

AGRO PRODUCTIVIDAD

Potential of the native **bamboo**

Guadua aculeata Rurp.
for the carbon
credit market

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Biopesticides for the control of whitefly (*Trialeurodes vaporariorum* Westwood) in tomato (*Solanum lycopersicum* L.) in greenhouse conditions

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ABSTRACT

Objective: To evaluate the effectiveness of biopesticides against whitefly (*Trialeurodes vaporariorum* Westwood) in greenhouse tomato (*Solanum lycopersicum* L.) cultivation.

Design/methodology/approach: The treatments evaluated included *Isaria javanica*, *Beauveria bassiana*, and a commercial insecticide. Evaluations were conducted at four post-application dates (DAA): 3, 5, 7, and 14 days. A factorial design was employed in randomized complete blocks with interaction, considering two factors: Factor 1: treatments, and Factor 2: DAA (3, 5, 7, and 14). The study variables included the number of surviving adults, nymphs, eggs, and the total number of surviving whitefly organisms.

Results: No statistical differences were observed among treatments in their effectiveness against whitefly eggs and adults. However, *Isaria javanica* and the commercial insecticide demonstrated a higher control percentage for nymphs. Regarding the total number of organisms, *Beauveria bassiana* showed a statistically significant difference compared to the commercial insecticide, whereas *Isaria javanica* showed no significant difference. The interaction between biopesticides and DAA did not yield significant differences across the variables studied.

Findings/conclusions: Biopesticides were shown to be efficient or better than the commercial insecticide for whitefly control in greenhouses.

Keywords: Microbial control, Entomopathogenic fungi, *Isaria javanica*, *Beauveria bassiana*.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables globally, with a *per capita* consumption of 20.2 kg. Mexico, recognized as the center of tomato domestication, exhibits extensive morphological diversity, making it one of the most widely cultivated crops in greenhouses (Pérez *et al.*, 2020). However, open-field tomato production faces growing challenges due to adverse environmental conditions and the prevalence of pests and diseases that significantly impact crop productivity (Carrillo *et al.*, 2003).



Conventional agriculture relies heavily on agrochemicals, including herbicides, insecticides, and fungicides, which contribute significantly to environmental pollution. A viable alternative to mitigate these impacts is sustainable agriculture (Cano *et al.*, 2004). Among the most promising strategies to reduce the environmental footprint of pest and plant disease control is the use of biological control agents (Hynes & Boyetchko, 2005).

Biological control of insect pests has emerged as one of the most effective strategies for sustainable pest management. This approach involves the use of predators, parasitoids, or pathogens to regulate insect populations. Insect pathogens, or entomopathogens, include viruses, bacteria, fungi, protists, and nematodes that infect and ultimately kill their insect hosts (Loreti *et al.*, 2020). Comparisons between entomopathogens and conventional chemical insecticides are typically centered on their efficacy and cost-effectiveness (Lacey, 2001).

The concept of biopesticides in the agrobiological industry refers to microbial biological products developed through formulation and industrial production for use in pest control. Biopesticides are effective in managing agricultural pests while minimizing environmental damage and avoiding further environmental pollution (González-Maldonado & García-Gutiérrez, 2012; Nava-Pérez *et al.*, 2012). Although the application of synthetic insecticides remains the most commonly employed method for controlling whitefly, their improper use has several drawbacks. These include increased production costs, the development of pest resistance, and heightened risks of environmental pollution, public health concerns, and food contamination (Eskenazi *et al.*, 2004; Hernández *et al.*, 2009; Pérez *et al.*, 2011). Biopesticides harness a diverse range of entomopathogenic microorganisms. Among the most commonly utilized fungal species, renowned for their broad-spectrum control capabilities, are *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* (Pacheco-Hernández *et al.*, 2019; Nava-Pérez *et al.*, 2012). These fungi exhibit variations in their infection mechanisms, replication sites, viability, and pathogenic processes. Additionally, differences among strains and varieties result in varying levels of efficacy (Nava-Pérez *et al.*, 2012).

B. bassiana is widely regarded as one of the most effective biological control agents in agriculture (Castruita-Esparza *et al.*, 2020; Malpartida-Zevallos *et al.*, 2013). Similarly, *Isaria javanica*, a well-studied and extensively utilized fungal species for insect management, has shown significant potential as an efficient microbiological agent for pest control (Flores *et al.*, 2013). Whitefly is one of the primary pests affecting crops, particularly solanaceous and cucurbitaceous plants (Basso *et al.*, 2001). The most prevalent species, *Trialeurodes vaporariorum*, adversely impacts plant growth by directly feeding on sap, transmitting viruses that cause diseases, and encouraging the development of sooty mold on leaves and fruits (Scotta *et al.*, 2014). Its feeding weakens plants by depleting nutrients (Toledo, 2019), often resulting in production losses of up to 100%, especially during spring, when whitefly populations reach their peak density (Ruiz & Aquino, 1996). The use of synthetic insecticides has increased substantially over the past century. However, the lack of regulation and excessive application of these chemicals have led to severe environmental damage and health risks (Ruiz *et al.*, 2018). Biopesticides have emerged as a viable alternative, offering economically attractive and ecologically sustainable solutions for pest management (Marchese & Filippone, 2018). This study was conducted in a greenhouse at the INIFAP-

Cotaxtla Experimental Station to evaluate the effectiveness of biopesticides in controlling whitefly in tomato cultivation under greenhouse conditions.

MATERIALS AND METHODS

This study was conducted at the INIFAP-Cotaxtla Experimental Station, located at km 34.5 on the Veracruz-Córdoba Federal Highway, in the municipality of Medellín, Veracruz, at an altitude of 15 meters above sea level, with a tropical sub-humid climate. The study was carried out in a 600 m² greenhouse with zenithal windows, anti-aphid mesh, and white plastic covering the upper part. Seeds of the hybrid tomato Atrevido F1 from Harris Moran[®] were used, which were initially sown in a nursery. One month after seedling emergence, transplanting was carried out into 1 m wide by 50 m long planting beds covered with white-black plastic mulch. A staggered topological arrangement was used, with 50 cm spacing between plants.

Experimental design and treatments

For the evaluation of biopesticides, a factorial randomized complete block design with interaction was used. Two factors were considered: factor 1, pesticides; and factor 2, days after application (DAA) of the pesticides 3, 5, 7, and 14 DAA. Two biopesticides, one synthetic insecticide, and a control (water) were evaluated, with the latter used only to account for natural mortality (Table 1). The experiment was conducted on two planting beds. Each bed contained two blocks, consisting of four plots with 12 plants arranged in double rows. In each plot, three plants in double rows were identified for evaluation.

The application of biopesticides was performed using electric spray backpacks (Hyunday[®] brand) equipped with full-cone nozzles. To prevent cross-contamination between treatments, a separate backpack was designated for each product. The spraying system was calibrated to generate very fine droplets at high pressure, producing a mist that ensured effective coverage and adherence across the entire plant surface. Applications were directed at both the adaxial and abaxial surfaces of the tomato leaves. Transparent plastic barriers were installed to isolate the four plants in each treatment and further eliminate the risk of cross-contamination. From each experimental plot, two central plants (usable plot) were sampled, and two leaves were taken from each plant, one from the upper part and the other from the middle part of the plant. The presence of whitefly by developmental stage was recorded. The first count focused on adults present on the abaxial side of the leaf, as their high flight capability required that the plant not be disturbed during counting. For counting eggs and nymphs, a paper template with a sampling area of 1.44 cm² was used.

Table 1. Treatments evaluated against whitefly in tomato cultivation under protected conditions.

Treatments	Active ingredient	Concentration	Dose
Movento ^{®a}	Spirotetramat	15%	1.25 mL L ⁻¹
<i>Beauveria bassiana</i> ^b	<i>Beauveria bassiana</i>	1 × 10 ⁸	2.5 mL L ⁻¹
<i>Isaria javanica</i> ^b	<i>Isaria javanica</i>	1 × 10 ⁸	2.5 mL L ⁻¹
Witness test	water		

^a Bayer, ^b Biotechnology ANDREB.

This template was placed on the abaxial side of the leaf along the second vein. The first sampling was conducted in the morning before applying the biopesticides, and subsequent samplings were repeated on days 3, 5, 7, and 14 after the application (DAA). The studied variable consisted of survival counts of organisms after the applications, considering the following response variables: the number of live adults, eggs, and nymphs. For pesticide evaluation, the sampling data were transformed into efficiency (%) using the formula by Henderson and Tilton (1955).

$$\text{Efficiency \%} = \left(\frac{n \text{ in } Co \text{ before treatment} * n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment} * n \text{ in } T \text{ before treatment}} \right) * 100$$

Where: n =number of insects, T =treated, Co =control.

For the statistical analysis, a univariate GLMM program was used with the statistical packages SPSS 20 and INFOSTAT, obtaining the analysis of variance for the independent factors and their corresponding interaction. The means test was performed using Tukey's test at 95% confidence.

RESULTS AND DISCUSSION

A statistically significant difference was observed in the efficiency of the pesticides for nymph control ($F_{2,452}=4.10$, $p=0.0171$) and the total number of organisms ($F_{2,642}=2.91$, $p=0.0550$). However, no statistical differences were observed for adults and eggs ($F_{2,468}=0.40$, $p=0.6714$) and ($F_{2,355}=2.64$, $p=0.0725$), respectively (Table 2).

A statistically significant difference was observed among the pesticides for the control of whitefly nymphs. *Isaria javanica* and Movento[®] exhibited the highest control percentages, with averages of 48% and 45%, respectively, both of which were statistically different from *Beauveria bassiana*. Regarding the total number of organisms, *B. bassiana* demonstrated superior efficacy, showing a statistically significant difference compared to Movento[®] and a similar effect to *I. javanica*. For adults and eggs, an efficiency exceeding 50% was recorded across the treatments. These findings align with those reported by Scorsetti *et al.* (2008b), who documented mortality rates ranging from 26.6% to 76.6% using entomopathogens against *T. vaporariorum*. Similarly, Quesada *et al.* (2006b) achieved mortality rates exceeding 50%, while Oreste *et al.* (2016) reported mortality rates of over 86%.

Table 2. Efficiency ($\bar{X} \pm ES$) of the treatments evaluated against whitefly in tomato cultivation under protected conditions.

Treatments	Adults	Nymphs	Eggs	Total organisms
<i>Isaria javanica</i>	69.72±3.32a	48.84±3.73 a	58.25±4.51a	48.80±2.98 ab
<i>Beauveria bassiana</i>	67.88±3.33a	34.00±3.85 b	65.19±4.00a	50.98±2.87a
Movento	72.16±3.46a	45.02±3.65 ab	52.35±3.90a	41.52±2.92b

Values corrected for natural mortality using the Henderson and Tilton (1955) formula.

\bar{X} =average. ES=Standard error. Means joined by the same letter indicate no statistical difference.

For the DAA (Days After Application) factor, no statistically significant differences were detected in the control of adults ($F_{3,468}=2.13$, $p=0.0951$), nymphs ($F_{3,452}=1.06$, $p=0.3673$), eggs ($F_{3,355}=1.37$, $p=0.2513$), or the total number of organisms ($F_{3,642}=0.49$, $p=0.6860$). Similarly, no significant differences were observed for the interaction between biopesticides and DAA across any of the variables studied: adults ($F_{6,468}=0.59$, $p=0.7419$), nymphs ($F_{6,452}=0.50$, $p=0.8092$), eggs ($F_{6,355}=0.62$, $p=0.7156$), or the total number of organisms ($F_{6,642}=0.38$, $p=0.8939$). These findings differ from those reported in other studies, which have documented significant effects on whitefly mortality at 7 DAA (Quesada *et al.*, 2006c; Oreste *et al.*, 2016b).

CONCLUSIONS

Isaria javanica and the commercial insecticide Movento[®] demonstrated greater efficiency in controlling whitefly nymphs compared to *Beauveria bassiana*. All evaluated treatments showed similar effectiveness in controlling whitefly eggs. Regarding the total number of organisms (sum of all developmental stages), *Beauveria bassiana* stood out as the most effective treatment, while *Isaria javanica* exhibited a similar level of control to Movento[®].

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State-level analysis of the relationship between agricultural production and prices in Mexico

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ABSTRACT

Objective: To determine the relationship between the quantity produced of the main agricultural product of each Mexican state and its price.

Design/Methodology/Approach: Sixty-two cointegration tests were conducted to examine the relationship between the production levels and the price of the main agricultural product of each state, with the exception of Baja California due to lack of data.

Results: Generally, the price does not influence the production of the main agricultural product in the 31 states analyzed over the long term. Only fifteen exceptions were found in the 62 tests conducted, which contradicts the existing literature. This lack of influence could be attributed to the specific characteristics of the crops studied, the available infrastructure, state-specific conditions, or the implementation of government programs. However, as mentioned, there are exceptions.

Study Limitations: The States were not characterized in detail.

Findings/Conclusions: Overall, there is no significant relationship between the price and the production levels of the main agricultural product of each Mexican state.

Keywords: agricultural production, agricultural sector, prices, production.

INTRODUCTION

There are various economic theories that attempt to explain the relationships between the economic agents in the agricultural sector, specifically between suppliers or producers and demanders or consumers. Among the main economic theories that seek to explain these relationships are the neoclassical, institutional, and critical theories (Hernández *et al.*, 2020, 2021; Cardona *et al.*, 2007).

The neoclassical theory suggests that the agents in the agricultural sector should be studied as if they were businesses. This implies that agricultural producers aim to maximize their profits, minimize costs, and adjust their production levels in response to price fluctuations (Hernández *et al.*, 2022 a, 2022 b; Cardona *et al.*, 2007).

Additionally, various studies indicate that agricultural production in several countries is influenced by product prices, supporting the supply and demand model of neoclassical theory. Therefore, it is suggested that an increase in the agricultural sector prices leads to a rise in production volume due to the incentives created (Tonconi, 2015; García, 2020; Benítez, 2022; OCDE-FAO, 2011; Brambila *et al.*, 2014; Hernández *et al.*, 2022 a, 2022 b; Hernández y González, 2022; Flores, 2014; Roitbarg, 2021). Similarly, Brambila *et al.*, (2014) points out that in Mexico, the relationship between price and production in the agricultural sector is positive, aligning with other studies that suggest producers are motivated to increase production when prices rise, primarily due to the potential of higher profits (Fernández, 2008; Márquez *et al.*, 2006, Cardona *et al.*, 2007).

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This aligns with the neoclassical microeconomics theory's supply and demand model, which posits that producers are rational and will seek to maximize profits and minimize costs. Consequently, when prices rise, producers are likely to respond by increasing their production levels (Tarza *et al.*, 2008; Jenneth, 2009; Cardona *et al.*, 2007; Roitbarg, 2021; Rivera, 2017; Pindyck and Rubinfeld, 2009). However, this price-production relationship is influenced by factors such as the type of producer. Producers with more economic and technological resources are less vulnerable to price decreases because they can offset income losses through increased competitiveness and productivity, a strategy not available to producers with limited resources (De Grammont, 2010; Guzmán *et al.*, 2012). Accordingly, the main agricultural products produced in 2021 by each of the 31 States, as shown in Figure 1, will be affected by price variations.

In Figure 1, it can be observed that in 41.9% of the Mexican states (that is, in 13 states), the main product produced in 2021 is sugarcane. According to neoclassical microeconomics theory, sugarcane should vary in response to price fluctuations. Furthermore, since it is the main product, the relationship between price and production should be evident (Tarza *et al.*, 2008; Jenneth, 2009; Cardona *et al.*, 2007; Roitbarg, 2021; Rivera, 2017; Pindyck and Rubinfeld, 2009).

Based on the points raised in this section, the objective of this research is to determine the relationship between the quantity produced of the main agricultural product in each state and its price in Mexico. It is also hypothesized that there is a relationship between the quantity produced of the main agricultural product in each state and its price in Mexico.

MATERIALS AND METHODS

The methodology used in this research aims to achieve the objective and test the hypothesis previously presented. A total of 62 cointegration tests were conducted (31 with a trend and 31 without a trend), which allowed us to determine the following: the existence of the relationship between the quantity produced of the main agricultural product produced in each state and its price; that this relationship is long term; and the relationship is not spurious (Gujarati and Porter, 2010; Wooldridge, 2010).

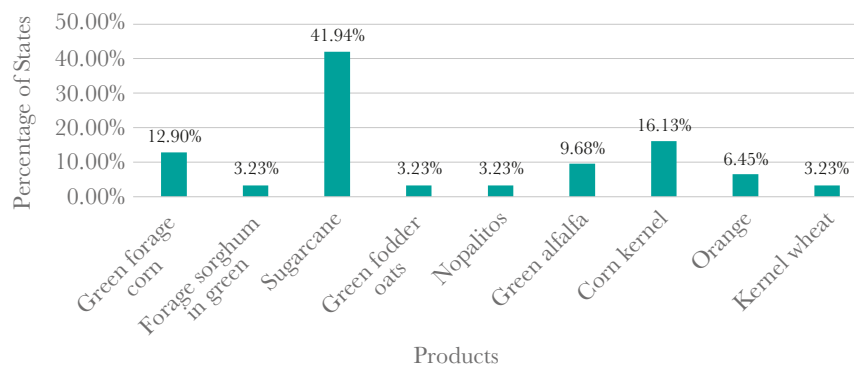


Figure 1. Main agricultural products produced in 2021 by each of the 31 states (the State of Baja California was excluded due to lack of data).

Source: Figure developed by the authors based SADER (2022) data.

The information used included the real price of crops per tons/hectares (deflated using the Índice Nacional de Precios al Consumidor published by the Instituto Nacional de Estadística y Geografía, 2022) and the quantity produced (production volume in tons related to the harvested area) of the main agricultural product of each Mexican state. The analysis period covered the years 1980 to 2021, and the data are annual (42 observations). This information was obtained from the databases on the website of the Secretaría de Agricultura y Desarrollo Rural (SADER, 2022).

Table 1 shows the main agricultural products produced in 2021 by each of the 31 states that were examined. It is worth mentioning that the state of Baja California was excluded from the study due to lack of information.

The States listed in Table 1 were analyzed through 62 cointegration tests, 31 with a trend and 31 without a trend, to provide a more comprehensive analysis. The goal was to determine if there is a long-term relationship between the quantity produced of each state's main agricultural product and its price, and to identify whether said relationship is spurious or not (Gujarati and Porter, 2010; Wooldridge, 2010). The models are based on the relationship between price and production as described and evidenced by several authors, including Tonconi (2015), García (2020), Benítez (2022), OECD-FAO (2011), Brambila *et al.*, (2014), Márquez *et al.*, (2006), Cardona *et al.*, (2007), and Roitbarg (2021), who suggest that producers seek to maximize their profits and, therefore, react to price variations.

In the cointegration tests conducted, price was treated as the exogenous variable, while the quantity produced was the endogenous variable, consistent with the supply and demand model of the neoclassical microeconomic theory. This approach also ensures that the model is free from endogeneity issues, as the price variable is not correlated with other unobserved variables (according to the cited literature).

Table 1. Products and States analyzed.

Federal entity	Main product produced	Represented by the abbreviations	Federal entity	Main product produced	Represented by the abbreviations
1. Aguascalientes	Green forage corn	A-MFV	16. Morelos	Sugarcane	Mor-CA
2. Baja California Sur	Forage sorghum in green	BCS-SFV	17. Nayarit	Sugarcane	N-CA
3. Campeche	Sugarcane	Ca-CA	18. Nuevo León	Orange	NL-N
4. Chiapas	Sugarcane	Chip-CA	19. Oaxaca	Sugar cane	O-CA
5. Chihuahua	Green fodder oats	Chih-AFV	20. Puebla	Sugar cane	P-CA
6. Ciudad de México	Nopalitos	CDMX-N	21. Querétaro	Green fodder corn	Que-MFV
7. Coahuila	Green alfalfa	Co-AV	22. Quintana roo	Sugarcane	QR-CA
8. Colima	Sugarcane	Col-CA	23. San Luis Potosí	Sugar cane	SLP-CA
9. Durango	Green fodder corn	D-MFV	24. Sinaloa	Corn kernel	Sin-MG
10. Estado de México	Corn kernel	EM-MG	25. Sonora	kernel wheat	Son-TG
11. Guanajuato	Green alfalfa	Gua-AV	26. Tabasco	Sugarcane	Tab-CA
12. Guerrero	Corn kernel	Gue-MG	27. Tamaulipas	Sugarcane	Tam-CA
13. Hidalgo	Green alfalfa	H-AV	28. Tlaxcala	Corn kernel	Tlax-MG
14. Jalisco	Sugarcane	J-CA	29. Veracruz	Sugarcane	V-CA
15. Michoacán	Corn kernel	Mich-MG	30. Yucatán	Orange	Y-N
			31. Zacatecas	Green fodder corn	Z-MFV

Source: Table developed by the authors based on SADER (2022) data.

