis of environmental variables with a sustainable phytosanitary management strategy aimed at strengthening stevia cultivation in the High Mountain Region of Veracruz.

Study site

The study was conducted in Orizaba, Veracruz, a municipality located in the central region of the state, at an approximate altitude of 1,230 m above sea level. This environment presents agroclimatic conditions of interest for evaluating the establishment and early development of *stevia rebaudiana* bertoni, since it is located above the altitudinal range commonly reported as optimal for this species.

The thermal variability characteristic of the region provides a relevant scenario for analyzing the adaptive capacity of the crop. According to Ramírez-Jaramillo and Lozano-Contreras (2016), altitudes above 1,200 m above sea level are considered unsuitable for the conventional establishment of *stevia rebaudiana*. Therefore, its evaluation in Orizaba represents a relevant setting for assessing its agronomic potential from a sustainable perspective.

Table 1. Agroecological requirements for the cultivation of *Stevia rebaudiana* Bertoni.

Parameter	Optimal	Suboptimal	Unsuitable
Climate	Tropical and subtropical	Temperate	Cold
Temperature (°C)	18–30	15–18; 30–43	Below 15; above 43
Precipitation (mm)	1000–1400	500–1000; 1400–2000	Below 500; above 2000
Altitude (m a.s.l.)	0–500	500–1200	Above 1200
Soil depth	Greater than 20 to 50 cm	Less than 20 cm	Deficient
Soil drainage	Good	Regular	Poor
Soil texture	Loam and sandy loam	Heavy	Heavy
Soil type	Luvisols, Nitisols, Regosols, and Fluvisols	Cambisols and Rendzinas	Solonchaks and Vertisols

Source: Adapted from Ramírez-Jaramillo and Lozano-Contreras (2016).

MATERIALS AND METHODS

Plant material and growing conditions

Stevia rebaudiana Bertoni seedlings obtained from shoots produced in a previous project were used as the plant material. These seedlings were gradually adapted to the conditions of the present study. An initial stock of 30 seedlings established in black soil was available. From this plant material, eight seedlings were randomly selected for the experimental evaluation associated with the factorial design.

The study period extended from October 2025 to February 2026. In addition to the results of the factorial analysis, the 30 seedlings established under direct planting conditions were monitored to assess their establishment and vegetative vigor. Complementarily, seven seedlings with visible *Planococcus citri* infestation were used in the phytosanitary trial to evaluate their response to the potassium soap bioinsecticide enriched with *Azadirachta indica* (neem) extract.

Irrigation was standardized by applying 800 mL of water per seedling every three days using a graduated cylinder to dose the supplied volume. From the third month onward, 17-17-17 NPK fertilizer was applied at a dosage of 10 g every 10 days as support for vegetative development during the experimental phase.

pH Measurement

The pH of the soil, the irrigation water, and the rainwater recorded during the experimental period was determined using the potentiometric method 4500-H⁺ B from Standard Methods (American Public

Health Association et al., n.d.). The inclusion of this variable was relevant because pH influences nutrient availability, the chemical equilibrium of the growing medium, and the conditions of the root environment, all of which affect the establishment and early development of seedlings.

Under nursery conditions, the soil had a pH of 5.7, whereas the irrigation water had a pH of 6.5. These values made it possible to characterize the chemical conditions of the growing medium and provided a complementary criterion for interpreting the initial development of the seedlings. Additionally, from days 84 to 105 of the trial, an average daily rainwater volume of up to 317 mL was recorded. This volume was determined using four rain gauges placed inside the nursery and measured with a graduated cylinder.

Variables evaluated

The evaluated variables were stem growth, number of leaves per seedling, incidence of *Planococcus citri*, light intensity in the nursery, and environmental conditions of temperature and relative humidity. Stem growth was determined by direct measurement in centimeters using a millimeter ruler for general evaluations; when greater precision was needed, a Mitutoyo digital caliper with a nominal resolution of 0.01 mm was used. The number of leaves was recorded by manual counting for each seedling.

The incidence of this pest was evaluated through direct manual counting of visible insects on the infested seedlings before and after the application of the bioinsecticide. To facilitate the observation of small individuals, a 10× BelOMO/Trilomo-type triplet magnifying glass was used. This procedure made it possible to compare pest abundance during the experimental period. The selection of this method was supported by methodological precedents in which direct counting of mealybugs on defined plant structures has been used as a sampling procedure in field studies on *P. citri* (Rossati et al., 2025).