According to Gujarati and Porter (2010) and Wooldridge (2010), before performing cointegration tests, it must first be established that the variables are non-stationary and of integration order one. To confirm that the variables are non-stationary, a unit root test of original order must be performed on each of the datasets analyzed. These authors suggest several unit root tests, one of which is the augmented Dickey-Fuller (ADF) test, known for its superior statistical properties compared to other tests like the Dickey-Fuller (DF), as it does not assume that the error term is uncorrelated.

It is important to note that the mention of 62 cointegration tests refers to the different conditions under which these tests were conducted. Each state's data (62 variables total) was tested both with and without a trend, leading to a total of 124 tests (62 with a trend and 62 without). These tests were further extended to analyze first differences, doubling the number to 248 tests, which ensures a comprehensive analysis of the variables under various scenarios.

To conduct the ADF test, Eviews software and the methodology described by Gujarati and Porter (2010) and Wooldridge (2010) were utilized. In total, 124 ADF tests were performed in their original order (62 with a trend and 62 without a trend). Specifically, four tests were conducted for each of the 31 States analyzed: two on the quantity produced of their main agricultural product (one with a trend and one without a trend) and two on the real price of the agricultural product (one with a trend and one without a trend). The ADF test in its original order is presented in Equation 1.

$$\Delta Y_t = \beta_1 + \beta_2 t + \delta Y_{t-1} + \sum_{i=1}^m \alpha_i \Delta Y_{t-i} + \varepsilon_t \quad (1)$$

Where: ε_t is a pure white noise error term; ΔY_{t-1} is the number of lagged difference terms that are included frequently.

In each of the ADF tests conducted in their original order, the Durbin-Watson statistic was first analyzed to ensure there were no autocorrelation issues. The Durbin-Watson statistic value needed to be above the point of significance (critical value) with an alpha of 5%, considering the respective values of k and n. Subsequently, the p values from the original order tests were analyzed. If the p value was greater than 0.05, the series had a unit root, meaning it was non-stationary; if it was less than 0.05, the series did not have a unit root, meaning it was stationary. Once it was determined that the variables analyzed were non-stationary in their original order, the order of integration was assessed.

To confirm that the analyzed variables were of integration order one, they needed to be stationary at the first difference. Thus, a second ADF test was conducted on all the variables, both with and without a trend, but this time using differences. Again, the Durbin-Watson statistic was analyzed to ensure there were no autocorrelation problems, followed by the analysis of the p values from these tests. This process involved performing 124 ADF tests, now with differences, to determine the order of integration. To verify that the variables analyzed were of integration order one, the series in the first difference had to be stationary, meaning that the p value of the ADF test needed to be less than 0.05.

If all the variables analyzed met the conditions of being non-stationary and of integration order one, the 62 cointegration tests were carried out. For this, the Eviews software was used. And following the methodology proposed by Gujarati and Porter (2010), and Wooldridge (2010), the augmented Engle-Granger (AEG) method was applied. This involved conducting 62 cointegration regressions using Eviews software, as presented in Equation 2.

$$Y_i = \beta_1 + \beta_2 X_{1i} + u_i \quad (2)$$

Where: Y_i =quantity produced from each state for a certain year i ; β_1 =intercept. β_2 =cointegration parameter; X_{1i} =real price for each of the 31 products for a certain year i ; u_i =estimated residuals from the cointegrating regression; u_i =year within the study period.

As mentioned by the authors, the residuals from the cointegrating regressions were obtained using Equation 2. Following this step, the AEG unit root test was applied to the 62 cointegration residuals to calculate the Engle-Granger tau statistic. This test is essential to determine whether the residuals are stationary and whether the variables are cointegrated. To do this, the p value of the Engle-Granger tau statistic is assessed. If the p value is less than 5%, it indicates that the cointegration residuals do not have a unit root and are therefore stationary, implying that the series are cointegrated in the long term. Conversely, if the p value of the Engle-Granger tau statistic is greater than 5%, it suggests that the cointegration residuals have unit roots and are non-stationary, indicating that the series are not cointegrated in the long term.

RESULTS AND DISCUSSION

The results of the 248 ADF unit root tests (conducted on the 62 variables analyzed from each of the 31 states, with and without trend in original order and with first differences) show no evidence of a positive serial correlation. In all cases, the value of the Durbin-Whatson statistic is above the critical point of significance (*i.e.*, the critical Durbin-Whatson value with its respective k and n values).

In the 124 ADF unit root tests conducted on the 62 variables analyzed, both with and without trend in original order, all p values are greater than 0.05, indicating that the series have a unit root. This means that the variables —real price and quantity produced— are non-stationary in their original order with an alpha of 5%. Similarly, in the 124 ADF unit root tests conducted on the 62 variables analyzed, both with and without trend and with first differences, the p values of all the variables are less than 0.05, indicating that the series do not have a unit root and are stationary. Therefore, the variables are of integration order one. Based on the above, Table 2 presents the outcome of the 62 AEG unit root tests applied to the 62 residuals of the cointegration regressions (with and without trend), corresponding to the quantity produced with the real price for each state.

As shown on Table 2, in fifteen of the 62 tests, the p values of the Engle-Granger tau statistic from the AEG test, applied to the residuals of the cointegration regressions, are less than 0.05. This indicates that, according to Gujarati and Porter (2010), and Wooldridge

Table 2. Test results of the 62 cointegration tests from the 31 States.

State	Cointegration test	P-value of the Engle-Granger tau statistic	Constant	Trend	Series are cointegrated
A	MFV price with MFV production in A with trend	0.002	0	0	Yes
	MFV price with MFV output at A without trend	0.943	0.012	-	No
BCS	SFV price with SFV production in BCS with trend	0	0	0	Yes
	SFV price with SFV production in BCS without trend	0.972	0.211	-	No
Ca	CA price with CA production in Ca with trend	0.949	0.715	0.013	No
	CA price with CA production in Ca without trend	0.757	0.131	-	No
Chiap	Price of CA with the production of CA in Chiap with trend	0.988	0	0	No
	CA price with CA production in Chiap without trend	0.518	0.004	-	No
Chih	AFV price with AFV production in Chih with trend	0.565	0.848	0.018	No
	AFV price with AFV production in Chih without trend	0.244	0	-	No
CDMX	Price of N with N production in CDMX with trend	0.981	0.088	0.056	No
	Price of N with N production in CDMX without trend	0.21	0	-	No
Co	AV price with AV production in Co with trend	0.366	0.006	0	No
	AV price with AV production in Co without trend	0.821	0.383	-	No
Col	CA price with CA production in Col with trend	0.509	0.097	0	No
	CA price with CA production in Col without trend	0.689	0.309	-	No
D	MFV price with MFV production in D with trend	0.970	0.977	0	No
	MFV price with MFV production in D without trend	0.959	0	-	No
EM	MG price with MG production in EM with trend	0.011	0	0.954	Yes
	MG price with MG production in EM without trend	0.002	0	-	Yes
Gua	AV price with AV production in Gua with trend	0.158	0	0	No
	AV price with AV production in Gua without trend	0.536	0	-	No
Gue	MG price with MG production in Gue with trend	0	0	0	Yes
	MG price with MG production in Gue without trend	0	0	-	Yes
H	AV price with AV production in H with trend	0.552	0.003	0	No
	AV price with AV production in H without trend	0.771	0	-	No
J	CA price with CA production in J with trend	0.007	0	0	Yes
	CA price with CA production in J without trend	0.384	0.165	-	No
Mich	MG price with MG production in Mich with trend	0	0.011	0	Yes
	MG price with MG production in Mich without trend	0.517	0	-	No
Mor	CA price with CA production in Mor with trend	0.726	0.002	0.239	No
	CA price with CA production in Mor without trend	0.565	0.006	-	No
N	CA price with CA production in N with trend	0.176	0	0	No
	CA price with CA production in N without trend	0.246	0	-	No
NL	N price with N production in NL with trend	0.183	0.118	0.079	No
	N price with N production in NL without trend	0.191	0	-	No
O	CA price with CA production in O with trend	0.207	0.002	0.553	No
	CA price with CA production in O without trend	0.082	0	-	No

Table 2. Continues.

State	Cointegration test	P-value of the Engle-Granger tau statistic	Constant	Trend	Series are cointegrated
P	CA price with CA production in P with trend	0.005	0	0	Yes
	CA price with CA production in P without trend	0.089	0	-	No
Que	MFV price with MFV production in Que with trend	0.803	0.967	0	No
	MFV price with MFV production in Que without trend	0.93	0.03	-	No
QR	CA price with CA production in QR with trend	0.009	0.003	0	Yes
	CA price with CA production in QR without trend	0.272	0.202	-	No
SLP	Price of CA with CA production in SLP with trend	0.007	0	0	Yes
	Price of CA with CA production in SLP without trend	0.063	0.221	-	No
Sin	Price of MG with MG production in Sin with trend	0.154	0.797	0	No
	Price of MG with MG production in Sin without trend	0.710	0	-	No
Son	Price of TG with TG production in Son with trend	0.041	0.061	0.001	Yes
	Price of TG with TG production in Son without trend	0.119	0	-	No
Tab	Price of CA with CA production in Tab with trend	0.186	0	0	No
	Price of CA with CA production in Tab without trend	0.489	0.321	-	No
Tam	Price of CA with CA production in Tam with trend	0.085	0	0.003	No
	Price of CA with CA production in Tam without trend	0.075	0.056	-	No
Tlax	Price of MG with MG production in Tlax with trend	0	0.264	0.001	Yes
	Price of MG with MG production in Tlax without trend	0	0	-	Yes
V	Price of CA with CA production in V with trend	0.002	0	0	Yes
	Price of CA with CA production in V without trend	0.625	0	-	No
Y	Price of N with N production in Y with trend	0.378	0	0.189	No
	Price of N with N production in Y without trend	0.223	0	-	No
Z	MFV price with MFV production in Z with trend	0.525	0.143	0	No
	MFV price with MFV production in Z without trend	0.715	0	-	No

Source: developed by the authors based on SADER (2022) data.

(2010), with an alpha of 5%, the variables do not have unit roots, so they are stationary, meaning they are cointegrated, both with and without trend. This implies a long-term relationship between the variables. These exceptions include the real price and production in the states of Aguascalientes, Baja California Sur, Jalisco, Michoacán, Puebla, Quintana Roo, San Luis Potosí, Sonora, and Veracruz (with trend), as well as the State of Mexico, Guerrero, and Tlaxcala (with and without trend). Additionally, in 47 of the 62 tests conducted, the p values are greater than 0.05, which implies that, with an alpha of 5%, the variables have a unit root and are therefore non-stationary, meaning they are not cointegrated, whether with or without trend. This indicates that there is no long-term relationship between the real price and production of the main agricultural product in each State.

The results of the cointegration tests suggest that, in most cases, the real price does not have a relationship with the quantity produced of the main agricultural product in each

Mexican state during the period from 1980 to 2021. In other words, the results indicate that, in general, the main agricultural product in each state does not correlate with its price. These findings contradict studies presented by various authors —Tonconi (2015), García (2020), Benítez (2022), OECD-FAO (2011), Brambila *et al.* (2014), Márquez *et al.* (2006), Cardona *et al.* (2007), Roitbarg (2021)— who argue that when prices rise, producers have incentives to increase production, and conversely, when prices fall, producers have incentives to decrease production.

Additionally, it is important to note that other research —such as that by De Grammont (2010) and Guzmán *et al.* (2012)— indicates that the relationship between price and production can be influenced by the type of producer, as producers with more economic and technological resources are less exposed to the effects of price decreases. The neoclassical microeconomic theory's supply and demand model also suggests that the price-production relationship may not materialize due to factors such as government intervention through subsidized programs. In Mexico, government programs like PROCAMPO may explain the lack of a relationship between price and production. Another reason for the lack of reaction in production to price changes could be that producers lack the economic and technological resources needed to take advantage of price increases (Guzmán *et al.*, 2012; Roitbarg, 2021; De Grammont, 2010; Guzmán *et al.*, 2012; Hernández *et al.*, 2022 a, 2022 b). It is also important to mention that the results of this research do not determine whether the support programs for the agricultural sector provided by the Mexican government distort the relationship between prices and production (Brambila *et al.*, 2014; Márquez *et al.*, 2006; Flores, 2014).

The implications of these results for policymakers designing government programs are that they must consider the impact of these programs on the relationship between production and product prices, as they can distort it. Furthermore, they must recognize that there are products in Mexico's agricultural sector that do not respond to price fluctuations, possibly due to the sector's infrastructure. Therefore, policies should focus on creating conditions that enable producers to better capitalize on price variations. On the other hand, producers should acknowledge that if their production does not respond to price fluctuations, they need to take measures to better position themselves to take advantage of these variations.

CONCLUSIONS

The results of the 62 cointegration tests conducted to determine the relationship between the quantity produced of the main agricultural product of each state and its price in Mexico indicate that, in most cases, there is no significant relationship. However, there were 15 exceptions where evidence of a relationship was found. These exceptions include the states of Aguascalientes, Baja California Sur, Jalisco, Michoacán, Puebla, Quintana Roo, San Luis Potosí, Sonora, and Veracruz with a trend, and State of Mexico, Guerrero, and Tlaxcala with and without trend.

This research does not allow us to pinpoint the specific factors that cause the price to have no relationship with production in these states. However, according to the literature review, this relationship could be influenced by the conditions of the producers, which

may prevent them from capitalizing on price variations, or by the government programs provided to the agricultural sector. These programs could potentially distort or even negate the relationship between price and production.

The research successfully achieved its objective, which was to determine the relationship between the quantity produced of the main agricultural product in each state and its price in Mexico. However, the hypothesis that there is a relationship between the quantity produced and its price is generally rejected, with the exception of the 15 cases noted earlier. Some limitations of the research include the fact that the conditions of the producers were not examined, and not all products by state were analyzed. As future lines of research, it is recommended to identify specific characteristics that cause the relationship between prices and production to be inconsistent.

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Agronomic response of experimental hybrids of poblano pepper under shade cloth in Coahuila, Mexico

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ABSTRACT

Objective: To evaluate the agronomic response of sixteen experimental hybrids of poblano pepper under shade cloth in Coahuila, Mexico.

Design/methodology/approach: The design of treatments and the statistical model was completely randomized with 16 treatments and four repetitions each, analyzed with an ANVA at $p \leq 0.05$ and Tukey's means test $p \leq 0.05$.

Results: In yield (kg plant^{-1}), the hybrids AN-103, AN-203, AN-204, AN-302, AN-401, AN-402 and AN-403 were superior; in average weight of the fruit the hybrids AN-203, AN-302, AN-402 and AN-304 stood out; the fruit length was greater in the hybrids AN-302, AN-304, AN-401, AN-402, AN-403, AN-404, AN-204 and AN-103; the number of fruits per plant was very similar in most of the hybrids; the hybrid AN-302 produced the widest fruits in its basal part near the peduncle, and it also stood out in the width at the center part of the fruit. The number of fruits per plant, average fruit weight and average fruit width are the attributes that contribute the most to yield, whose Pearson correlation is 0.74, 0.55 and 0.44.

Findings/Conclusions: The hybrids that showed a favorable agronomic response in most of the attributes evaluated were AN-401, AN-402, AN-203, AN-302. The agronomic response of the experimental hybrids was variable in most of the traits evaluated; therefore, the genetic potential of each of the hybrids is different and is reflected in their phenotypic response under the specific conditions of the environment in which they developed.

Keywords: *Capsicum annuum*, phenotype, attribute, quality, performance.

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INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the native species from Mexico, and Olvera *et al.* (1998) point out that there are records of its cultivation since the year 7,000



to 2,555 BC, primarily in the states of Puebla and Tamaulipas. The production of this crop is a fundamental part of the economy in the local, regional and national scope. In Mexico, in the agricultural year 2022, the planted surface of green chili pepper was 158,000 hectares, of which 16,700 hectares correspond to poblano type chili pepper (16,091 were grown in the open, 553 under greenhouse, and 46 under shade cloth), and average a yield of 25.03 tons per hectare. When it comes to dehydrated ancho chili pepper, the planted surface exceeds 15,000 hectares, and it is precisely its forms of trade that confer it versatility in the chili pepper market, whether in green fruit as “poblano chili pepper” or dehydrated as “ancho chili pepper”. The states that lead the production of poblano pepper are Zacatecas, Guanajuato and Sinaloa, and for ancho chili pepper they are Zacatecas and San Luis Potosí (SIAP, 2022). However, the use of criollo seed is still frequent, particularly with small-scale producers, which sometimes generates low yields due to inadequate selection and obtainment of seed (Marín *et al.*, 2013), and the problem represented by diseases, particularly in the soil (Pérez *et al.*, 2016; Macías *et al.*, 2009). There is also a lack of economic resources, high cost of inputs, and scarcity of water resources (Galindo *et al.*, 2002). In addition, changes in the agrometeorological conditions are favoring adverse situations, which induce problems in production, generating economic loss, primarily for small-scale farmers (Joukhadar and Walker, 2020). It is precisely the lack of adaptability of the varieties to such environmental changes that impact their productivity most (Kumari *et al.*, 2019); therefore, it is important to conserve and to improve the broad diversity and genetic variability of this crop (Pérez *et al.*, 2016), in order to foster its adaptation to adverse environmental and increasingly less predictable conditions (Aguilar *et al.*, 2010).

One of the alternatives to face the problems cited before is plant breeding, which has favored the generation of new varieties with higher yield, of better commercial and nutritional quality, greater resistance to adverse abiotic and biotic factors, and greater adaptation to different environmental conditions (Torres, 2018; Aguirre and Muñoz, 2015). Hybridation is the technique that allows the creation of improved hybrid varieties, with greater genetic potential in every sense (Acquaah, 2012), and which at the same time contribute to the sustainability of the agricultural and livestock systems (Camarena *et al.*, 2014). On the other hand, protected agriculture, which is defined as the type of agriculture where crops are protected under any type of cover with the aim of controlling radiation, temperature and relative moisture (SADER, 2020), has increased in Mexico at a rate of 14% annually since 2015 to date. Of the 51,852 protected hectares, 20,800 correspond to shade cloth, where tomatoes, chili peppers and cucumbers are primarily cultivated (SIAP, 2022); hence, the importance of understanding the agronomic behavior of new hybrids or varieties under specific conditions, mainly under protected environments (Maboko and Du Plooy, 2018).

The diversity of chili peppers found in the country (Mercer and Perales, 2010) represents an area of opportunity for the improvement of their agronomic traits through geneticist methods (Toledo *et al.*, 2016). In addition, the growth rate in protected agriculture sets the standards, and therefore, the objective of this study was to assess the agronomic behavior of experimental hybrids of poblano pepper under conditions of shade cloth in Coahuila.

MATERIALS AND METHODS

Location of the experiment

The study was conducted under shade cloth located in the “El Bajío” experimental field of Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, Mexico, located on 25° 21’ 24” LN and 101° 02’ 05” LW, at 1762 masl. The mean annual precipitation is 400 mm and the mean annual temperature 18 to 22 °C. The field work was carried out in the period of May to December in 2021.

Plant material

Sixteen experimental hybrids of poblano pepper were used, developed in the Centro de Capacitación de Desarrollo en Tecnología de Semillas del Departamento de Fitomejoramiento (Table 1), of Universidad Autónoma Agraria Antonio Narro.

Seedbed stage

Seeds from the hybrids were planted in polystyrene trays of 200 cavities, and the germination substrate consisted of peat moss and perlite in a proportion of 70:30%, respectively. A commercial formula of 20-30-10 added with microelements was used for seedling nutrition, 0.5 g L⁻¹ five days after the emergence, 0.75 and 1 g L⁻¹ at 15 and 30 days after the corresponding emergence until before the transplant.

Establishment in the field and crop management

The transplant was conducted 64 days after having sown the seeds, and it was carried out in a loam soil with the characteristics described in Table 2. It was during the 2022 fall-winter cycle, the cultivation beds were 25 cm high, the distance between the beds was 1.80 m, and the distance between plants was 30 cm in a single row.

Irrigation was carried out through a dripping irrigation system, which consists in an irrigation strip with caliber 6000 Aquatrax[®], with droppers at 20 cm and expenditure of 0.75 liters per hour; the soil was supplied with daily irrigation until reaching the field capacity, the irrigation water quality is described in Table 3. The total nutrition applied via fertirrigation during the cultivation cycle was 170-80-270 of N-P₂O₅-K₂O.

Table 1. Plant material of the study evaluated under conditions of shade cloth in Coahuila, Mexico.

Identification code	Origin	Identification code	Origin
AN-102	Coahuila	AN-301	Coahuila
AN-103	Coahuila	AN-302	Coahuila
AN-104	Coahuila	AN-304	Coahuila
AN-101	Coahuila	AN-303	Coahuila
AN-201	Coahuila	AN-401	Coahuila
AN-203	Coahuila	AN-402	Coahuila
AN-204	Coahuila	AN-403	Coahuila
AN-202	Coahuila	AN-404	Coahuila

Table 2. Physicochemical characteristics of the analysis of soil fertility, where experimental hybrids of poblano pepper were established under shade cloth.