Temperature and relative humidity were monitored using an Elitech RCW-360 monitor, with wireless transmission and real-time data visualization through a Wi-Fi connection to a computer. As a complementary environmental reference source, the NASA POWER platform was used, which provides meteorological and solar data derived from satellite observations and models (NASA POWER, n.d.). During the monitoring period, the records obtained with the local equipment and the data consulted from NASA POWER showed an approximate variation of -1 to -2 °C in temperature and 0.2 to 0.3 units in relative humidity, which made it possible to corroborate a general trend of consistency between both sources.

In addition to morphological growth, environmental conditions may modify the physiological and biochemical responses of *Stevia rebaudiana*, including processes related to the biosynthesis of steviol glycosides. Therefore, the interpretation of early vegetative development should be linked to the cultivation environment and to the physiological response of the plant under specific local conditions (Sharma et al., 2026).

Light intensity in the nursery was determined using a Steren photocell lux meter equipped with a silicon photodiode sensor and optical filter, with a measurement capacity of up to 10,000 lux. All measurements were performed on the same experimental units, using the same instruments, under uniform observation criteria and at consistent intervals throughout the study period.

Statistical analysis

The study considered a replicated 2^2 factorial experimental design, in which the effects of temperature and relative humidity on the early growth of the seedlings were analyzed. Stem height was considered the main response variable, Y1, for the analysis of morphological performance.

The data generated during the experimental period were analyzed by analysis of variance (ANOVA) using Minitab 2023, with the purpose of determining the effects of temperature, relative humidity, and their interaction on the early growth of *Stevia rebaudiana* Bertoni seedlings. This analysis made it possible to identify the factors with a significant effect on the evaluated response and provided quantitative support for interpreting the agronomic behavior of the crop.

RESULTS AND DISCUSSION

Effects of Temperature and Relative Humidity on Stem Growth

The replicated 2^2 factorial experimental design made it possible to identify the influence of environmental conditions on the morphological performance of *Stevia rebaudiana* seedlings. An analysis of variance performed in Minitab 2023 revealed a positive and significant effect of relative humidity on stem growth, with a coefficient of determination of $R^2 = 94.51\%$. These results indicate that a high proportion of the variation observed in the response variable Y1 was explained by the factors included in the experimental model.

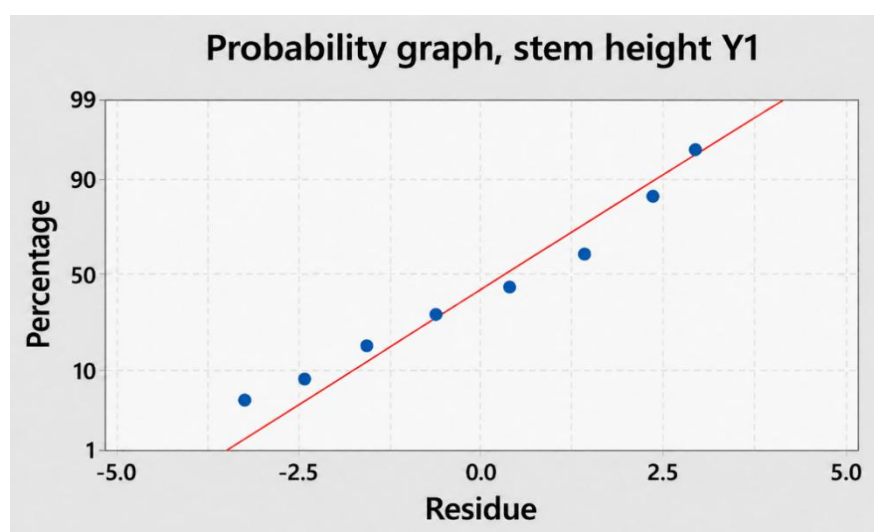


Figure 1. Probability graph (stem height).

Although temperature and relative humidity are closely related environmental variables, their interaction was not statistically significant in the evaluated factorial model. Nevertheless, the combined interpretation of these variables remains agronomically relevant, since previous studies have highlighted their influence on the adaptation and development of *Stevia rebaudiana* under specific cultivation conditions (Gutiérrez-González et al., 2025). Therefore, relative humidity should be considered a key factor in future crop management and adaptation strategies in high-altitude regions.

The critical reference line in the Pareto chart was located at 2.776, corresponding to the significance criterion established at $\alpha = 0.05$. Effects B and A exceeded this value and were therefore considered statistically significant for response Y1. In contrast, the AB interaction did not exceed the critical value, indicating that it was not statistically significant under the evaluated experimental conditions. Among the significant factors, relative humidity had the greatest effect, as shown in Figure 2.