Physical-chemical characteristics of the soil							
Textural class	Apparent density (g.cm ⁻³)	pH (1:2 water) alkaline	Total carbonates (%)	Salinity (CE extract 1:2 water) Ds/m	SP (%)	FC (%)	PWP (%)
Loam	1.25	8.61	8.25	1.1	40	21.3	12.7
Macronutrients in parts per million (ppm)							
N - NO ₃ ⁻	P-Olsen	S	Cl	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺
39.7	65	55.9	ND	658	3995	321	106
Micronutrients in parts per million (ppm)							
Fe ³⁺	Mn ²⁺	B ³⁺	Zn ²⁺	Cu ⁺	Mo ²⁺		
2.07	3.11	1.31	4.95	0.51	ND		

SP=Saturation point, FC=Field capacity, PWP=Permanent wilting point, pH=Hydrogen potential, ND=Not determined.

Table 3. Characteristics of irrigation water that was used for water and nutrient supply to the crop.

Macronutrients in Milliequivalents L ⁻¹								
NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	Cl ⁻	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	HCO ₃ ³⁻
0.41	ND	1.61	2.2	0.1	5.57	2.42	3.22	7.56
Micronutrients in parts per million (ppm)								
Fe ³⁺	Mn ²⁺	B ³⁺	Zn ²⁺	Cu ⁺	Mo ²⁺	pH	CE (Ds/m)	RAS
0.0118	0.0047	0.4	0.0891	0.0122	ND	7.5	1.15	1.61

pH=Hydrogen potential, EC=Electric conductivity, SAR=Sodium absorption rate.

The poblano pepper plants were not pruned, so they required support during their growth to avoid breaking of the branches from the weight of fruits; the tutor used to keep the plants upright was the Spanish or “strapped” type, the distance between logs was five meters, and four tutoring actions were carried out throughout the cultivation cycle.

For pest control (white fly, trips, paratriosis), weekly applications of Spirotetramat at 15.3%, Spiromesifen at 23.1% and Imidacloprid 17%+betacyflutrin 12% at a rate of 1 ml L⁻¹ were made. The weeds were eliminated with mattock and manually every 15 days.

Quantification of the response variables evaluated

Only one harvest was conducted in the cultivation cycle, and it was 90 days after the transplant, which took place on August 20, 2022, and it was conducted manually at the time when the fruit reached its commercial maturity. The yield (g plant⁻¹) resulted from adding the weight of the fruits from each plant, which were weighed in a digital precision scale Sartorius (TS 1352Q37, Gottingen, Germany). After weighing the fruits, the number of fruits from each plant (NFP) was counted, while the average fruit weight (AFW) was calculated by dividing the total weight of fruits by the total number of fruits from each plant; meanwhile, the base width, medium width, and fruit length (BW, MW and FL) were estimated taking eight fruits randomly per repetition, and a Truper[®] brand digital Vernier (CALDI-6MP, Atlacomulco, Mexico) was used. The plant height was

determined with a Truper[®] brand flexometer calibrated in centimeters (PRO-5MEC Atlacomulco, Mexico), while the stem thickness was determined at the time of the harvest in four plants per repetition with a Truper[®] brand digital Vernier (CALDI-6MP, Atlacomulco, Mexico), the calyx depth, length of the peduncle, and thickness of the mesocarp (in mid-cut of the fruit), were determined in the same fruits that were used to quantify BW, MW and FL, with a Truper[®] brand digital Vernier (CALDI-6MP, Atlacomulco, Mexico).

Description of treatments and statistical analyses

Sixteen experimental hybrids of poblano pepper were assessed under a completely randomized experimental arrangement and since all the treatments were under shade cloth in an area of 450 m², whose soil analysis was described in Table 2, it allowed minimizing the environmental variability. Meanwhile, the statistical analysis was carried out with the INFOSTAT 2019 software; first the normality test was conducted, so it was not necessary to perform data transformation, and their analysis was carried out with the completely random linear statistical model with 16 treatments and four repetitions each (ANVA $p \leq 0.05$), each repetition with four measurable and quantifiable useful plants. For the means comparison, Tukey's test was conducted (Tukey $p \leq 0.05$). The correlation analysis was carried out with Pearson's methodology. The data obtained were analyzed with the Infostat[®] version 2019 statistical software.

RESULTS AND DISCUSSION

According to Tukey's means test $p \leq 0.05$, in the variable of yield in kilograms per plant, it was observed that the hybrids AN-103, AN-203, AN-204, AN-302, AN-401, AN-402 and AN-403 are statistical equal, although superior to the rest of the tested hybrids; however, among them the AN-402 stands out; while the hybrids with lowest yield were AN-303, AN-104 and AN-301, the rest of the hybrids kept a range of yield between 0.35 and 0.45 kg plant⁻¹ (Figure 1A). The differential statistical response observed in the variable of number of fruits per plant indicates that most of the hybrids produced a very similar number of fruits. Except for the hybrids AN-104, AN-301, AN-304, AN-303 and AN-404, all of them were surpassed by more than 65% by the hybrid AN-401 (Figure 1B), which stood out among those of best response under the conditions of assessment under shade cloth. In the average fruit weight, significant statistical responses were observed between the hybrids tested, and the hybrid AN-203 stands out for this variable, followed by AN-302, AN-402 and AN-304, although the hybrids AN-103, AN-201, AN-204, AN-401, AN-401 and AN-403 are also in the same statistical group; in addition, the hybrid AN-203 exceeded by more than 35% the hybrids that statistically produced the fruits with least mean weight (Figure 1C).

According to Tukey's means test $p \leq 0.05$, significant statistical differences were observed in the variable of fruit length, where the hybrids that produced the shortest fruits were AN-102, AN-101, AN-201, AN-203, AN-202, AN-301 and AN-303; the rest of the hybrids showed a similar statistical response, although higher than those mentioned before (Figure 1D). It is important to point out that the fruit length is of utmost importance,

since this variable is defining for the destination market and generally fruits of 17-18 cm are preferred, ranges where most of the hybrids tested were found. On the other hand, in the variable of basal width of the fruit, statistical differences were observed according to Tukey's means test $p \leq 0.05$, where the hybrid AN-302 produced the widest fruits in their basal part close to the peduncle; while the fruits of less width in the base were produced by the hybrid AN-404, in the rest of the hybrids a trend was observed of intermediate response among the ones mentioned before (Figure 1E), although the hybrid AN-302 exceeded by more than 10% all the hybrids tested. In the variable of width of the central part of the fruit, significant statistical differences were also detected according to Tukey's mean test $p \leq 0.05$, where similar to the width of the base, the hybrid AN-302 produced the widest fruits at the center part, although in the same statistical group there were the hybrids AN-103, AN-104, AN-204, AN-202, AN-302, AN-304, AN-401, AN-402 and AN-403; meanwhile, the fruits of less width at center were produced by the hybrids AN-404, AN-301, AN-101 and AN-102 (Figure 1F). The differential statistical response observed between the hybrids, in the yield variables and components, is attributed to the genetics of each hybrid, in response to the combination of two different parents (Pech *et al.*, 2010), since when they are cultivated in the same site and under the same conditions of shade cloth, the uniformity of the assessment environment is assumed except for the soil, and what is expressed is the genetic differences of each material (Rangnamei *et al.*, 2017). This translates into its phenotypic performance in response to the environmental condition under which they develop (Loatournerie *et al.*, 2015; Maboko and Du Plooy, 2018), allowing to clearly identify the hybrids of greatest potential for those response variables. Combined with adequate management practices under conditions of shade cloth, they could increase their productive potential and the cultivation cycle could be prolonged allowing more trade windows; however, the yield produced by each plant is of greater importance when new materials, hybrids or varieties are tested (Echandi, 2005). In addition, the yield is a good indication of the genetic potential of a hybrid, since such a variable is related with the response of tolerance of plants to adverse factors of biotic and abiotic type and in the best of cases to new agroclimatic conditions or specific environments (Zewdie and Bosland, 2000). Although the new perspectives indicate that the generation of hybrids is accelerated and could be more accurate when combining traditional techniques of genetic improvement with the biotechnology of molecular markers (Nar, 2023), it is the presence of the consumer which in the end determines the genetic material that is produced and traded (Goldman *et al.*, 2023). Outstanding experimental hybrids ought to be tested in different producing areas of the country, with the aim of corroborating their stability, productivity and quality (Zewdie and Bosland, 2000), for the farmers to validate such results and so that they can be eventually produced and traded in those regions.

In the trait of calyx depth, differential statistical responses between the hybrids tested were observed, where excepting the hybrids AN-101, AN-202, AN-302, AN-303, and AN-404, the other hybrids showed a statistically similar behavior for that variable (Figure 2A), where AN-201 surpassed by up to 45% the hybrids that produced the fruits with lowest calyx depth. This response variable is of great importance, since the calyx depth

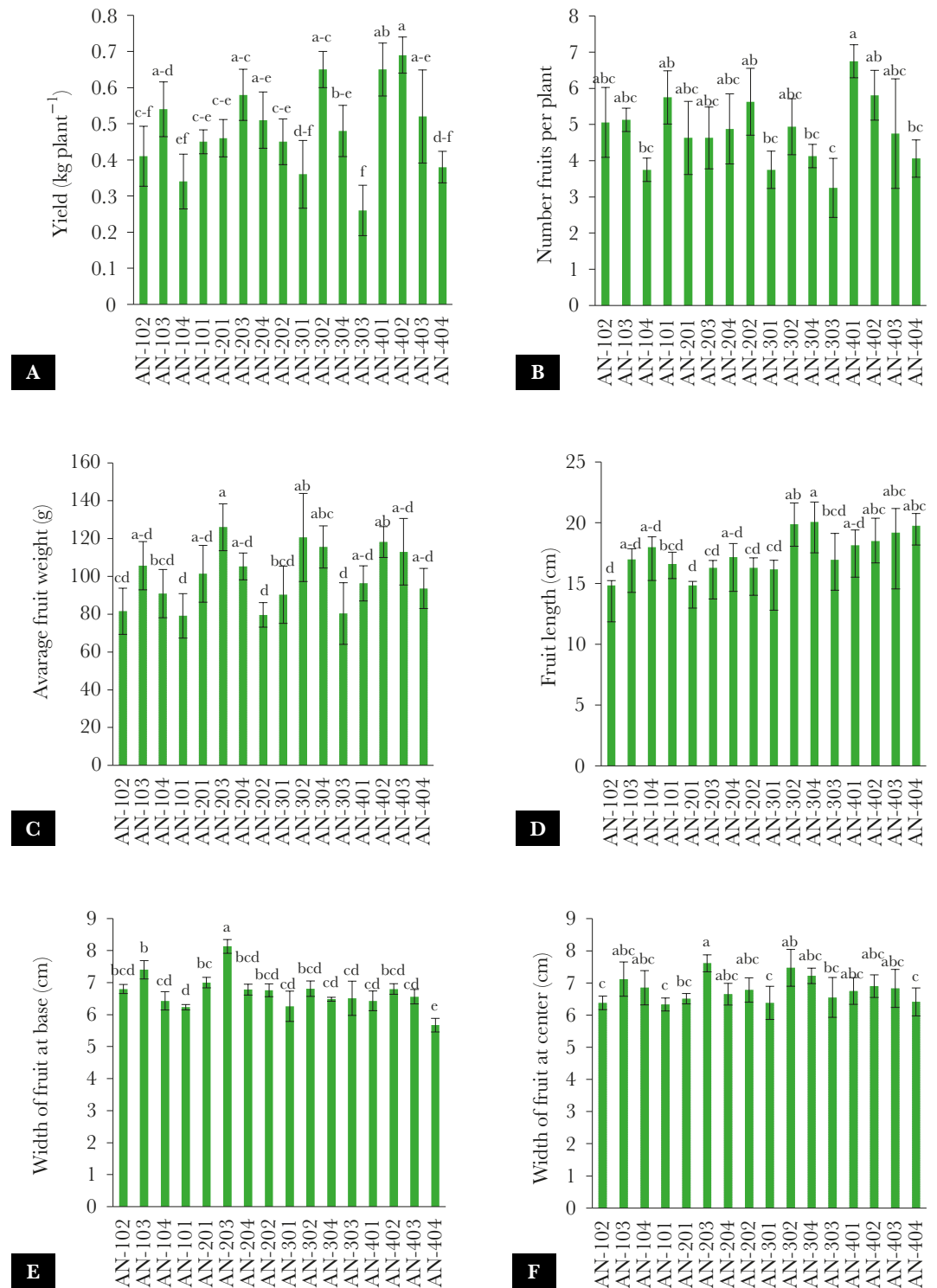


Figure 1. Response of the experimental hybrids of poblano pepper in the yield (A), number of fruits per plant (B), average weight of the fruit (C), fruit length (D), width of the fruit at base (E), and width of the fruit at center (F). ANOVA $p \leq 0.05$, Tukey's means test ($p \leq 0.05$), vertical bars corresponded to standard deviation.

is generally associated with a better fruit quality; however, a greater calyx depth is directly related with the proliferation of pathogens in zones of high frequency of precipitation, induced by the water accumulation in that structure. In length of fruit peduncle, statistical differences were also detected according to Tukey's means test $p \leq 0.05$, where except for the hybrids AN-202, AN-201, AN-102 and AN-204, the rest of the hybrids showed a statistically similar behavior although higher than the ones cited before (Figure 2B). Regarding this variable, it is important to mention that as the fruit length peduncle increases the harvesting tasks are eased, although it is also associated with the fall of fruits when frequent tasks are carried out in the crop, particularly tutoring. The differential statistical response (Tukey $p \leq 0.05$), observed in the variable final height of the plant

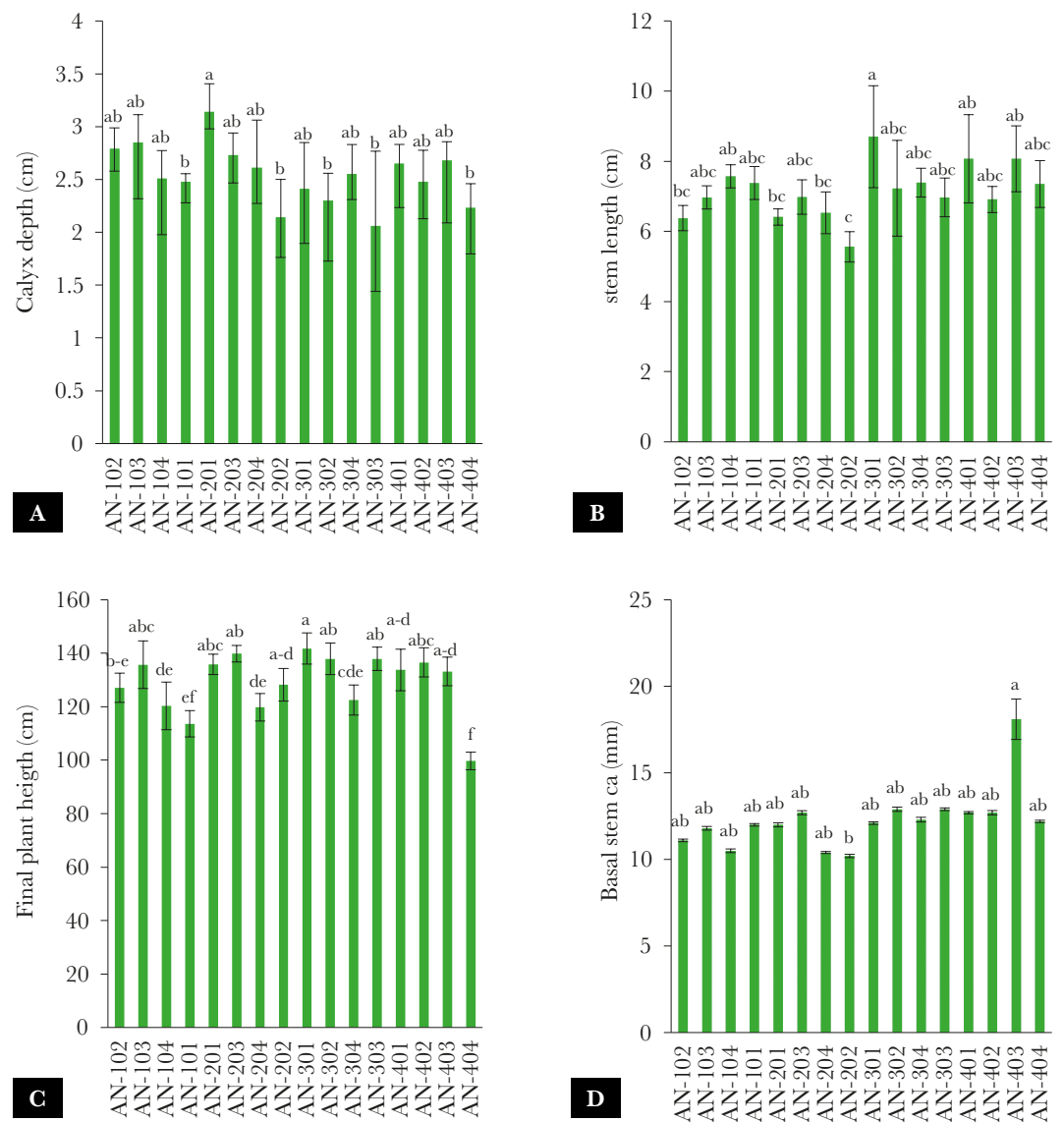


Figure 2. Response of the experimental hybrids of poblano pepper in the calyx depth (A), length of the peduncle (B), final height of the plant (C), basal stem thickness (D). ANVA $p \leq 0.05$, Tukey's means test ($p \leq 0.05$), vertical bars correspond to standard deviation.

indicates that the hybrids behaved differently under the conditions tested, with the hybrids AN-301, AN-302, AN-303, AN-401, AN-402, AN-403, AN-201, AN-203 and AN-103 being higher than the rest, while the hybrid of lowest final height of the plant was the hybrid AN-404. Likewise, the differential statistical response (Tukey $p \leq 0.05$), showed by the variable of basal stem thickness, indicates that with the exception of the hybrid AN-202, all the hybrids showed a similar response under to conditions tested, although the hybrid that stands out among all of them is AN-403. The plant height and the stem thickness are two indicators of the plant vigor, and they are generally associated with the final productivity, so it is important to consider them at the time of choosing a hybrid for commercial production.

Table 4 shows, from Pearson's coefficients of correlation, that the number of fruits per plant, average weight of the fruit and width at center of the fruit, are the traits that influence most in a positive way in the yield of poblano pepper with correlations of 0.74, 0.55 and 0.44, respectively, and similar trends are reported by Parvin and Arifuzzaman (2023). Therefore, these traits must be considered by farmers at the time of choosing or selecting new varieties or hybrids for their agricultural areas, although the stem thickness and the plant height also collaborate with 0.25 and 0.24. The width at center of the fruit, fruit length, and basal width of the fruit are the variables that most contributed in the accumulation of average weight of the fruit with 0.63, 0.49 and 0.44, respectively; without a doubt, these are parameters to take seriously into consideration when new hybrids or varieties are selected. The basal width of the fruit is related positively with the plant height with 0.53 of positive correlation; that is, with a greater plant height the fruit is wider, or at least in the hybrids and the conditions tested. The fruit length is negatively correlated with the basal width of the fruit with -0.29 , which indicates that as the fruit becomes longer its width decreases. The stem thickness is associated with the length of the peduncle with 0.35 of positive correlation.

Table 4. Pearson's coefficient of correlation of variables evaluated in poblano pepper grown under shade cloth.

	Yield	NFP	AFW	FL	WBF	ACF	CD	PL	FHP
NFP	0.74**								
AFW	0.55**	-0.13							
FL	0.25	-0.11	0.49**						
WBF	0.36**	0.09	0.44**	-0.29*					
WCF	0.44**	0.03	0.63**	0.39**	0.62*				
CD	0.20	0.09	0.20	-0.2	0.35*	0.08			
PL	0.04	-0.09	0.17	0.21	-0.13	0.08	0.11		
FPH	0.24*	0.04	0.25*	-0.16	0.53*	0.25*	0.12	0.09	
TBS	0.26*	0.16	0.15	0.18	-0.02	0.10	-0.01	0.35*	0.19

*=significant ≤ 0.05 , **=highly significant $p \leq 0.01$. Yield=Yield, NFP=Number of fruits per plant, AFW=Average fruit weight, FL=Fruit length, WBF=Width at base of the fruit, WCF=Width at center of the fruit, CD=Calyx depth, PL=Peduncle length, FPH=Final plant height, and TBS=Thickness of basal stem.

CONCLUSIONS

The agronomic response of the experimental hybrids tested under shade cloth was variable in most of the variables evaluated, so a different genetic potential is inferred in each one of them, in response to the conditions of shade cloth under which they developed. The hybrids that showed a favorable agronomic response in most of the attributes evaluated were AN-401, AN-402, AN-203, AN-302.

In poblano pepper, the variables that most contributed to the yield are the number of fruits per plant, average weight of the fruit, width of the fruits at the base and the center, and to a lesser extent the stem thickness and plant height.

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Yield of Five Alfalfa Varieties (*Medicago sativa* L.) Under Two Cutting Frequencies

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ABSTRACT

Objective: To determine the yield and morphological composition of five alfalfa varieties under two defoliation frequencies during the spring-summer period.

Design/Methodology/Approach: A randomized block design was employed, following a 5×2×2 factorial arrangements. The factors considered were five alfalfa varieties, two cutting frequencies (CF), and two seasons of the year, resulting in a total of 20 treatments with three replicates. The evaluated variables included dry matter yield (DMY), plant height, leaf-to-stem ratio (L:S), leaf area index (LAI), and morphological composition (MC). A factorial analysis was conducted using the FACTORIAL ANOVA and PROC GLM procedures, along with a mean sensitivity test via Tukey ($\alpha=0.05$), utilizing the SAS statistical software.

Results: The San Miguel variety exhibited the highest DMY (2,150 kg DM ha⁻¹) and plant height (31 cm). A light CF (35 days) resulted in higher yield (1,256 kg DM ha⁻¹), greater height (29 cm), and a higher LAI (3.3), while also favoring the L:S ratio (1.07). Summer yielded higher production (2,280 kg DM ha⁻¹), whereas spring showed a higher leaf proportion (1.05). Regardless of variety and season, MC displayed a greater percentage of leaves and stems under a light cutting frequency.

Study Limitations/Implications: No limitations were identified; however, further evaluations are needed during the autumn-winter period.

Findings/Conclusions: Cutting frequency was the determining factor influencing the evaluated variables. Its interaction with the season largely defined the proportion of morphological components and overall pasture quality.

Keywords: *Medicago sativa*, dry matter, temperature, defoliation, cultivar.

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most widely used legume for the production of plant-based protein in forage crops, making it the most important forage in agricultural production systems. It is also the most utilized crop for biomass production due to its dry matter yield and nutritional quality (Rojas-García et al., 2017; Rivas et al., 2020). The time required for a cultivated species to recover the harvested biomass, whether through animal or mechanical defoliation known as the defoliation interval, recovery period, or regrowth pattern is influenced by multiple environmental factors. Among these, ambient

temperature has been identified as the primary determinant of a plant's photosynthetic capacity and, consequently, its yield (Montes *et al.*, 2016; Gaytán *et al.*, 2019). In addition to these findings, other studies indicate that during spring and summer, climatic conditions optimally stimulate the growth and development of alfalfa plants, leading to greater biomass accumulation, increased height, a higher leaf area index (LAI), and greater stem density per tiller, all of which determine overall yield (Teixeira *et al.*, 2008; Álvarez-Vázquez *et al.*, 2023). Therefore, understanding the seasonal productive behavior and regrowth patterns of different alfalfa varieties cultivated in the region is essential for establishing optimal defoliation frequencies. This, in turn, enables more efficient forage production depending on the cultivated varieties (Rivas *et al.*, 2020). Several studies have reported that cutting frequencies of every four to five weeks during spring and summer stimulate the formation of new stems and leaf tissue, which enhances yield, persistence, quality, and overall productivity of alfalfa pastures (Montes *et al.*, 2016; Gaytán *et al.*, 2019; Álvarez-Vázquez *et al.*, 2023). The aim of this study was to evaluate the productive performance of five alfalfa varieties under different cutting frequency schemes during the spring-summer period. The research hypothesis proposed that the San Miguel and Oaxaca alfalfa varieties would exhibit higher yield under severe cutting frequencies during spring.

MATERIALS AND METHODS

Study area

The study was conducted during the spring-summer season of 2023 at the Instituto Tecnológico del Valle de Oaxaca (ITVO) in Xoxocotlán, Oaxaca (17° 01' 20.40" N, 96° 44' 51.50" W) at an altitude of 1,530 meters. The region has a warm semi-arid climate with summer rainfall (García, 2004). The soil exhibited a sandy loam texture with a pH of 7.5 and an organic matter content of 1.3%. Temperature and precipitation data (Figure 1) were obtained from the POWER Data Access Viewer database (NASA, 2024).

Establishment and experimental design

The establishment of the pastures took place in December 2022, using viable pure seed with an average germination rate of 92% and a seeding density of 30 kg ha⁻¹. Each

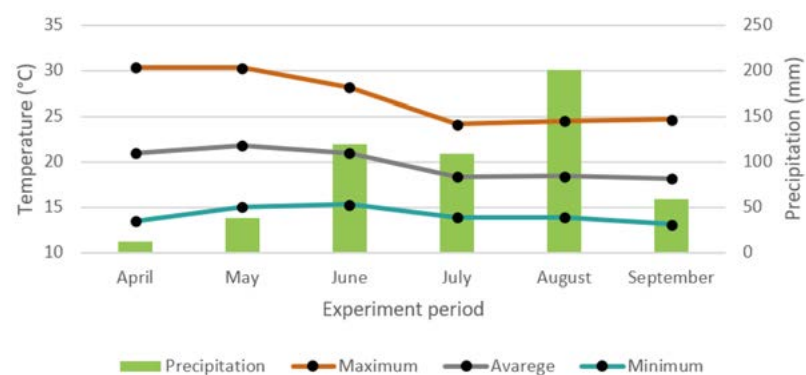


Figure 1. Maximum, average, and minimum temperature, along with monthly accumulated precipitation during spring and summer of 2023.

plot measured 42 m² per variety. Irrigation was applied every 15 days, and no fertilizers or agrochemicals were used for pest or weed control. A randomized block design was employed, following a 5×2×2 factorial arrangement. The factors considered were alfalfa varieties (Aragón, Atlixco, San Miguel, Victoria, and Oaxaca), two cutting frequencies (severe: every four weeks; light: every five weeks), and two seasons (spring and summer), resulting in a total of 20 treatments with three replicates.

In March, a uniformity cut was performed to minimize the residual forage covariate effect. Subsequent cuts were adjusted according to the designated cutting frequency, maintaining a residual height of 5 cm above ground level.

Variables evaluated

Height

Before each defoliation, the height of 20 plants was recorded using a measuring tape placed parallel to the plant, measuring from its base at ground level to the uppermost young vegetative tissue.

Dry matter yield (DMY)

The fixed-frame method was used, in which all the forage within a 0.25 m² square was harvested. The forage was then washed and weighed fresh using a scale (Model B-15, Brand: IBN). A 100 g subsample was taken and placed in a forced air drying oven (Model H-B2, Brand: Riossa) at 55 °C until a constant weight was reached to determine moisture content.

Leaf-to-stem ratio (L:S)

The leaf-to-stem ratio was estimated by dividing the dry weight of the leaves by the dry weight of the stems from the 100 g subsample.

Leaf area index (LAI)

Approximately 10% of the subsample weight was used. Leaf area was determined using the ImageJ[®] software, and with the obtained leaf area data and the sampled surface, the leaf area index (LAI) was estimated.

Morphological composition (MC)

A second 100 g subsample was separated and weighed by morphological components (leaf, stem, inflorescence, and dead material). Each component was placed in separate paper bags for subsequent drying.

Statistical analysis

The data obtained for the evaluated variables were grouped by cutting frequency and season. They were analyzed using Bartlett's test, considering that the data followed a normal distribution, with a significance level greater than $P < 0.05$. This confirmed the normal distribution of the data, eliminating the need for transformation. Therefore, the

data were used for mean analysis (Tukey, $P < 0.05$), main effects, and interactions through the Factorial ANOVA and GLM procedures in SAS 9.0 for Windows.

RESULTS AND DISCUSSION

Forage yield

The DMY values (Table 1) indicate that the San Miguel variety outperformed Victoria by 15% ($P < 0.05$), while no significant differences were observed among the remaining varieties ($P > 0.05$). A light cutting frequency (CF) produced 8% more forage than a severe cut, and summer accumulated 23% more forage than spring. Studies have shown contrasting responses among varieties. Rojas-García *et al.* (2017) and Álvarez-Vázquez *et al.* (2018) reported San Miguel as the highest and lowest-yielding variety in their respective studies. Regarding cutting intervals, the results obtained with a light CF contrast with those of Álvarez-Vázquez *et al.* (2023), who found no significant differences ($P > 0.05$) between harvesting every four or five weeks in both seasons. Conversely, Gaytán *et al.* (2019) demonstrated that cutting alfalfa every four weeks resulted in higher DMY, regardless of pasture establishment age. The light CF allowed for a longer recovery period between cuts, optimizing radiation and temperature utilization by the canopy in both seasons (Teixeira *et al.*, 2008; Hernández *et al.*, 2012). The higher summer yield aligns with findings reported by Álvarez-Vázquez *et al.* (2018), Rojas *et al.* (2019), and Rivas *et al.* (2020).

These yields may be due to the fact that during this period there are rains and warm temperatures that favor the optimal development of alfalfa (Teixeira *et al.*, 2008).

Height

La The San Miguel variety exhibited the greatest height (31 cm) among the evaluated varieties ($P < 0.05$), with a 6 cm difference compared to Victoria, which had the shortest

Table 1. Forage dry matter yield, growth rate, height, leaf-to-stem ratio, and leaf area index under different varieties, cutting frequencies, and seasons.

Factor		DMY kg MS ha ⁻¹	Height (cm)	Leaf-to-stem ratio (L:S)	LAI
Variety	Aragón	2126 ab	28 b	0.99 a	3.3 a
	Atlixco	2114 ab	28 b	1.03 a	3.2 a
	San Miguel	2150 a	31 a	1.01 a	3.2 a
	Victoria	1874 b	25 c	1.00 a	3.0 a
	Oaxaca	2100 ab	28 b	1.04 a	3.1 a
	SE	94	0.7	0.02	0.10
Frequency	Severe	1989 b	27 b	1.07 a	3.0 b
	Mild	2156 a	29 a	0.96 b	3.3 a
	SE	59	0.4	0.01	0.06
Season	Spring	1865 b	28 a	1.05 a	3.2 a
	Summer	2280 a	28 a	0.98 b	3.1 a
	SE	59	0.4	0.01	0.06

Means with the same letter in each column do not present statistical differences ($P > 0.05$); *=($P < 0.05$), **=($P < 0.01$); NS=($P > 0.05$); DM=dry matter, LAI=leaf area index.

stature. However, no significant differences were observed among the remaining varieties ($P > 0.05$). Allowing one additional week of growth resulted in a 2 cm increase compared to harvesting every four weeks ($P < 0.05$). Seasonal variation did not significantly affect this variable ($P > 0.05$). Sánchez *et al.* (2019) also documented slight differences in plant height among evaluated varieties. Regarding cutting frequency (CF), the results contrast with those reported by Gaytán *et al.* (2019), who recorded plant heights of 36 cm with four-week cutting intervals, as well as with Montes *et al.* (2016), who reported differences between cutting frequencies in spring. The height differences among varieties can be attributed to their genetic characteristics and environmental response (Sánchez *et al.*, 2019). The greater height observed with light cuts corresponded to harvesting plants at a more advanced phenological stage. Meanwhile, the minimal height variation between seasons could be due to an average temperature difference of only 3 °C between them (Figure 1). Under optimal conditions, growth remained constant, with temperature being the most influential environmental factor affecting this variable (Teixeira *et al.*, 2008).

Leaf-to-stem ratio (L:S)

No significant differences were found among the varieties ($P > 0.05$). However, harvesting every four weeks and during the spring season resulted in a higher leaf-to-stem ratio (Table 1). Regarding cutting frequency (CF), these results contrast with those of Gaytán *et al.* (2019), who, using the Oaxaca variety, found no statistical differences between four- and five-week cutting intervals. However, their reported L:S values exceeded 2. Similarly, the findings align with those of Rojas-García *et al.* (2017), who observed no variations among varieties but did find seasonal differences under a four-week cutting frequency, though their reported values were lower than those in this study. Conversely, these results differ from Álvarez-Vázquez *et al.* (2018), who reported significant differences among varieties with four-week cuts but no seasonal differences. In contrast, Rivas *et al.* (2020) found significant differences both among varieties and between seasons, suggesting that this variable is strongly influenced by these two factors. Cutting every four weeks (> 1) led to a higher leaf proportion due to the plant's age at the time of harvest. This proportion directly impacts the forage's nutritional value, which is correlated with the L:S ratio (Montes *et al.*, 2016; Gaytán *et al.*, 2019). The greater leaf presence in spring can be attributed to radiation and temperature, which enhance photosynthetic rates and accelerate leaf tissue turnover (Teixeira *et al.*, 2008).

Leaf area index (LAI)

No significant differences were observed among varieties or between seasons ($P > 0.05$). However, implementing a light cutting frequency increased solar capture by alfalfa leaves by 10% ($P < 0.05$). These results differ from those reported by Sánchez *et al.* (2019), where summer exhibited the highest leaf area per stem. Extending the cutting interval to five weeks increased the LAI, allowing the canopy to capture more light, thereby enhancing regrowth capacity and yield (Teixeira *et al.*, 2008; Rivas *et al.*, 2020).

Morphological composition (MC)

The Figure 2 illustrates the proportion of leaves, stems, and senescent material by variety under two cutting frequencies across the evaluated seasons. Among the varieties, Oaxaca, Atlixco, and San Miguel exhibited a higher average percentage of leaves under both cutting frequencies and seasons. With a light cut (Figure 3B), the leaf percentage was significantly higher regardless of variety or season ($P < 0.05$). The percentage of stems was greater in summer ($P < 0.05$), while senescent material increased in spring and under a severe cutting frequency ($P < 0.05$). A higher leaf presence is commonly associated with four-week cutting intervals (Álvarez-Vázquez *et al.*, 2018). However, these results contrast with previous findings, as a five-week cutting interval allowed the varieties to recover more effectively, facilitating efficient mobilization of stored nitrogen and carbohydrates from the crown and roots (Teixeira *et al.*, 2008). No significant seasonal differences were observed in leaf proportion, consistent with the findings of Rojas *et al.* (2019).

Stem presence was higher in summer and under a light cutting frequency, which was reflected in the DMY. As cutting frequency increased, the stem percentage in the morphological composition also increased. Although the Oaxaca variety is considered the local landrace of the region, San Miguel demonstrated superior parameters in this experiment. Therefore, it could serve as a viable production alternative for farmers. This finding supports the need for further evaluation of this variety on a larger cultivation scale, allowing for direct comparisons with the local landrace in terms of actual production.

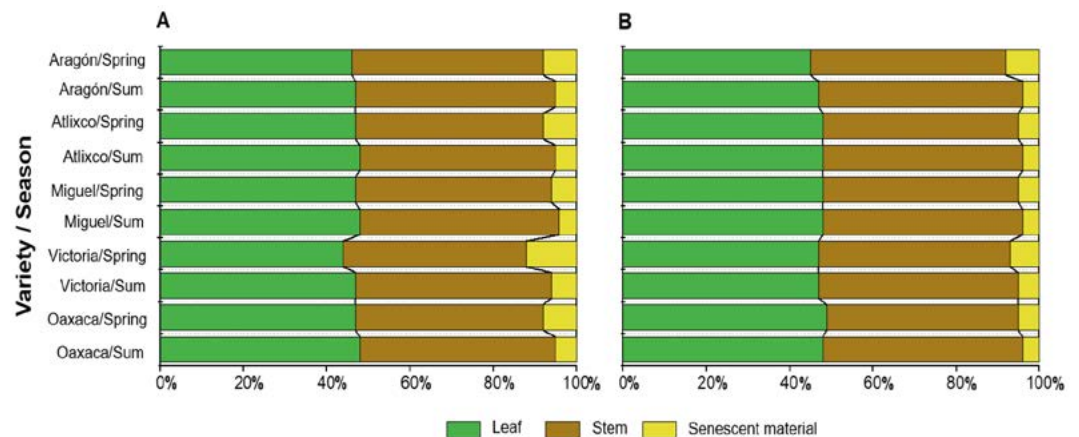


Figure 2. Changes in the morphological composition (%) of five alfalfa varieties. A=Severe CF, B=Light CF. Spr=Spring, Sum=Summer.

CONCLUSIONS

Cutting frequency was the key factor influencing the behavior of the studied variables. Its interaction with the season significantly affected the proportion of morphological components and overall pasture quality. The differences among varieties were attributed to their response to the site's edaphoclimatic conditions.

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Effects of juice of ten variants of prickly pear (*Opuntia* spp.) on the energy metabolism of Wistar rats with induced diabetes

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ABSTRACT

The effects of juice of 10 variants of prickly pear fruit on blood sugar level and related metabolic parameters were evaluated on glycostatic (GS) and hyperglycemic (HG) Wistar rats. The selection of variants was based on their abundance and the economic potential of their fruit in the state of San Luis Potosi, their humanization degree, and fruit colors. The experimental design used was completely randomized with an 11×2 factorial arrangement, where factor A was the juice of the pear variant (10 variants and water for control), and factor B was the health condition of the rats (GS and HG). In most of the treatments there was an increase in weight, except for GS animals which received juice from Tapón rojo variant. HG rats which received water showed higher plasma levels of glucose ($p < 0.05$) than those which received juice from the variant Sangre de toro, as well as all GS rats. Although both wild and cultivated prickly pears are seasonal fruits, they have a wide variety of colors and contain different compounds that, after regular consumption, benefit glucose and lipid metabolism. Prickly pear juices significantly modified the concentration of glucose in the blood of Wistar HG and GS rats.

Keywords: *Opuntia* spp., glucosa, diabetes, Wistar rats.

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INTRODUCTION

The epidemic of diabetes mellitus (DM) is recognized as a global threat by the World Health Organization (WHO). It is estimated that worldwide 180 million people suffer from diabetes, and that this figure will be more than double by 2030 (Shaw *et al.*, 2010; WHO, 2018; IDF, 2023). In 2005, 1.1 million deaths were recorded due to diabetes, of which around 80% occurred in low-to-medium income nations, the majority of which are less prepared to face this epidemic (NOM-015, 2010). In Mexico, DM takes first place in



the number of deaths per year; mortality rates in both men and women show an increasing tendency, with more than 70,000 deaths and 400,000 new cases every year (WHO, 2014; NOM-015, 2010; INEGI 2019; SSA, 2019).

DM type 2 (DM2) corresponds to what used to be known as DM non-insulin-requiring diabetes or adult DM, but currently it is occurring in young people too. Its importance lies in the multiple disorders it produces because of the relationship between insulin secretion and the sensitivity of the receptors to this hormone's action in the complicated glucose homeostasis (ElSayed *et al.*, 2023). This metabolic disease modifies the cellular transmission signals, partially blocks the action of the surface receptors and the expression of specific genes, it diminishes adenine dinucleotide phosphate (NADPH) and the levels of glutathione, which increases oxidative stress (Calabrese *et al.*, 2012). Other important effects related with DM are: auto-oxidation of glucose and cholesterol associated with low density lipoprotein (cLDL), the increase of myeloperoxidase activated by protein kinase C, and the increase in free radicals (Mansoor *et al.*, 2022).

In view of the complexity of this condition, the Mexican Secretariat of Health, in its Effective Medical Practice Bulletin, proposes a plan to treat DM2, in which it suggests identifying the etiology, delimiting risk factors, establishing criteria for its diagnosis, and carrying out clinical evaluations to establish pharmacological treatment and develop an adjuvant nutrition plan that includes foods rich in antioxidants, low in sugar and with considerable concentrations of soluble fiber (Mao *et al.*, 2021). The consumption of soluble fiber improves glucose control, reduces hyperinsulinemia, as well as plasmatic concentrations of lipids in individuals with DM2 (Li *et al.*, 2020). Soluble dietary fiber has the capacity of absorbing water and forming gels, producing a laxative effect, reducing carbohydrate absorption, and generating a satiety sensation (Silveira, 2003; Chawla and Patil, 2010).

The fruit of the prickly pear (*Opuntia* spp.) in addition to containing a considerable amount of soluble fiber (mucilage and pectin), has compounds of biological, technological and commercial interest such as simple sugars, antioxidants like betalains, phenolic compounds, flavonoids as well as important amounts of vitamin C (Zenteno *et al.*, 2015). All of these compounds are appreciated as very favorable in terms of a healthy diet and also as ingredients for the design of new functional foodstuffs (Sáenz *et al.*, 2007). Several investigations have credited the hypoglycemic effect of the *Opuntia* spp. cladode in animals with streptozotocin induced diabetes, or in patients with DM2 (Andrade and Wiedenfeld, 2011; Madrigal *et al.*, 2022; García *et al.*, 2024). In addition to the cladodes, the diversity of the nopal fruits can be used to take advantage of and encourage the consumption of both commercial varieties and other less known varieties but with biological and commercial potential (Reyes *et al.*, 2009, 2011). Furthermore, the prickly pear fruit has lately drawn attention because the fructose it contains can be an alternative way to provide energy to cells incapable of receiving glucose, thus avoiding the activation of other metabolic actions which cause complications, especially in diabetic patients (Kaur *et al.*, 2012; Gouws *et al.*, 2019; Sabtain *et al.*, 2021).

Thus, the objective of this study was the evaluation of the effect of consuming juice from 10 different variants of prickly pear fruit on body weight and blood glucose levels on

Wistar rats with normal levels of glucose or with streptozotocin induced hyperglycemia, as part of the evaluation of prickly pear juice as a functional foodstuff. Due to our previous results on the nutritional content of a wide diversity of wild and cultivated prickly pears, we hypothesize that after regular consumption, metabolic health benefits will be obtained in an optimal and diabetic state.