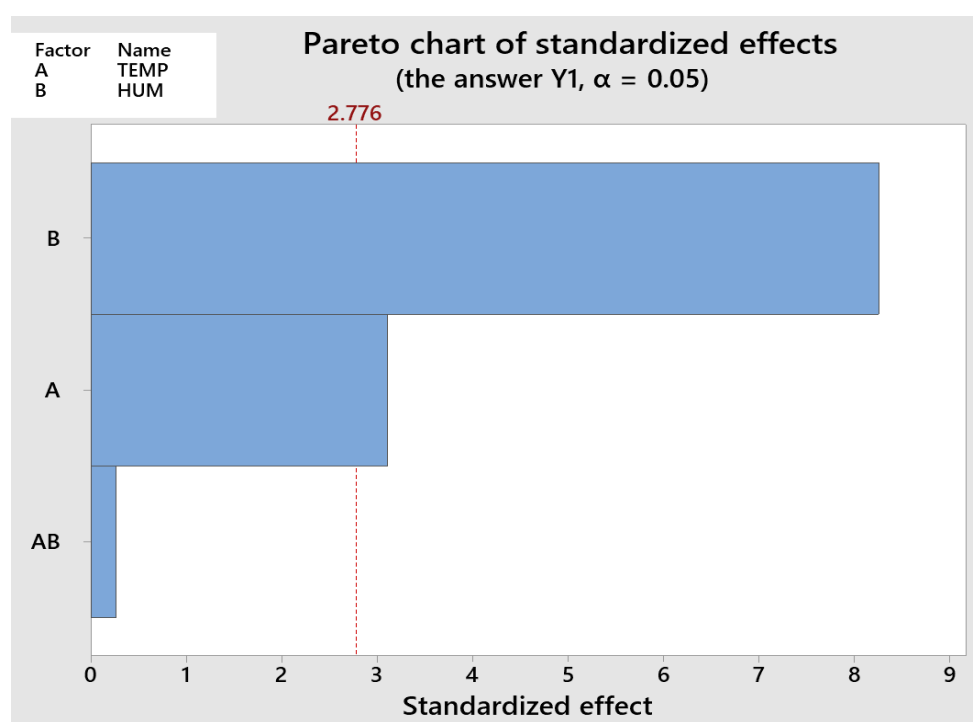


Figure 2. Pareto chart for temperature and humidity.

Relative humidity had the greatest effect, suggesting that atmospheric moisture availability was the most important component during the early stage of development. This behavior may be related to plant water balance, leaf expansion, and the maintenance of cell turgor. Although temperature also contributed to the crop response, its effect was lower than that observed for relative humidity.

Compared with seedlings previously managed in pots, those established in black soil showed better establishment and greater vegetative vigor. The average leaf development was approximately 378 leaves per seedling, with representative values ranging from 315 to 383 leaves in specimens 17 to 22. This variable is a relevant agronomic indicator in *Stevia rebaudiana*, since leaves are the main productive organ because of their relationship with the accumulation of steviol glycosides. In this context, the evaluation

of agromorphological, physiological, and biochemical traits allows a more accurate assessment of crop responses under specific production environments (Thakur et al., 2025).

From an agronomic perspective, these results suggest that soil cultivation favored a more stable vegetative response. Compared with previous trials, seedlings established in black soil developed approximately 11% more leaves, which may be attributed to better interactions between the root system and the substrate. Soil conditions and water management influenced not only the physical establishment of the seedlings but also their ability to sustain active leaf growth, a key aspect of a species whose agronomic value is closely associated with the accumulation of leaf biomass.

Complementarily, sustained seedling growth was observed throughout the monitoring period. The simultaneous increase in stem height and leaf development reinforces the interpretation that relative humidity plays a decisive role during the early stages of establishment by promoting the physiological processes associated with vegetative growth.



Figure 3. Adapted *Stevia rebaudiana* Bertoni seedlings grown in black soil under nursery conditions at TecNM, Orizaba Campus.



Figure 4. General view of *Stevia rebaudiana* Bertoni seedlings established under direct planting conditions at TecNM, Orizaba Campus.

Phytosanitary response of *S. rebaudiana* Bertoni seedlings to neem-based bioinsecticide treatment

Direct counting per seedling made it possible to quantify the response of *Planococcus citri* to the evaluated bioinsecticide treatment. In seedling 21, the visible insect population decreased from 53 individuals at the beginning of the application period to 7 individuals on day 13, corresponding to an 86.8% reduction. These results suggest that a potassium soap formulation enriched with neem may contribute to the early management of this pest under nursery conditions.