MATERIALS AND METHODS

Selection of variants and juice extraction

According to Reyes *et al.*, (2009), the selection of all these variants was based on three criteria: (a) abundance and economic potential of the fruit in the state of San Luis Potosi, (b) degree of humanization (cultivated and wild), and (c) diversity of colors of the prickly pear fruit.

The prickly pear fruit of the 10 different variants were picked at full ripeness; six of these are cultivated: Rojo Pelón (*Opuntia ficus-indica*), Blanca (*O. albicarpa*), Amarilla Monteza, Pico Chulo, Torreoja and Sangre de Toro (*O. megacantha*), and four are wild: Cardona (*O. streptacantha*), Charola (*O. streptacantha* ssp. *aguirrana*), Tapona and Tapón Rojo (*O. robusta*). The fruit were picked or harvested in the municipality of Villa de Arriaga, located in the high plain of the state of San Luis Potosi, Mexico (21° 55' 00" N; 101° 22' 59" O). Peel and glochids (slender, hair-like thorns) were removed from the fruit; the juice of the flesh was extracted with a stainless-steel blender (International LI-12-106, Mexico); the seeds were separated with a conventional 8 mesh filter. The filtered juice was stored in sterile containers at -20 °C until it was used.

Animal model

Male Wistar rats (263±38.6 g) provided by the Bioscience Center of the Faculty of Agronomy, UASLP, were used as the experimental units. Each animal was kept in individual polypropylene cages in a conditioned room in the Instituto de Investigación de Zonas Desérticas, with continuous ventilation, controlled temperature and relative humidity conditions and with a circadian cycle inverted to 12 h light/12 h dark. The study was conducted following the animal care guidelines specified in accordance with the corresponding institutional and local ethical regulations and recommendations for care and maintenance and with an approved institutional bioethical protocol (NOM-051, 1995; NOM 062, 1999).

The animals were subjected to a seven day adaptation period with standard rodent feed (Lab Diet[®] Rodent Chow 5001, USA) and *ad libitum* water. Subsequently, they were randomly distributed into two different health conditions: normal levels of glucose (GS) and hyperglycemic streptozotocin-induced (HG). The experimental period was 12 weeks, during which body weight change of the animals were recorded.

Preliminary trial to estimate the dosage of the prickly pear juice

In order to establish the dosage of prickly pear juice for each variant that would be administered to the experimental units, a preliminary trial was conducted with normal rats. According to our previous studies (Zenteno *et al.*, 2015; 2018) and based on the

amount of total sugars in the juice of each prickly pear variant, the variants with the most and the least sugar concentration were selected (Tapón Rojo with 15.13% and Tapón with 10.91%). The two juices of these prickly pears, and two beverages of reference (commercial brand cranberry juice and water) were evaluated, in doses equivalent to 150, 300 and 450 mL intended for a healthy person of 65 kg of body weight, but adjusted to the weight of the each rat, were administered through esophageal cannula in order to generate glucose tolerance curves. For this purpose, blood glucose levels were measured with glucometer (Accu-Check[®] Performa, USA) at 0, 30, 60 and 120 min after the administration of the corresponding beverages (n=3 per group).

Based on the glucose tolerance curves, the dose of 300 mL was selected, since after 60 min, the blood glucose of the rats that received prickly pear juice was similar to that of rats that were administered only water or commercial brand juice; in this way metabolic alterations in the rats were avoided. Once the dosage was selected, the volume of prickly pear juice was calculated for each animal, considering the percentage of total sugars in the juice of each variant, the weight of the animal, and the total sugars in the recommended portion of the commercial brand cranberry juice. In this way, altering the normal blood sugar levels of healthy rats was avoided, since simple sugars immediately impact the levels of blood glucose.

The feed and thawed and homogenized prickly pear juices were administered every day at the beginning of the dark period.

Induction of hyperglycemia

Sixty six rats with normal levels of glucose (GS) were considered as healthy rats. In week one of the experimental period and after an 8 h fasting, 66 experimental units were administered on a single occasion and intraperitoneally 50 mg streptozotocin (Sigma Aldrich[®] SO130, USA) per kg body weight dissolved in sodium citrate buffer (0.1 M, pH 4.5). Six experimental units of treatment (water) were administered by the same route only with citrate buffer. After one week of streptozotocin induction and with 8 h of fasting, the hyperglycemia status was assessed by caudal vein puncture of the animals and measurement with glucometer (Accu-Check[®] Performa, USA) and they were considered as experimental units with DM2 when presented fasting glucose levels >200-450 mg/dL. In order to maintain the hyperglycemic state of these rats, similar to the state of a patient with DM2 without therapeutic control (200-300 mg/dL), the rats received an individual dosage of insulin (1-4 U) of insulin (Lantus[®], Sanofi, France) every 12 h by subcutaneous administration during all the experimental period.

Biochemical evaluation

At the end of the experimental period for slaughter, the animals were fasted for 8 hr, weighed and given an individualized overdose of sodium pentobarbital (Sedalpharma[®] Pet's Pharma, Mexico) intraperitoneally according to established ethical protocols (NOM-033, 2014).

Intracardiac whole blood was obtained from all experimental units for serum collection by resting (10 min) and centrifugation at 2500 rpm/10 min (Solbat J-40, Mexico) for

biochemical analyses of glucose with commercial enzyme kits (Bayer, Sées, France) and semiautomated chemistry analyzer (Excel™, Stanbio, USA).

Experimental design and statistical analysis

The experiment was conducted according to a completely randomized experimental design with an 11×2 factorial arrangement of treatments (n=6) where each animal was considered as an experimental unit. The factor A was the juice of the cactus pear variant (10 variants and water), and factor B was the health condition of the rats (glycostatic and hyperglycemic). The data obtained from the evaluated variables were tested for normality using the Shapiro-Wilk test ($p>0.43$), which indicated that the information was normal, allowing a variance analysis to be carried out for a design completely randomized, using the PROC GLM of the SAS program version 9.2 (SAS Institute, Inc., Cary, North Carolina, USA), when ANOVA indicated differences between treatments, it was applied a Tukey multiple means test, setting a significance level of $p<0.05$.

RESULTS AND DISCUSSION

The effect of prickly pear juice on the body weight of glycostatic rats (GS) (Figure 1) and hyperglycemic rats (HG) (F=2.82, P=0.0054) (Figure 2) are shown. In the majority of treatments there was an increase in weight, except in GS animals which received juice of variant Tapón Rojo and HG animals which received juice from variants Rojo Pelón, Charola and Blanca, which actually lost weight; although, there was only significant difference in the final weight between HG rats which received juice from Pico Chulo variant, in GS and HG rats with Sangre de Toro juice, and in HG rats with juices from Charola and Blanca variants. It is important to note that, out of the 10 evaluated variants, the juice from variant Pico Chulo had the highest content of ascorbic acid and total phenolic compounds, and this was the only juice registering the presence of protocatechuic acid (Zenteno *et al.*, 2015). It has been demonstrated that this phenolic acid reduces oxidative stress by inhibiting the

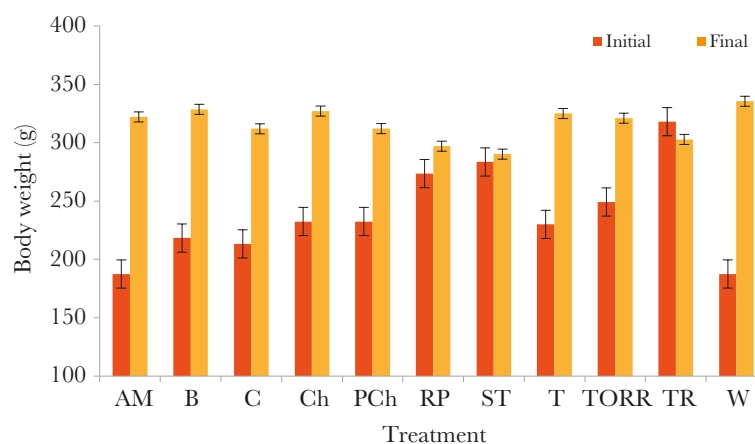


Figure 1. Initial and final weight of glycostatic rats supplemented with juice of 10 variants of prickly pear (*Opuntia* spp.). (Means \pm standard error). AM: Amarilla Monteza, B: Blanca, C: Cardona, Ch: Charola, Pch: Pico Chulo, RP: Rojo Pelón, ST: Sangre de Toro, T: Tapona, TO: Torreja, TR: Tapón Rojo, W: water.

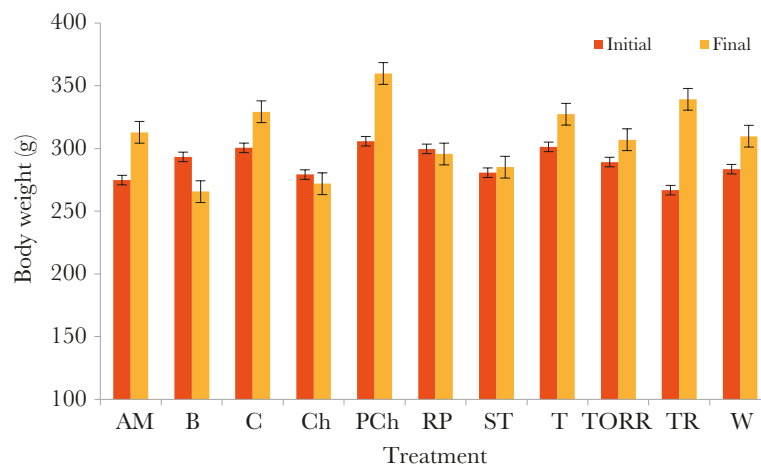


Figure 2. Initial and final weight of hyperglycemic rats supplemented with juice of 10 variants of prickly pear (*Opuntia* spp.). (Means \pm standard error). AM: Amarilla Monteza, B: Blanca, C: Cardona, Ch: Charola, Pch: Pico Chulo, RP: Rojo Pelón, ST: Sangre de Toro, T: Tapona, TO: Torreja, TR: Tapón Rojo, W: water.

enzymatic NADPH oxidase system, a multiproteic complex that generates free radicals, especially superoxide radical (de Faria *et al.*, 2012).

In general, glycostatic rats that received prickly pear juice had a lower body weight, which coincides with Trejo *et al.*, (1996), Fernandes *et al.*, (2009) and Fatma and Hassan (2011), who administered prickly pear juice from *O. fuliginosa* and from *O. ficus-indica* to healthy rats and observed a lesser weight gain than in diabetic rats.

The increase of plasmatic levels of glucose is the best metabolic indicator of DM2 (NOM-015, 2010). HG rats that received water showed higher plasmatic levels of glucose than HG rats that received juice from variant Sangre de Toro and all GS rats (Table 1); nonetheless, in HG rats receiving juices from variants Blanca, Charola, Rojo Pelón, and Tapona ($P \geq 0.05$), plasmatic glucose values can be observed within the normal amplitude for Wistar rats, that is, from 98 to 152 mg/dL (Bolani, 1990).

The concentration of plasmatic glucose in GS rats receiving the juices is within the normal amplitude of reference. It is important to note that HG rats receiving the juice of variant Sangre de Toro showed concentrations of plasmatic glucose similar ($P \geq 0.05$) to those of GS rats receiving juice of the same variant. This may be due to the fact that such variant showed lower quantities of sugar and higher content of total phenol compounds, which reduce the absorption of carbohydrates and regulate the enzymes involved in the metabolism of glucose (Chun *et al.*, 2007; Ovaskainen *et al.*, 2008), and an important amount of soluble fiber, which reduces hyperglycemia, cardiovascular risks, body weight and improves sensibility of insulin receivers (Weickert *et al.*, 2006; Qi *et al.*, 2006). In spite of its moderate content of flavan 3-oles, the Sangre de Toro juices show the highest amount of procyanidine b, which inhibits the biosynthesis of lipids (Vidal *et al.*, 2005), reduces the biosynthesis and secretion of apolipoproteins, and prevents the esterification of cholesterol and the production of lipoproteins (Sugiyama *et al.*, 2007); additionally, the juices of Sangre de Toro contain intermediate quantities of betalains and ascorbic acid, and showed good antioxidant capacity (Zenteno *et al.*, 2015). In general, polyphenols have favorable

Table 1. Effect of prickly pear juice on the concentration of metabolic blood indicators (mg/dL) in hyperglycemic (HG) and glucostatic (GS) Wistar rats at the end of the experimental period.

Variant	Glucose		Cholesterol		Triglycerides	
	GS	HG	GS	HG	GS	HG
Rojo Pelón	140.5±20.1 ^b	151.1±67.0 ^{ab}	88.4±7.2 ^{abcd}	112.0±7.2 ^a	95.1±8.4 ^{abc}	57.8±10.7 ^c
Blanca	114.4±9.6 ^b	158.6±90.0 ^{ab}	104.2±8.8 ^{ab}	54.8±7.2 ^{cdef}	156.5±14.8 ^{abc}	178.8±77.3 ^{abc}
Amarilla Monteza	141.4±11.7 ^b	211.5±121.9 ^{ab}	81.7±8.8 ^{abcde}	50.4±7.2 ^{def}	83.5±8.5 ^{bc}	191.1±62.0 ^{abc}
Pico Chulo	105.0±10.1 ^b	174.9±55.4 ^{ab}	55.9±8.8 ^{cdef}	56.5±7.2 ^{cdef}	60.6±7.6 ^c	201.1±99.6 ^{abc}
Torrejoja	116.6±10.9 ^b	231.5±94.6 ^{ab}	58.2±8.8 ^{cdef}	92.5±7.2 ^{abc}	75.4±26.1 ^{bc}	79.2±29.4 ^{bc}
Sangre de Toro	124.2±3.8 ^b	127.0±42.8 ^b	68.8±8.8 ^{bcdef}	59.8±7.2 ^{cdef}	81.0±25.7 ^{bc}	66.8±11.0 ^c
Cardona	103.5±9.9 ^b	168.3±78.3 ^{ab}	43.2±7.9 ^{ef}	68.1±7.2 ^{bcdef}	53.8±23.8 ^c	242.8±204.0 ^a
Charola	97.1±13.0 ^b	155.8±75.1 ^{ab}	75.2±7.2 ^{abcde}	33.5±8.8 ^f	67.4±8.7 ^c	228.0±110.2 ^{ab}
Tapona	115.3±6.9 ^b	145.3±47.6 ^{ab}	63.0±8.8 ^{bcdef}	60.5±7.2 ^{cdef}	97.2±39.1 ^{abc}	74.3±39.9 ^{bc}
Tapón Rojo	121.3±8.0 ^b	222.7±67.8 ^{ab}	75.8±8.8 ^{abcde}	90.3±7.2 ^{abcd}	108.4±24.3 ^{abc}	94.7±44.7 ^{abc}
Water	125.6±6.6 ^b	292.9±194.7 ^a	64.4±7.9 ^{bcdef}	49.8±7.2 ^{def}	110.3±19.5 ^{abc}	186.6±39.2 ^{abc}
F	4.62	13.53	2.88	4.11	12.71	3.64
p-value	≤0.001	≤0.001	0.0042	0.0002	≤0.001	0.0006

Means ± standard error. Means with different literals are statistically different ($P \geq 0.05$).

effects on the cardiovascular system of diabetic individuals, which is probably due to their metabolic regulation of lipids and apolipoproteins (Vidal *et al.*, 2005), and their stimulation of insulin secretion (Bahadoran *et al.*, 2013).

CONCLUSIONS

With the consumption of prickly pear juice of various variants, regardless of their color or whether they were wild or cultivated, a maintenance of body weight gain and diminish the concentration of glucose in healthy rats, oscillating in optimal and adequate ranges for healthy rats, in addition to a considerable benefit on the glycemic of rats with induced diabetes. Therefore, more biochemical and genetic studies are needed to understand the specific mechanisms of action by which prickly pear juice exerts its beneficial actions in the body.

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Declaration of competing interest

The authors declare no actual or potential conflict of interest, including financial, personal or relationship with other organizations.

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Impact of Point Sources on the Water Quality of the Camalote River, Oaxaca, Mexico

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ABSTRACT

Objective: This study assesses the water quality of domestic wastewater discharges into the Camalote River, Oaxaca, Mexico, and evaluates their environmental impact. The findings aim to support the development of preventive and mitigation measures to benefit both the environment and the communities relying on this resource.

Design/methodology/approach: Seven domestic wastewater discharges into the river were analyzed following the methodology outlined in NOM-001-ECOL-1996 for water sample collection and analysis.

Results: The analysis shows that the discharges exceed the maximum permissible limits established by NOM-001-SEMARNAT-2021, particularly for total suspended solids (256.67 mg L^{-1}) and biochemical oxygen demand (392.22 mg L^{-1}) in discharge 4, as well as total coliforms ($27,333,333.33 \text{ NMP } 100 \text{ mL}^{-1}$) and fecal coliforms ($10,000,000.00 \text{ NMP}/100 \text{ mL}$) in discharge 6.

Limitations/implications: The findings indicate significant risks to public health and aquatic ecosystems. Continuous monitoring of the river is strongly recommended, particularly during drought periods when the river's dilution capacity decreases, leading to higher pollutant and organic matter concentrations and anaerobic conditions that harm aquatic biodiversity.

Findings/conclusions: The study underscores the urgent need to implement preventive and mitigation strategies, including wastewater treatment and revegetation of key areas, to reduce pollution and safeguard water quality for the benefit of the environment and the local communities dependent on this vital resource.

Keywords: Discharges, pollution, water quality indicators, monitoring.

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INTRODUCTION

The integrity of water sources refers to maintaining their structure and functionality by preserving the natural balance of their physical, chemical, and biological conditions (Qadri *et al.*, 2020). Protecting and/or restoring these water bodies requires a thorough understanding of their quality (Kroll *et al.*, 2019). Spatial and temporal variations in surface water quality are influenced by both natural factors and human activities (Chen *et al.*, 2023). Analyzing how these relationships vary across time and space, along with identifying the sources of elements that alter water properties and their behavior in rivers,

is essential for implementing effective basin-level strategies for water quality conservation and management (Hamid *et al.*, 2020). Factors affecting water quality can be categorized into two types based on their origin: point sources, such as direct discharges of domestic wastewater, and non-point sources, which transport pollutants through runoff and surface drag, contaminating water bodies (Chakraborti, 2021). Rivers possess a natural self-purification capacity that plays a critical role in enhancing water quality (Darji *et al.*, 2022). In Mexico, official data on river water quality remain limited. Nonetheless, several studies have examined these water bodies and the associated contamination processes, aiming to identify major pollution sources and generate data to support the development of management and conservation strategies (Rodríguez-Nuñez *et al.*, 2022). These studies are vital for improving water resource management and protecting both the environment and the communities that depend on these resources (Casillas-García *et al.*, 2021). Due to the above, this research evaluates the quality of domestic wastewater discharges into the Camalote River, Oaxaca, Mexico, and assesses their environmental impact. The findings may inform relevant authorities in the development of preventive and mitigation measures to safeguard the environment and the communities reliant on this vital resource.

MATERIALS AND METHODS

The Camalote River is situated in the state of Oaxaca, within the municipality of Acatlán de Pérez Figueroa, between the coordinates 18° 32' 16.48"–18° 23' 48.78" N and 96° 33' 20.69"–96° 26' 43.28" W. It forms part of the Papaloapan Basin. The region receives an average annual precipitation of 1,786.6 mm, predominantly concentrated during the wet season, which occurs from June to September. The study area is characterized by two dominant vegetation types: tropical evergreen forest, which covers 18.41% of the region, and secondary shrub vegetation of the tropical evergreen forest, accounting for 10.04% of the total area. Land use in the region is primarily divided between urban zones and agricultural activities (INEGI, 2010).

Sampling and analysis of discharges into the Camalote river

Seven water discharges were identified and mapped with the assistance of municipal authorities in Acatlán de Pérez Figueroa, who provided insights into significant sites and nearby activities relevant to the study. These discharges are located between coordinates 759638.28 E, 2048191.42 N and 760404.58 E, 2047433.41 N, at elevations ranging from 105 to 107 meters above sea level (masl). A subsequent field survey along the river was conducted to confirm the identified sampling locations.

A composite sample was collected from each wastewater discharge, comprising four grab samples taken at three-hour intervals (Figure 1). Sampling was performed in compliance with the methodology established in NOM-001-ECOL-1996, the regulation for domestic wastewater discharges. The sampling campaign was conducted during both the rainy and dry seasons of 2017.

The samples were collected and stored in glass and high-density polyethylene containers, properly labeled, preserved at 2 °C, and transported to the laboratory for analysis. The

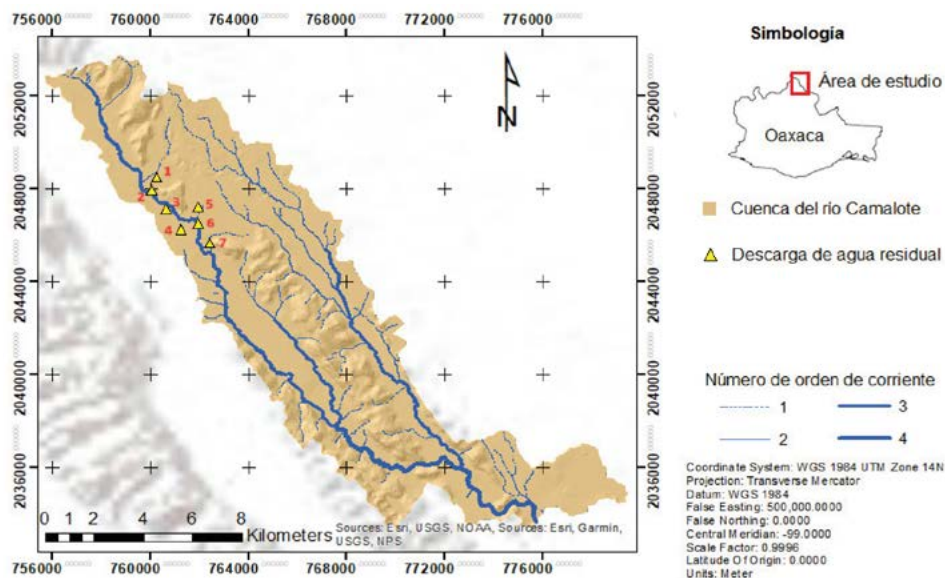


Figure 1. Camalote river basin and water discharges.

Table 1 outlines the physical, chemical, and biological parameters evaluated in the wastewater discharges, along with the corresponding analysis methods.

Data Analysis

A nested experimental design with two factors season (rainy and dry) and discharge sites as levels, each with three repetitions was employed for data analysis. Normality (Shapiro-

Table 1. Physical, chemical, and biological parameters evaluated in wastewater discharges.

Parameter	Method
1. Temperature ⁽¹⁾	Electrometric
2. pH ⁽¹⁾	Electrometric
3. Flow ^{(1)*}	Sampling port
4. Fats and oils ^{(2)(A)}	Soxhlet Extraction
5. Total suspended solids ⁽³⁾	Quantitative measurement
6. Sedimentable solids ⁽²⁾	Imhoff
7. Biochemical oxygen demand ⁽²⁾	Winkler
8. Total nitrogen ⁽²⁾	Kjeldahl
9. Total phosphorus ⁽²⁾	Spectrophotometric
10. Total coliforms ⁽²⁾ Fecal coliforms ⁽²⁾	Most probable number in multiple tubes
11. Helminth eggs ⁽²⁾	Microscopic observation
12. Copper, Nickel, Lead, Zinc, Cadmium, Chromium, Mercury ⁽²⁾	Atomic absorption spectrophotometry

Note: (1) Data obtained in the field. (2) Data obtained in the Environmental Biotechnology Laboratory, DEIS in Soils, Universidad Autónoma Chapingo. (3) Data obtained in the laboratories of ABC Química, Investigación y Análisis, S. A. de C. V. (A) Sample adjusted in the field to pH<2 with 37% HCl.

Wilk) and homogeneity of variance (Levene) tests were performed. Upon satisfying these assumptions ($p > 0.05$), an analysis of variance (ANOVA) and Tukey's mean comparison test ($p < 0.05$) were conducted to identify significant differences between seasons (rainy and dry) and among discharges within each season. The statistical model used for the experimental design was as follows:

$$Y_{ijk} = \mu + p_i + P_{j(i)} + e_{ijk}$$

where: Y_{ijk} = response obtained from the determinations, μ = general mean, p_i = effect of the i -th season, $P_{j(i)}$ = effect of the j -th discharge within the i -th season, e_{ijk} = random error associated with observation Y_{ijk} . The statistical analysis was carried out using InfoStat software, free version 2020 (Di Rienzo *et al.*, 2017).

RESULTS AND DISCUSSION

Water quality of the discharges into the Camalote river. Analysis of variance of the determinations conducted by period

The analysis of variance indicated that the variables P, As, and Cd did not show statistically significant differences between the rainy and dry seasons, nor among the discharges within each period. However, the other variables evaluated did show significant differences by period and by discharge within each period (Table 2), indicating sensitivity to temporal and spatial conditions. The observed variations suggest that water quality in the study area is influenced by rainfall and anthropogenic factors.

During the dry season, an increase in Biochemical Oxygen Demand (BOD_5), total suspended solids, and fats and oils was observed, reflecting an accumulation of pollutants due to the river's reduced dilution capacity. BOD_5 reached 239.32 mg L^{-1} during the dry season, nearly doubling the value recorded during the rainy season (119.55 mg L^{-1}). Total coliforms ($6,823,238 \text{ MPN}/100 \text{ mL}$) and fecal coliforms showed a considerable increase during the dry season, posing a higher risk to public health. Additionally, there was an increase in the presence of helminth eggs (1.48 eggs L^{-1}) and in the concentrations of heavy metals such as chromium, mercury, and lead, which were significantly higher during the dry season. These results suggest that drought conditions exacerbate pollution risks in the Camalote river, highlighting the need to consider both temporal and spatial factors when assessing water quality, as corroborated by previous studies like that of Taher *et al.* (2021).

Average daily flow, ph, settleable solids, total suspended solids, fats and oils

During the rainy season (Table 3), discharge six recorded the highest flow rate (20.56 L s^{-1}), making it the most significant source of water discharged into the river by volume. Discharge three showed the highest amount of settleable solids (2.02 mL L^{-1}), indicating an increased accumulation of material on the riverbed. Discharge four exhibited the highest total suspended solids concentration (256.67 mg L^{-1}), exceeding the daily maximum permissible limit (MPL) of 72 mg L^{-1} (NOM-001-SEMARNAT-2021) by 3.5 times. High turbidity levels, as noted by Kowe *et al.* (2023), can reduce light

Table 2. Analysis of variance of the determinations conducted in the discharges.

Parameter	P value		Period average	
	Period	Discharge	Rain	Dry
Average Daily Flow (L/s)	0.0001	0.0001	7.13 a	3.58 b
Average Daily Temperature (°C)	0.0001	0.0420	26.44 b	29.40 a
pH	0.0001	0.0001	7.48 a	7.18 b
Total Coliforms (MPN 100 mL ⁻¹)	0.0001	0.0001	3972523.81 b	6823238.10 a
Fecal Coliforms (MPN 100 mL ⁻¹)	0.0001	0.0001	3733952.38 a	3038142.86 b
Helminth Eggs (Eggs L ⁻¹)	0.0080	0.0001	1.14 b	1.48 a
Settleable Solids (mL L ⁻¹)	0.0001	0.0001	1.61 a	0.40 b
Total Suspended Solids (mg L ⁻¹)	0.0001	0.0001	159.33 a	86.14 b
Fats and Oils (mg L ⁻¹)	0.0001	0.0001	3.18 b	6.36 a
BOD ₅ (mg L ⁻¹)	0.0001	0.0001	119.55 b	239.32 a
N total (mg L ⁻¹)	0.0001	0.0001	4.45 b	9.20 a
Fósforo (mg L ⁻¹)	0.2873	0.4652	1.00 a	1.00 a
As (mg L ⁻¹)	0.2921	0.2001	1.2E-03 a	1.3E-03 a
Cd (mg L ⁻¹)	0.3343	0.3476	0.00015 a	0.00015 a
Cu (mg L ⁻¹)	0.0001	0.0001	2.5E-03 b	0.06 a
Cr (mg L ⁻¹)	0.0001	0.0001	3.1E-04 b	0.03 a
Hg (mg L ⁻¹)	0.0001	0.0001	2.7E-05 b	2.0E-04 a
Ni (mg L ⁻¹)	0.0001	0.0001	0.01 b	0.02 a
Pb (mg L ⁻¹)	0.0001	0.0001	0.01 b	0.05 a
Zn (mg L ⁻¹)	0.0001	0.0001	0.35 b	0.73 a

* Different letters per row indicate significant differences ($p < 0.05$). Tukey's Mean.

penetration and harm aquatic ecosystems. Discharge six recorded the highest fats and oils concentration (17.79 mg L⁻¹), just below the MPL of 18 mg L⁻¹ (NOM-001-SEMARNAT-2021). These contaminants, as highlighted by Adetunji and Olaniran (2021), can inhibit water oxygenation and negatively affect aquatic flora and fauna. During the dry season (Table 3), discharge 6 again showed the highest flow rate (10.13 L/s), which was less than half the volume observed in the rainy season. According to Vione *et al.* (2023), lower flow during dry periods reduces the river's dilution capacity, resulting in elevated pollutant concentrations. Discharge four continued to exhibit the highest total suspended solids concentration (183.00 mg L⁻¹), exceeding the MPL by 2.5 times (NOM-001-SEMARNAT-2021), indicating persistent turbidity. Discharge 3 recorded the highest settleable solids concentration (0.94 mL L⁻¹), reflecting the diminished transport capacity of particles during the reduced flow. Discharge 6 maintained the highest fats and oils concentration (34.25 mg L⁻¹), surpassing the MPL by 1.8 times (NOM-001-SEMARNAT-2021), signaling an ongoing source of contamination.

This study confirms that domestic discharges degrade water quality, consistent with Boyd (2019), who asserts that anthropogenic pollution, particularly via runoff, increases turbidity and sedimentation, thereby adversely affecting aquatic ecosystems.

Table 3. Average flow rate, daily average temperature, pH, settleable solids, and fats and oils for the different discharges.

Discharge	Average daily flow rate (L s ⁻¹)	Average daily temperature (°C)	pH	SS (mL L ⁻¹)	SST (mg L ⁻¹)	Fats and oils (mg L ⁻¹)
Rainy season						
1	1.87 f	26.27 a	7.5 b	1.16 e	122.33 d	0.24 d
2	5.43 cd	25.93 a	7.3 b	1.22 de	127.00 d	1.70 b
3	4.53 cd	26.50 a	8.0 a	2.02 a	208.67 b	0.04 h
4	7.49 b	26.40 a	7.3 b	1.70 c	256.67 a	1.60 b
5	3.76 e	25.70 a	7.5 b	1.33 d	143.33 c	0.04 d
6	20.56 a	27.60 a	7.4 b	1.86 b	203.67 b	17.79 a
7	6.68 bc	26.67 a	7.3 b	1.99 a	53.67 e	0.88 c
Dry season						
1	0.98 c	27.87 c	7.13 b	0.05 e	7.67 d	0.66 c
2	3.57 b	28.70 bc	7.13ab	0.10 e	65.33 c	3.51 b
3	1.15 c	29.43 ab	7.83 a	0.94 a	101.33 b	0.24 c
4	3.63 b	29.93 ab	7.23ab	0.20 d	183.00 a	2.63 b
5	0.97 c	29.93 ab	7.00 b	0.65 c	61.00 c	0.34 c
6	10.13 c	30.90 a	7.00 b	0.80 b	167.00 a	34.25 a
7	4.66 b	29.07 bc	6.83 b	0.10 e	17.67 d	2.86 b

BOD₅, total nitrogen, coliforms, and helminth eggs

During the rainy season, BOD₅ varied significantly among discharges. Discharge 7 showed the highest concentration, at 189.76 mg L⁻¹ (Table 4), exceeding the MPL (180 mg L⁻¹ daily average) set by NOM-001-SEMARNAT-2021. Total suspended solids exceeded the MPL by up to 3.5 times, highlighting the need to minimize them to prevent further increases in BOD₅ after their release (Donald *et al.*, 2022). Discharge 6 exhibited the highest concentration of total coliforms (14,000,000 MPN/100 mL) and fecal coliforms (18,666,666.67 MPN/100 mL), far exceeding the MPL (500 MPN/100 mL) established by NOM-001-SEMARNAT-2021.

During the dry season, BOD₅ increased across all discharges (Table 4), with the highest value recorded in discharge 4 (392.22 mg L⁻¹), exceeding the MPL of 180 mg L⁻¹ daily average (NOM-001-SEMARNAT-2021). This indicates a higher organic load associated with domestic wastewater, a concerning situation as the presence of organic matter promotes oxygen consumption by bacteria during oxidation, leading to anaerobic conditions that hinder the survival of aquatic organisms (Yoon *et al.*, 2024). Muñoz-Nava *et al.* (2012) noted that settlements near rivers contribute to increased BOD₅ levels. Discharge 6 (Table 4) maintained high levels of contamination by total coliforms (27,333,333.33 MPN/100 mL) and fecal coliforms (10,000,000.00 MPN/100 mL), far exceeding the MPL of 500 MPN 100 mL⁻¹ (NOM-001-SEMARNAT-2021). This fecal contamination, attributed to untreated wastewater discharges, poses risks to both aquatic life and human health (Bhatt *et al.*, 2024). Regarding the number of

Table 4. Biochemical oxygen demand, total nitrogen, coliforms, and helminth eggs in the different discharges.

Discharge	BOD ₅ (mg L ⁻¹)	Total N (mg L ⁻¹)	Total coliforms (NMP 100 mL ⁻¹)	Coliformsf Fecal (NMP 100 mL ⁻¹)	Helminth eggs (eggs L ⁻¹)
Rainy season					
1	28.14 g	0.13 d	100000.00 e	8333.33 d	1.00 b
2	154.78c	1.73 c	180000.00 e	186666.67 d	1.33 b
3	135.26d	1.64 c	1933333.33 d	1966666.67 c	1.00 b
4	180.64b	2.59 c	8333333.33 b	866666.67 dc	1.00 b
5	98.16 e	6.73 b	3333333.33 c	4400000.00 b	1.67 a
6	50.10 f	6.43 b	14000000.00 a	18666666.67 a	1.00 b
7	189.76a	11.88 a	17666.67 e	42666.67 d	1.00 b
Dry season					
1	91.44 e	0.57 f	22666.67 d	21000.00 d	1.33 bc
2	299.91b	3.58 de	206666.67 d	2433333.33 c	1.67 abc
3	223.47c	2.73 e	3066666.67 cd	2300000.00 c	1.00 bc
4	392.22a	4.33 d	10333333.33 b	2066666.67 c	2.00 ab
5	182.58d	16.02 b	4366666.67 c	4400000.00 b	2.67 a
6	121.77e	14.44 c	27333333.33 a	10000000.00 a	0.67 c
7	392.22a	22.73 a	2433333.33 cd	46000.00 d	1.00 bc

helminth eggs, discharge five recorded the highest concentration (2.67 No. H/5L). Although Mexican regulations do not specify MPLs for these pathogens in river waters, their presence poses a health risk for residents who use the water directly. Helminth eggs are considered indicators of microbiological contamination (Téllez *et al.*, 2023).

Heavy metal concentration

During the rainy season, Zn was the only metal that showed significant differences among the seven discharges ($p < 0.05$), with the highest concentrations found in discharges 6 (0.56 mg L⁻¹) and 4 (0.55 mg L⁻¹), without exceeding the MPLs established by NOM-001-SEMARNAT-2021 (Table 5). During the dry season, significant differences ($p < 0.05$) were observed in all heavy metals evaluated in the river water, although none exceeded the MPLs according to NOM-001-SEMARNAT-2021. Discharge 1 recorded the highest concentrations of Hg (5.6E-04 mg L⁻¹) and Ni (0.07 mg L⁻¹), while discharge 7 had the highest concentrations of Cu (0.28 mg L⁻¹), Pb (0.17 mg L⁻¹), and Zn (0.93 mg L⁻¹).

Although heavy metals did not exceed the MPLs, continuous monitoring of water quality in the Camalote river is recommended, particularly during the dry season, as Cr, Hg, and Zn have toxic effects and can bioaccumulate in aquatic food chains (Liu *et al.*, 2021). Additionally, Ni and Zn negatively affect the growth and reproduction of aquatic organisms, such as macroinvertebrates (Mancilla-Villa *et al.*, 2023).

Table 5. Heavy metal concentrations in the different discharges.

Discharge	Cu	Cr	Hg	Ni	Pb	Zn
	mg L ⁻¹					
Rainy season						
1	2.5E-03 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.38 c
2	2.5E-03 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.05 d
3	2.5E-03 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.37 c
4	2.5E-03 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.55 a
5	2.5E-03 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.09 d
6	2.5E-03 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.56 a
7	2.5E-06 a	3.1E-04 a	2.7E-05 a	0.01 a	0.01 a	0.47 b
Dry season						
1	2.5E-03 c	3.1E-04 e	5.6E-04 a	0.07 a	0.01 b	0.90 a
2	2.5E-03 c	3.1E-04 e	5.7E-04 a	0.01 d	0.01 b	0.48 c
3	0.10 b	0.01 d	2.7E-05 b	0.03 b	0.13 a	0.74 b
4	0.04 bc	0.08 a	6.6E-05 b	0.01 d	0.01 b	0.98 a
5	2.5E-03 c	0.04 c	6.6E-05 b	0.01 d	0.01 b	0.30 d
6	2.5E-03 c	0.05 b	9.5E-05 b	0.02 c	0.01 b	0.76 b
7	0.28 a	3.1E-04 e	2.7E-05 b	0.01 d	0.17 a	0.93 a

CONCLUSIONS

The water quality of domestic discharges entering the Camaloter River during both the rainy and dry seasons exceeds the maximum permissible limits (MPLs) for settleable solids, total suspended solids, BOD, and total and fecal coliforms as established by NOM-001-SEMARNAT-2021. These exceedances pose significant risks to public health and aquatic ecosystems. Although heavy metal concentrations remained below the MPLs, continuous monitoring is essential due to their potential for bioaccumulation and adverse effects on the food chain. This study underscores the urgent need for mitigation and prevention measures, such as enhancing wastewater treatment systems and implementing revegetation in strategic areas, to reduce pollution and safeguard water quality for the benefit of the environment and the communities that rely on the river.

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Ex situ morphological characterization of the native chili Cha'hua (*Capsicum annuum* L.) cv. Chahua

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ABSTRACT

Objective: To morphologically characterize Cha'hua chili (*Capsicum annuum* L.), a native variety from the Yucatán Peninsula.

Design/methodology/approach: Seeds collected from Cha'hua chili in the state of Yucatán were sown in 200-cavity polystyrene trays. After transplanting, the plants were placed in hydroponic bags and morphologically characterized using the descriptor for the genus *Capsicum* from the International Plant Genetic Resources Institute (IPGRI).

Results: The plant reached a height of 118 cm and a width of 34.8 cm, with white flowers and light green fruits at the early stage, turning red at maturity. The fruits measured 7.42 cm in length and 2.32 cm in width. The plant exhibited tolerance to viral infections caused by whiteflies. Capsaicin and dihydrocapsaicin contents were determined at 10.10 mg g⁻¹ and 3.03 mg g⁻¹, respectively.

Study limitations/implications: The morphological characterization of the Cha'hua variety will generate valuable knowledge to enhance its adaptation to large-scale production systems.

Findings/conclusions: This plant possesses morphological characteristics suitable for genetic improvement aimed at commercial exploitation, given its market acceptance both as fresh and dried fruit, supported by competitive market prices.

Keywords: capsaicin, horticulture, *Capsicum annuum*, native crop, hydroponics.

INTRODUCTION

The diversity of genetic resources forms the foundation of agriculture and food production. Their conservation is essential for ensuring their continuity, which in turn provides products to humanity and supports food and nutritional security (Becerril, 2013).



Mexico possesses a vast array of *Capsicum* spp. phylogenetic resources. Chili peppers are among the most widely used plants worldwide, holding significant culinary, economic, and sociocultural importance in Mexico (Ramírez *et al.*, 2000). In particular, the Yucatán Peninsula hosts a great diversification of landrace chili peppers from the species *C. annuum*, *C. chinense*, and *C. frutescens*, yet comprehensive knowledge of the native germplasm remains limited (Latournerie *et al.*, 2002; Pardey *et al.*, 2006; Castillo-Aguilar *et al.*, 2023). In the Yucatán Peninsula, chili cultivation (*Capsicum* spp.) occurs both in open fields and controlled environments such as greenhouses and shade houses, as seen with habanero chili (*Capsicum chinense* Jacq). However, native landrace varieties are also cultivated in traditional milpa systems and backyard gardens, with the most frequently grown varieties being Ixcat Ik, Dulce, Maax, Yaax Ik, and Cha'huá, all belonging to *Capsicum annuum*, as well as Pico Paloma (*Capsicum frutescens*). These varieties are cultivated regionally for local consumption and are integral to the peninsula's gastronomy (González-Estrada *et al.*, 2012; Castillo-Aguilar *et al.*, 2023). In Maya communities, 70% of Cha'huá chili (*C. annuum* cv. Chahua) plants are managed in small plots, cultivated under rainfed conditions in milpa systems due to their drought tolerance (Molina Suárez *et al.*, 2021).

Cha'huá chili, also known as Chawa (González-Estrada *et al.*, 2012), is a native landrace from the Yucatán Peninsula resistant to drought, with fruit size varying but averaging 4.4 cm in length and 2.1 cm in diameter. It is commercially distributed at the regional level and primarily used for condiment production. This type of chili can be marketed both fresh and dried (Aguilar-Rincón *et al.*, 2010). Given the importance of Cha'huá chili for Maya communities in the Yucatán Peninsula and its potential for genetic improvement, this study aimed to conduct an *ex situ* morphological characterization to optimize its management and enhance its production scale in regional agriculture.