Table 2. Visible count of *Planococcus citri* on seedling 21 during bioinsecticide treatment.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Planococcus citri</i>	53	48	41	45	43	38	34	31	28	25	23	11	7

Note: The percentage reduction was calculated on the basis of the initial count and the count recorded on day 13.

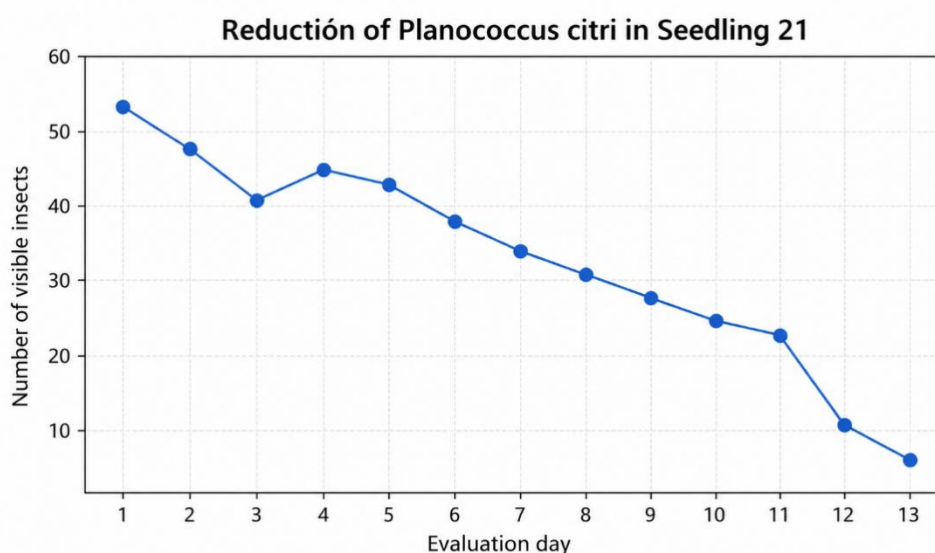


Figure 5. Reduction in the visible population of *Planococcus citri* in seedling 21 during the bioinsecticide treatment period.

From the perspective of sustainable agronomic management, these findings are relevant because they show that the use of bioinputs may represent a lower-environmental-impact alternative to conventional pesticides. In addition, its effectiveness during an early stage of infestation supports its potential use as both a preventive and corrective tool within integrated pest management schemes.

Overall, the results revealed that the early growth of *Stevia rebaudiana* under the conditions of the High Mountain Region of Veracruz was strongly influenced by relative humidity, whereas the use of bioinputs contributed to the development of more sustainable phytosanitary management strategies. From a sustainability perspective, the initial management of this crop can be strengthened through biocompatible strategies that promote plant growth and reduce dependence on chemical inputs. In this

context, beneficial plant–microorganism interactions have been identified as alternatives for improving the growth, yield, and sustainability of stevia cultivation (Abdelsattar et al., 2023). The integration of both dimensions, vegetative growth and ecological pest control, represents one of the main contributions of this pilot trial, as it allows the transition from a conceptual proposal for sustainable management to an experimental basis with regional agronomic relevance.

CONCLUSIONS

A pilot trial conducted in Orizaba, Veracruz, confirmed that the initial establishment of *Stevia rebaudiana* Bertoni in black soil was influenced mainly by relative humidity, which was identified as the variable with the greatest effect on seedling vegetative growth. These results show that crop success during its early stage depends largely on its response to local environmental conditions.

The factorial analysis revealed that both relative humidity and temperature significantly affected stem height. However, relative humidity presented the greatest magnitude of effect, while the interaction between the two factors was not statistically significant under the evaluated conditions. These findings support the relevance of environmental monitoring as a complementary tool for the initial management of the crop.

The potassium soap bioinsecticide enriched with neem showed potential for the early control of *Planococcus citri*, reducing the visible insect population in seedling 21 from 53 to 7 individuals over a 13-day period. These results support its use as a sustainable, low-environmental-impact alternative within early phytosanitary management strategies.

As a relevant contribution, this study suggests that *Stevia rebaudiana* may show promising initial adaptation to altitudinal and thermal conditions different from those commonly reported as optimal for its conventional establishment. Overall, this work provides an experimental and methodological basis for future agronomic evaluations of crops of regional interest exposed to variable environmental conditions.

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To the BioSecuraNet International Network—International Network for Biotechnology and Innovative Strategies for Global Food Security.

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