MATERIALS AND METHODS

The morphological characterization of Cha'huá chili was part of the research project "Collection, Morphological Characterization, Molecular Typification, Conservation, and Sustainable Utilization of the Capsicum Genus in the State of Campeche." The study was conducted at the Lol'ic (Chili Flower) nursery, located along the former Mérida-Motul highway, in front of Hacienda San Antonio Holactún, in the community of Conkal, Yucatán. Sowing took place on August 22, 2023, in 200-cavity polystyrene trays, using peat moss (Peat Moss Sunshine 3[®]) as the substrate. The seeds of *Capsicum annuum* cv. Chahua were collected in the community of Conkal, Yucatán. Transplanting occurred 50 days after sowing, when the plants had reached an average height of 20 cm. They were transferred to hydroponic plastic bags (600-gauge), measuring 50 cm in width and 40 cm in length. The substrate used consisted of 40% Nitisol soil, 40% Rendzina soil, and 10% agricultural perlite (Multiperl[®]). The plants remained in a protected system for three months before being moved to open-field conditions. An integrated management approach was applied for fertilization and pest and disease control, following the recommendations of Soria *et al.* (1999). Morphological characterization was performed on 10 randomly selected plants with three replications. The evaluation included seedling, plant, flower, and fruit characteristics, using the *Capsicum* descriptor from the International Plant Genetic Resources Institute

(IPGRI, 1995). Additionally, spiciness level was determined using high-performance liquid chromatography (HPLC). The morphological characterization of quantitative variables was analyzed through descriptive statistics, including mean, standard deviation, and coefficient of variation.

RESULTS AND DISCUSSION

Seedling

Cha'huá chili exhibited a 50% seedling emergence rate at 8 ± 2 days after sowing (DAS), with full plant emergence at 10 ± 12 DAS. The seedlings had a white coleoptile, scarce hypocotyl pubescence, green cotyledon leaves, and oval-shaped cotyledon leaves with an average length of 10.12 ± 2.57 mm and a width of 1.72 ± 0.28 mm. Leaf width was the trait with the lowest coefficient of variation (CV) at 16.27%.

These results are important considering that there is no existing morphological description of the seedling stage of Cha'huá chili. Therefore, the observed values represent a valuable contribution to morphological knowledge. This nursery growth stage is crucial, as nursery managers must be well aware of the morphological characteristics of the variety or ecotype they are working with to ensure varietal purity, leading to the expected field results.

Plant

The Cha'huá chili plant was characterized by an erect and rustic growth habit, a stem with no anthocyanins, lanceolate leaves with wavy margins (Figure 1). Additional characteristics related to the plant's growth stage are shown in Table 1.

The qualitative and quantitative characteristics observed in Cha'huá chili allowed for a morphological definition of the plant during its vegetative growth stage. Some of these findings align with the descriptions provided by Aguilar-Rincón *et al.* (2010) and Escalera-Ordaz *et al.* (2019), although their morphological characterizations are limited. Based on field observations, the qualitative traits that contributed most to the morphological



Figure 1. From left to right: Leaf shape and color of Cha'huá chili; stem shape, color, and pubescence of Cha'huá chili. Photographs taken in the field by the authors.

Table 1. Qualitative and quantitative characteristics of the plant growth stage of the Cha'huá chili (*Capsicum annuum* cv. Chahua).

Feature	Value	Feature	Value
Stem color	green	tillering	dense
Anthocyanins at the node	absent	Leaf density	dense
Stem shape	angular	Leaf color	green
Pubescence on the stem	scarce	Leaf shape	lanceous
Plant growth habit	erect	Leaf margin	wavy
Plant height (cm)	58.6±8.51	Leaf pubescence	medium
Plant width (cm)	53.6±0.51	Leaf length (cm)	8.72±1.13
Branching density	high	Leaf width (cm)	3.48±0.66

description were leaf shape, leaf margin, and pubescence level. These results partially agree with those reported by Latournerie *et al.* (2002), who identified leaf shape as a key element in chili characterization.

Regarding the quantitative traits, the coefficients of variation for the evaluated characteristics ranged between 10% and 20%, similar to those reported by Escalera-Ordaz *et al.* (2019). These coefficients indicate a low to moderate variation in plant characteristics. Based on this, it was determined that the Cha'huá landrace variety has lost certain wild-type chili traits over time, particularly in terms of growth habit and plant stature. Unlike wild chilis, which exhibit a determinate growth pattern and are highly dependent on environmental conditions for crop management, Cha'huá chili has begun to show increased susceptibility to pests and diseases (Castillo-Aguilar *et al.*, 2023).

Flower

The flowers of Cha'huá chili were characterized as solitary and pendulous, with a white, round corolla, white filaments, blue anthers, and an exerted stigma. These traits align with the descriptions provided by Aguilar-Rincón *et al.* (2010) in his morphological study of Mexican chili varieties. Additional characteristics can be observed in Figure 2 and Table 2.

**Figure 2.** Flower of the Cha'huá chili (*Capsicum annuum* L.) cv. Chahua, showing its shape, color, position, anthers, and exposed style. Photographs were taken in the field by the authors.

Table 2. Qualitative and quantitative characteristics of the Cha'huá chili flower (*Capsicum annuum* L.) cv. Chahua.

Feature	Value	Feature	Value
Number of flowers per axil	1	Filament color	white
Position of the flower	earring	Style length (mm)	1.75 ± 0.21
Color of the corolla	white	Corolla size (mm)	21.47 ± 1.10
Corolla shape	round	Exsertion (stigma)	exserted
Corolla length	1.5 a 2.5	Calyx pigmentation	absent
Anther color	blue	Calyx margin	dentate
Anther length (mm)	3.22 ± 0.50	Calyx construction	absent

A differential morphological characterization of the Cha'huá chili flower must consider both qualitative and quantitative traits, as several floral morphological characteristics are identical to those of other chili types, such as Ixcat Ik and Yaax Ik chili (Castillo-Aguilar *et al.*, 2023).

Fruit

Qualitatively, the fruit was observed to be light green in its immature stage, orange at the intermediate stage, and red when fully mature, with slight transverse wrinkling (Figure 3). It is an elongated fruit with an average length of 9.9 cm and a width of 1.1 cm, values that fall within the ranges reported by González-Estrada (2000) and Aguilar-Rincón *et al.* (2010). The fruit had an average weight of 4.39 g and a pericarp thickness of 1.35 cm (Table 3).

A more detailed description than the one provided in this study is not available, as this chili is considered a native landrace variety of the Yucatán Peninsula, cultivated on a small scale for regional culinary purposes. The qualitative characteristics that best described the Cha'huá chili fruit were its slight transverse wrinkling and its pale green coloration, which

**Figure 3.** Cha'huá chili plants (*Capsicum annuum* L.) cv. Chahua with fruits at different stages of maturity. Photographs taken in the field by the authors.

Table 3. Qualitative and quantitative characteristics of the fruit of the Cha'huá chili (*Capsicum annuum* L.) cv. Chahua.

Feature	Value	Feature	Value
Anthocyanin spot	absent	Fruit weight (g)	4.39±1.66
Fruiting period (days)	60 days	Pedicle length (cm)	28.16±3.85
Fruit color stage I	Light green	Fruit wall thickness (cm)	1.35±0.27
Fruit color intermediate stage II	orange	Shape of fruit at junction with pedicel	obtuse
Fruit color mature III	red	Neck at base of fruit	absent
Fruit shape	elongated	Shape at apex of fruit	pointed
Fruit length (cm)	9.91±2.01	Flowering vestige	absent
Fruit width (cm)	1.10±0.20	Transverse wrinkling of fruit	mild

producers refer to as white. The fruit gradually transitions through two additional color shades before reaching its final red hue (Figure 3).

The morphological characteristics of the fruit exhibited moderate to high variability. Based on the obtained coefficient of variation (CV) values, the variation was greater than that reported by Escalera-Ordaz *et al.* (2019) in their morphological characterization of cultivated varieties of *Capsicum pubescens*. Among the quantitative traits, the pedicel length showed the lowest CV at 13.67%, while fruit weight was the most variable trait, with a CV of 37.81%, a value considered high. This variability is partially explained by the fact that Cha'huá chili is a landrace variety (Castillo-Aguilar *et al.*, 2023). In addition to the morphological characterization of the Cha'huá chili landrace, its pungency was determined, yielding values of $10.1057 \pm 0.922 \text{ mg g}^{-1}$ of capsaicin per gram of dry matter and $3.0317 \pm 0.2766 \text{ mg g}^{-1}$ of dihydrocapsaicin per gram of dry matter, equivalent to a total of 211,520.289 Scoville Heat Units (SHU). These values can be considered high, taking as a reference habanero chili (*Capsicum chinense* Jacq.), which has an average total Scoville rating of 250,000 SHU (Castillo-Aguilar *et al.*, 2023).

CONCLUSIONS

The most distinctive morphological traits of Cha'huá chili included the absence of anthocyanins in the seedling stage, coleoptile coloration, and leaf shape. During vegetative growth, the most notable characteristics were stem shape and pubescence, as well as leaf morphology. In the flowering stage, flower position stood out as a defining trait. Regarding the fruit, its sequential coloration throughout development was particularly distinctive, especially its light green hue in the early stages. The obtained morphological data should be considered preliminary due to the variation among Cha'huá chili ecotypes and potential environmental influences that may affect the expression of different morphological traits. The capsaicinoid content of Cha'huá chili proved to be significant when compared to values reported for other chili varieties in the Yucatán Peninsula, reinforcing its versatility for use in fresh, mature, and dried forms.

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Green manures (Fabaceae) and bokashi: An alternative to replace inorganic fertilizers in corn cultivation

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ABSTRACT

Objective: To determine the production of forage, green corn and corn grain depending on the bokashi (BOK) and green manures - *Crotalaria juncea* (CROJUN), *Canavalia ensiformis* (CAJCAJ) and *Cajanus cajan* (CANENS) used exclusively or combined with native efficient microorganisms (NEM) and arbuscular mycorrhizal fungi (AMF), in order to identify the best alternatives to replace inorganic fertilizers (IF) in the H-520 corn cultivation.

Design/methodology/approach: The following treatments were evaluated: 28 t ha⁻¹ of BOK+NEM+AMF, CROJUN, CAJCAJ, CANENS, CROJUN+NEM+AMF, CAJCAJ+NEM+MI, CANENS+NEM+AMF 100 and 50% of the IF 62-00-00 kg/ha of N-P₂O₅-K₂O (IF100 and IF50) and one unfertilized treatment.

Results: The biomass produced by CROJUN exceeded that of CAJCAJ, fertilization treatments had different effects on forage production, corn characteristics and yields of corn, totemoxtle, ears and grains, with BOK+NEM+AMF, CROJUN and CANENS standing out.

Limitations on study/implications: The study demonstrates that BOK and green manures are alternatives to replace IF. However, further research is needed to confirm these findings, adjust the amount of bokashi or to include other green manures, either exclusively or in combination with NEM and AMF.

Findings/conclusions: BOK combined with NEM and MI, as well as the green manures *C. juncea* and *C. ensiformis* with a biomass production of 2.1 or 6.3 t ha⁻¹ together with the biomass of local weeds, are agroecological alternatives that can promote forage, green corn and corn grain yields similar or higher than those obtained with IF.

Keywords: C/N ratio, H-520, Nitrogen, Soil fertility.

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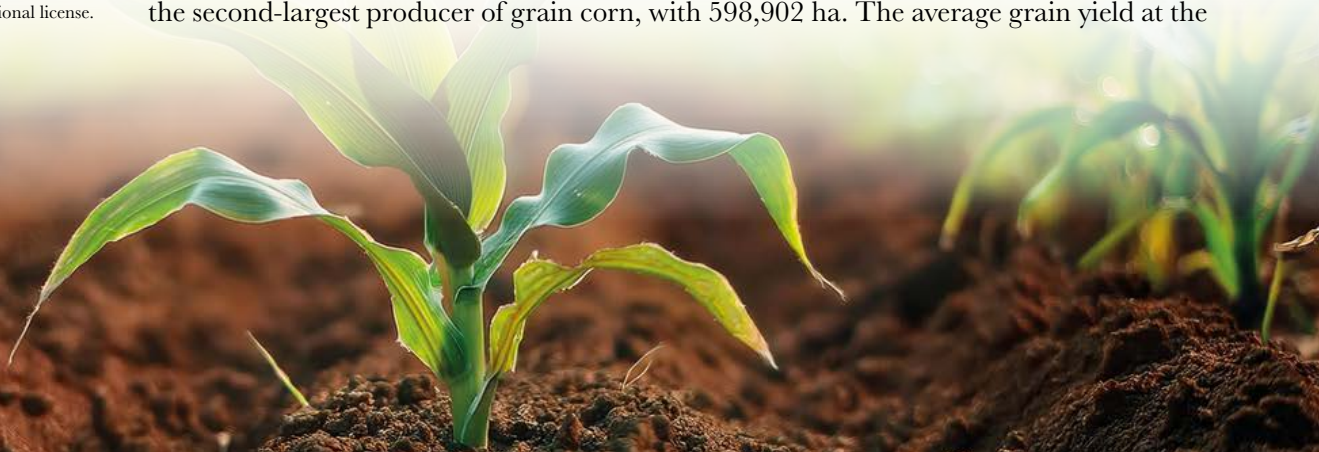
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INTRODUCTION

Corn (*Zea mays* L.) is the main agricultural crop in Mexico, with 6.4 and 0.5 million hectares dedicated to grain and forage production, respectively. The state of Veracruz is the second-largest producer of grain corn, with 598,902 ha. The average grain yield at the



national level and in Veracruz is 4.3 and 2.3 t ha⁻¹, respectively (SIAP, 2023). Regarding forage, Veracruz reports an area of 2,228 ha with an average yield of 20 t ha⁻¹, which is lower than the national average yield of 34 t ha⁻¹.

The 69% of corn is produced under rainfed conditions, while the remaining 31% is grown in irrigated areas (SIAP, 2023). Corn is primarily produced through a conventional system that includes the use of agrochemicals and inorganic fertilizers (IF). In Veracruz, it has been reported that for every ton of grain produced, the plant extracts 27, 11, and 23 kg of nitrogen (N), phosphorus (as P₂O₅), and potassium (as K₂O), respectively (Vásquez Hernández *et al.*, 2014). These nutritional requirements can be met in some regions by the nutrients already present in the soil, as in the case of potassium (K), while others must be supplied through an external source, such as nitrogen (N) and phosphorus (P). Fertilization rates reported for the state of Veracruz include 156-46-30 and 129-23-23 kg ha⁻¹ of N-P-K (Palafox-Caballero *et al.*, 2005; Capetillo-Burela *et al.*, 2021).

The production of plant-based foods through an agroecological system is a current necessity; therefore, it is essential to improve technologies focused on the nutrition of agricultural crops. Among the alternatives currently available to replace IF are the use of bokashi organic fertilizer (BOK), green manures (GM), native efficient microorganisms (NEM), and arbuscular mycorrhizal fungi (AMF).

BOK is a fertilizer that allows for the utilization of plant residues, manure, and regional minerals. There is no single recipe; therefore, the amount of nutrients it can provide to the soil will vary in each production unit. The GM technology involves using plants (mainly Fabaceae) as an exogenous source of N. The Fabaceae species form a symbiosis with nitrogen-fixing bacteria, and the fixed nitrogen is transferred to the Fabaceae plants. Once these plants are cut and deposited in the soil, the mineralization process begins. At this point, the nutrients become available to be utilized by the crop of interest (Matías-Ramos *et al.*, 2023). Both the effects of BOK and GM depend on the amount of nutrients they supply to the soil and the synchronization between the release of nutrients and the nutritional demand of the agricultural crop (Watthier *et al.*, 2023). NEM and AMF are microorganisms that help plants tolerate biotic and abiotic stress due to the production of secondary metabolites and the extension of the root system. They also contribute to the mineralization of organic residues, increasing the availability of nutrients for plants (Gómez-Leyva *et al.*, 2023; Oberholzer *et al.*, 2024). The effect of agroecological nutrition alternatives is directly related to climate behavior, and for rainfed conditions, the main factor is rainfall. Water availability will affect the mineralization process and nutrient transport. Therefore, production under rainfed conditions remains a challenge for corn cultivation, as nearly 70% of production occurs under these conditions. The objective of the study was to determine the production of forage, ear, and grain corn depending on the use of BOK and different species of GM applied in combination with NEM and AMF, with the aim of identifying the best alternatives to replace IF in corn production under rainfed conditions in the central coastal region of Veracruz, Mexico. The initial hypothesis is that at least one agroecological alternative will be identified that allows for a 100% replacement of IF in corn cultivation.

MATERIALS AND METHODS

Area of Study

The experiment was conducted at the Cotaxtla Experimental Field, belonging to the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), located in Medellín, Veracruz, Mexico (18° 49' 59" N; 96° 22' 59" W). The soil had a pH of 5.62, with 2.88% organic matter, and a loam texture (11, 41, and 48% clay, sand, and silt, respectively). The concentrations of N (nitrate), P-PO₄⁻², S-SO₄⁻², K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Cu²⁺, Mn⁺, and B³⁺ were 106.6, 60.0, 20.0, 680.0, 1990.0, 510.0, 45.3, 4.6, 5.3, 18.6, and 0.52 mg kg⁻¹, respectively. Temperature and precipitation data were recorded throughout the duration of the experiment (Figure 1).

Experimental design and treatments

The treatments consisted of the exclusive use of the green manures *Cajanus cajan* (CAJCAJ), *Canavalia ensiformis* (CANENS), and *Crotalaria juncea* (CROJUN), or their combination with NEM and seeds inoculated with the AMF *Rhizophagus intraradices* (40 spores g⁻¹) (CAJCAJ+NEM+AMF, CANENS+NEM+AMF, and CROJUN+NEM+AMF); BOK combined with NEM and AMF (BOK+NEM+AMF); IF applied at 100% and 50% of the recommended dose (IF100 and IF50); as well as a non-fertilized treatment (Control).

An experimental design in randomized blocks with three replications was used. The experimental unit consisted of 15 furrows, each 6 meters long. The IF dose was estimated using the rational method (Mata *et al.*, 2015). The target yield was 9 t ha⁻¹, with the assumption that for every ton of grain, 27, 11, and 23 kg of N, P₂O₅, and K₂O would be extracted (Vásquez Hernández *et al.*, 2014), which is equivalent to a requirement of 243, 103, and 206 kg ha⁻¹ of N, P₂O₅, and K₂O. Considering a soil bulk density of 1 g cm³ and a depth of 0.2 m, the soil availability of N, P₂O₅, and K₂O was estimated at 212, 275, and 1638 kg ha⁻¹, respectively. The inorganic fertilization included only the application of N, with a relative efficiency of 50%. The 100% and 50% doses corresponded to 62 and 31 kg ha⁻¹ of N.

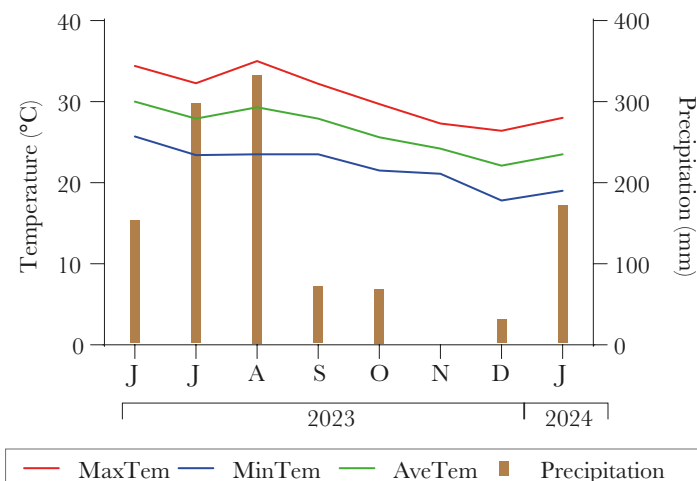


Figure 1. Behavior of maximum temperature (MaxTem), minimum temperature (MinTem), average temperature (AveTem), and precipitation from June 2023 to January 2024, in Medellín, Veracruz, Mexico.

Green manure

Soil preparation consisted of mechanical weeding, two harrowing passes, and furrowing. The distance between furrows was 0.8 m. For *C. juncea* and *C. cajan*, 4 grams of seed were used per linear meter (50 kg/ha). For *C. ensiformis*, 10 seeds were used per linear meter (125 kg/ha). The three GM species were sown on the same date (06/16/2023). No pest, disease, or weed control was carried out. Irrigation was solely dependent on rainfall. The cutting of above-ground biomass from the GM and weeds was carried out 56 days after sowing, using a mechanical weeder. In each experimental unit, the fresh weight of the GM and weeds was recorded in a 2 m² area.

To determine the dry biomass, representative samples of the GM and weeds were taken and dried in a forced air oven (at 70 °C) until a constant weight was reached. Using the data from the GM and weeds, the amount of plant biomass produced per hectare was estimated.

Corn establishment

Seven days after the biomass cutting, six passes of harrowing were performed to shred and incorporate the plant residues into the first 20 cm of soil. After 20 days of incorporating the biomass, two more passes of harrowing were carried out, and then the field was plowed. The distance between rows was 0.8 m. The corn H-520 was established (14/09/2023) at a density of 50,000 plants ha⁻¹ (with a plant spacing of 0.25 m). Two supplementary rolling irrigations were performed during the vegetative stage and at the beginning of corn flowering, which corresponded to 12 and 51 days after corn sowing (DAS). The control of lepidopteran pests was carried out through an application of the active ingredient cypermethrin at a commercial dose, 20 DAS.

Corn fertilization

FI100 and FI50 were applied foliarly in three applications (15, 25, and 35 DAP). In each application, 1 or 0.5 g of urea (46% N) per plant was used, diluted in approximately 8 ml of water, corresponding to a volume of 400 L ha⁻¹. The BOK was prepared (on 11/02/2023) using chicken manure, cow manure, pangola grass, soil, and sand, in a 2:2:2:1:1 (volume/volume) ratio, with a mixture of molasses and water (10% molasses). The characteristics of the BOK were as follows: 19.52% organic matter and 11.32% C; pH of 6.61, a C/N ratio of 12.58, and the following concentrations: 0.9% total N, 1.17% P₂O₅, 0.84% K₂O, 2.67% CaO, 0.68% MgO, 0.95% Fe²⁺, 0.03% Zn²⁺, 0.00% Cu²⁺, 0.05% Mn²⁺, and 0.03% B²⁺. The amount of BOK applied was determined based on its N concentration and a relative efficiency of 25%. To supply 62 kg ha⁻¹ of N, an equivalent of 28 t ha⁻¹ of bokashi in dry weight (551 g plant⁻¹) was applied. The BOK was applied once, 5 DAS, near the corn stalk and incorporated into the soil at the time of hilling.

The NEM used were prepared according to Rebolledo *et al.* (2012), using microorganisms obtained from local grass weed species. For every 150 L of water, the following inputs were used: molasses (15 L), grass residues (2 kg), corn flour (2 kg), wheat bran (2 kg), and charcoal (0.5 kg). For the treatments that included the application of NEM, a 5% concentration was

used, applying 8 mL per plant of the solution every 10 days, starting 5 DAS and concluding at the flowering stage (a total of five applications).

Forage and Corn Ear Production

At 82 DAS, in each experimental unit, plant density per hectare was estimated (in five linear meters), along with fresh biomass of forage, fresh weight of leaves, stems, and corn ears with husk. To determine corn ear quality, measurements were taken for ear diameter, fresh weight of ears with husk, ear length, number of grain rows, and estimated ear yield per hectare. Representative samples of each maize plant organ were collected and dried in a forced-air oven at 70 °C until reaching a constant weight. These data were used to estimate the dry weight of maize forage.

Corn Yield

At 152 DAS, grain harvest was carried out. In each experimental unit, the number of plants and the number of ears per plant were counted in 13 linear meters. In 26 ears, measurements were recorded for ear weight with husk, ear weight without husk, husk weight, cob weight, and grain weight.

Data Analysis

The data were analyzed using the statistical software InfoStat. Analysis of variance and Tukey's mean comparison test were performed, both at a 95% probability level ($p \leq 0.05$).

RESULTS AND DISCUSSION

Regarding the vegetative biomass of GM, the dry matter of CROJUN was similar to that of CANENS+ME+AMF and CROJUN+ME+AMF but superior to the others. Similar dry matter of weeds and total dry matter were detected among treatments (Table 1). The yield of fresh and dry forage detected with BOK+NEM+AMF was similar to that of CANENS, CROJUN, CANENS+NEM+AMF, and CROJUN+NEM+AMF but superior to that of CAJCAJ (Table 1). Lower fresh weight, diameter, length, and yield of corn ears were detected with CAJCAJ compared to CANENS, CROJUN, and BOK+NEM+AMF (Table 2). At the time of corn harvest, the totomoxtle yield of CROJUN+NEM+AMF was higher than that of FI50. The cob and grain yield obtained with BOK+NEM+AMF was similar to that of CROJUN and CROJUN+NEM+AMF but higher than the rest of the treatments (Table 2). The maximum forage yields achieved with CANENS, BOK+NEM+AMF, CROJUN, CROJUN+NEM+AMF, and CAJCAJ+NEM+AMF (35, 34, 31, 29, and 28 t ha⁻¹, respectively) are similar to or even higher than the 22-30 t ha⁻¹ reported by Sánchez Hernández *et al.* (2013; 2019) using the same planting density and variety, with the fertilization dose of 161-46-00 kg ha⁻¹ of N-P-K. Regarding the ear, Ortiz-Torres *et al.* (2013) and Fernández González (2015) report that the desirable characteristics are a weight between 370 and 480 g, yields between 14 and 18 t ha⁻¹, diameters of 5 cm, and lengths of 18 to 20 cm, with 15 to 18 rows of grain. These values are reported for ear varieties such as V-525 and A7573. In this study, none of the treatments reached the desired parameters, which is attributed to the variety used,

Table 1. Dry matter (DM) of green manures and weeds, as well as green and dry forage of corn managed with 100% and 50% inorganic fertilizers (IF), or with bokashi (BOK) and weed residues, *Cajanus cajan* (CAJCAJ), *Canavalia ensiformis* (CANENS), and *Crotalaria juncea* (CROJUN), either exclusively or combined with Native Effective Microorganisms (NEM) and seed inoculated with arbuscular mycorrhizal fungi (AMF), in Medellín, Veracruz.

Treatments	DM of green manure (t ha ⁻¹)	DM of weeds (t ha ⁻¹)	Total DM (green manure + weeds) (t ha ⁻¹)	Corn green forage yield (t ha ⁻¹)	Corn dry forage yield (t ha ⁻¹)
1. CAJCAJ	0.8 b	7.2 a	8.0 a	20 c	6 c
2. CANENS	2.1 b	6.7 a	8.8 a	35 a	10 ab
3. CROJUN	6.3 a	5.8 a	12.1 a	31 ab	8 abc
4. CAJCAJ+NEM+AMF	0.8 b	8.6 a	9.4 a	23 bc	7 bc
5. CANENS+NEM+AMF	2.4 ab	6.9 a	9.3 a	28 abc	8 abc
6. CROJUN+NEM+AMF	4.8 ab	4.0 a	8.8 a	29 abc	8 abc
7. BOK+NEM+AMF	-	6.5 a	6.5 a	34 a	11 a
8. IF100	-	5.2 a	5.2 a	24 abc	7 bc
9. IF50	-	8.7 a	8.7 a	23 bc	7 bc
10. Control	-	7.2 a	7.2 a	26 abc	8 abc
Coefficient of variation	80.51	52.34	46.03	31.53	32.09

Different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

as genetically, the size of the ear of the H-520 is smaller compared to ear varieties (Ortiz-Torres *et al.*, 2013; Fernández González, 2015).

Regarding grain yield, the maximum values detected in this study (4.4-5.0 t ha⁻¹) with the application of BOK+NEM+AMF, CROJUN, CROJUN+NEM+AMF, CANENS, and CANENS+NEM+AMF are higher than the national average of 4.28 t ha⁻¹ and the state of Veracruz's average of 2.29 t ha⁻¹. However, these values align with the average yield of the Veracruz District (5.4 t ha⁻¹), where production follows a conventional system that incorporates the use of mineral fertilizers (SIAP, 2023).

In this research, green manures, BOK, NEM, and AMF were alternatives used for agroecological maize nutrition. According to the results, the combination of BOK with NEM and AMF promoted the best outcomes, as 34 and 11 t ha⁻¹ of green forage and dry forage surpassed or were similar to those obtained with mineral fertilization (23-24 and 7 t ha⁻¹ of green and dry forage, respectively). Similarly, they promoted ears with greater weight (238 g) and size (5 and 16 cm in diameter and length), consequently achieving the highest yield of ears and grain per hectare (13 and 5 t ha⁻¹, respectively). The best effect of BOK is attributed to it being the treatment that supplied the greatest amount and diversity of nutrients to the soil. With 28 t ha⁻¹, it supplied approximately 4888, 2830, 225, 293, 210, 668, 170, 238, 13, 8, and 8 kg ha⁻¹ of organic matter, C, total N, P₂O₅, K₂O, CaO, MgO, Fe²⁺, Mn²⁺, Zn²⁺, and B²⁺, respectively. With these amounts, an increase of 0.24% in organic matter levels is expected in the top 20 cm of soil, as well as an increase in

Table 2. Characteristics of the ear and yield of ear, husk, cob, and grain of corn managed with 100% and 50% inorganic fertilizers (IF), or with bokashi (BOK) and residues from weeds, *Cajanus cajan* (CAJCAJ), *Canavalia ensiformis* (CANENS), and *Crotalaria juncea* (CROJUN), either alone or combined with Native Effective Microorganisms (NEM) and seed inoculated with arbuscular mycorrhizal fungi (AMF), in Medellín, Veracruz.

Treatments	Fresh weight of ear with bractea (g)	Diameter of ear with bractea (cm)	Ear length (cm)	Number of rows kernels of the ear	Yield (t ha ⁻¹)			
					Ear	Bractea	Cobs	Grain
1. CAJCAJ	128 c	3.8 b	13 b	12 a	7 b	0.7 ab	0.6 d	3.3 d
2. CANENS	218 a	4.9 a	16 a	12 a	12 a	0.7 ab	0.8 bcd	4.1 bc
3. CROJUN	221 ab	5.0 a	16 a	13 a	11 a	0.8 ab	0.9 ab	4.7 ab
4. CAJCAJ+NEM+AMF	173 abc	4.5 a	14 ab	13 a	9 ab	0.7 ab	0.8 bcd	3.8 cd
5. CANENS+NEM+AMF	191 abc	4.6 a	14 ab	13 a	10 ab	0.8 ab	0.8 bcd	4.0 bc
6. CROJUN+NEM+AMF	216 ab	4.9 a	15 ab	12 a	11 a	0.9 a	0.8 bcd	4.4 abc
7. BOK+NEM+AMF	238 a	5.0 a	16 a	13 a	13 a	0.8 ab	1.0 a	5.0 a
8. IF100	169 bc	4.4 ab	14 ab	13 a	9 ab	0.7 ab	0.7 cd	3.3 d
9. IF50	168 bc	4.4 ab	14 ab	12 a	9 ab	0.6 b	0.6 d	3.3 d
10. Control	180 abc	4.5 a	15 ab	12 a	9 ab	0.8 ab	0.7 cd	3.6 d
Coefficient of variation	27.57	12.17	13.18	12.98	33.53	60.14	33.60	37.35

Different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

cation exchange capacity. Improving these two soil characteristics has a positive impact on nutrient efficiency, as it ensures better moisture retention and enhances the soil's ability to retain nutrients in the rhizosphere zone (Martins Neto *et al.*, 2020). Despite these agronomic advantages of BOK, it is important to mention that this alternative also has limitations that producers should consider. The first limitation is the high amount of input required per hectare and per production cycle. In this study, during the preparation of BOK, an approximate cost of \$1,150.00 MXN (USD\$57.50) per ton was estimated, considering the 28 t ha⁻¹ used. This represents an investment of \$32,000.00 MXN (USD\$1600.00). In addition to this production cost, transportation and application expenses should also be taken into account. Therefore, this technology is recommended for small-scale producers who cultivate less than one hectare. It is also recommended for livestock producers or companies that have surplus manure and other vegetal and mineral resources, allowing them to produce their own inputs for forage production.

The positive effect of a GM increases as the amount of plant biomass produced rises, particularly when there is synchronization between nutrient release into the soil and the crop's nutritional demand (Wathier *et al.*, 2023). For this study, the results indicate that in the central coastal region of Veracruz, *Crotalaria juncea* and *Canavalia ensiformis* are promising options for use under rainfed conditions. Considering the production of green forage, dry forage, ear yield, and grain yield, *Crotalaria juncea* applied alone or in combination with ENF and AMF achieved values of 29-31, 8, 11, and 4.4-4.7 t ha⁻¹; in the case of *C. ensiformis*, applied alone or in combination with ENF and AMF, the values were 28-35, 8-10, 10-12, and 4.0-4.1 t ha⁻¹, respectively. The values achieved were

statistically similar to those obtained with BOK, and specifically for grain production, they were higher than those obtained with IF and unfertilized plants. The better effect of *C. juncea* and *C. ensiformis* is attributed to their greater growth capacity. In descending order, the total plant biomass supplied was 8.8-12.1, 8.8-9.3, and 8.0-9.4 t ha⁻¹ with *C. juncea*, *C. ensiformis*, and *C. cajan*, respectively. The total plant biomass produced in the different areas showed no statistical differences; therefore, the varying effects promoted by the green manures are mainly attributed to the amount of biomass produced by each Fabaceae. It is important to note that the weeds mainly consisted of non-leguminous species, with prominent grasses such as *Sorghum halepense* and *Cyperus rotundus*, which are characteristic of tropical regions in Mexico (González-Elizondo *et al.*, 2018; Herrera Solano *et al.*, 2023). Since they do not belong to the Fabaceae family, the main contribution of the weeds was the provision of organic matter and nutrient recycling. Therefore, the only exogenous nutrient was nitrogen, supplied through the biomass of the Fabaceae. Considering that the amount of nitrogen supplied to the soil depends on the amount of biomass produced (Watthier *et al.*, 2023), the nitrogen contribution was in the following descending order: *C. juncea* > *C. ensiformis* > *C. cajan*, with biomass production of 4.8-6.3, 2.1-2.4, and 0.8 t ha⁻¹, respectively. For the same geographical region, Ávila-Escobedo *et al.* (2022) report that for every ton of dry biomass produced by *C. juncea*, *C. ensiformis*, and *C. cajan*, 18.3-18.4, 24.7-26.1, and 14.9-15.1 kg of nitrogen are supplied to the soil, respectively. Therefore, for this study, it is estimated that nitrogen contributions were 88-116, 52-63, and 11.9-12.1 kg ha⁻¹, respectively.

The synchronization between nutrient release and the nutritional demand of the crop mainly depends on the C/N ratio of organic residues and climatic conditions. A lower C/N ratio results in a faster mineralization rate (Watthier *et al.*, 2022). Among the different organic residues used, the lowest C/N ratio was 12.6, corresponding to BOK. In contrast, for *C. juncea*, *C. ensiformis*, and *C. cajan*, the C/N ratios detected in the same study area were 21.6-24.3, 17.0-18.0, and 31.7-33.7, respectively, 75-89, 69-90, and 118-124 days after sowing, corresponding to the flowering stage (Ávila-Escobedo *et al.*, 2022). Considering that in this study, the green manure biomass was cut at 56 DAS, it is expected that the C/N ratio in the biomass of the three green manures was lower than that found during the flowering stage, due to reduced lignification of the plants. In the first three months following the incorporation of the plant residues, the temperature ranged between 21 and 35 °C, with an accumulated precipitation of 480 mm. Therefore, it is assumed that the environmental conditions were favorable for the mineralization of nutrients present in both the BOK and the plant residues, which helps explain the positive effects observed with these two technologies. Currently, the main limitation for the extensive use of GM technology is the availability and cost of seeds. For this study, the seed quantities used per hectare were 50 kg for *C. juncea* and 125 kg for *C. ensiformis*. The regional market price for the seeds is approximately \$130.00 and \$95.00 MXN (USD\$6.5-4.75), representing acquisition investments of \$6,500.00 and \$11,875.00 MXN (USD\$325-593.75), respectively. This value should be supplemented by the cost of soil preparation for the planting of these green manures. One advantage of green manures is that producers can grow their own seeds, reducing investment for subsequent cycles. When comparing GM and BOK from

an economic and operational standpoint, green manures emerge as a better option for producers, as they not only require lower investment but also simplify fieldwork. The focus is on soil preparation, planting, and incorporating the green manures, with the possibility of using agricultural machinery for all tasks. Therefore, for future work, it is recommended to consider the combined application of green manures with low doses of BOK.

CONCLUSIONS

In the central coastal region of Veracruz, under rainfed conditions, bokashi applied at a dose of 28 t ha⁻¹, along with indigenous efficient microorganisms and seeds inoculated with mycorrhizal fungal spores, as well as green manures such as *Crotalaria juncea* and *Canavalia ensiformis* with biomass production of 2.1 or 6.3 t ha⁻¹, along with local weed biomass, are alternatives that can promote forage, cob, and grain yields of *Zea mays* H-520 similar to or greater than those obtained with mineral fertilization (62-00-00 kg ha⁻¹ of N-P₂O₅-K₂O). Based on these results, the initial hypothesis of the research is accepted.

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Origin, evolution, currents of thought, and methodological implications of the agroecosystem concept: a review

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ABSTRACT

The objective of the research was to analyze the different currents of thought and to describe the origin and evolution of the concept of agroecosystems across different studies.

Methodology. A state-of-the-art analysis of the agroecosystem concept was conducted using the Web of Science platform, considering 225 articles with the TITLE “Agroecosystem” AND KEYWORDS “Agroecosystem.” Metrics such as year, country, sustainable development goals (SDGs), language, summary, and conclusions were also considered. A bibliometric analysis was performed using VOSviewer software, and graphs were created to visualize the bibliographic connections between the documents obtained from the Web of Science database.

Results. Articles from 1991 to 2024 were identified, with over 90% published from 2018 to the present. The top countries publishing this type of research are the USA, China, Canada, France, Germany, and Italy, accounting for 82% of the publications. The remaining 18% are spread across 44 countries worldwide. The predominant language is English (96%), followed by Spanish (3.1%) and Russian (1%).

Conclusions. The concept of agroecosystems is embedded in the social, cultural, political, and economic contexts, as all these aspects are directly related to agriculture, livestock, fishing, and other essential activities for feeding humanity. These activities are carried out within agroecosystems managed by humans for both commercial and self-consumption purposes, aiming to satisfy society’s demand for food, goods, services, and inputs.

Keywords: Society, crops, environment, ecology, soil health

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INTRODUCTION

The term ecology was coined by the German biologist Ernest Haeckel in 1870, referring to it as the study of the economy of nature; and today, many scientists define it as the science that studies the interrelationships between living organisms and their environment.

In a generalized way, agroecosystems (AES) can be defined as environmental systems modified and managed by humans to produce goods and services in a structured and diversified manner. Therefore, their conceptualization and application are adaptable to most of the living ecosystems on our planet. The idea behind agroecosystems is to conceive agriculture as a living, complex system that seeks to imitate natural processes and leverage ecological principles to increase productivity sustainably, conserving natural resources and promoting biodiversity (Stephen, 2015). It is known that agroecosystems integrate traditional knowledge of farmers with scientific advances in ecology, agronomy, and other disciplines to design agricultural systems that are resilient, efficient, and environmentally friendly. Additionally, they aim to promote crop diversification, the use of agroecological practices such as crop rotation, integrated pest management, organic fertilization, and the incorporation of biodiversity elements into agricultural systems (Méndez *et al.*, 2015).

The word agroecosystem is composed of two terms: agro-ecosystem; where agro refers to land, soil as a source of production; agriculture, derived from the Latin *ager* and *cultura*, is defined as the activity in which humans, in a given environment, manage natural resources, the quality and quantity of energy and information to produce and reproduce plants and animals to satisfy predominantly anthropocentric needs (Hernández, 1977). The concept of agroecosystems began to be used with the emergence of the journal *Agro-Ecosystem* (Harper, 1974). Three years later, the first seminar on Agroecosystems of Mexico was held in Mexico (Hernández, 1977). According to Hart (1979), an agroecosystem is an ecosystem that at least includes a population of agricultural utility; that is, it encompasses a biotic community and a physical environment with which this community interacts, and it typically includes populations of plants and animals. In this regard, it is important to mention that agroecosystems differ from natural ecosystems due to the presence of agricultural populations, and that the performance of an agroecosystem is regulated by human intervention. This intervention is generally planned, meaning that the farmer has a purpose to fulfill within the system and manages it by following a preconceived management plan that theoretically allows them to achieve specific objectives. Today, there are a number of definitions of agroecosystems because this term is a conceptual construct that refers to physical spaces that have been modified by humans to produce various social satisfiers, both material and non-material (Pérez, 1996). Agroecosystems, like ecosystems, include both biotic and abiotic components and the interactions between them. In this regard, Hernández (1977) mentions that an agroecosystem is an ecosystem modified to a greater or lesser extent by humans to use natural resources in the processes of agriculture, livestock, forestry, and wildlife production. Odum (1984) states that an agroecosystem is a domesticated ecosystem, with intermediate forms existing between natural ecosystems (such as grasslands, forests, etc.) and artificial ecosystems like cities. Conway (1987) mentions that an agroecosystem is an ecological system modified by humans to produce fiber or agricultural products. Marten (1988) states that an agroecosystem is a complex of air, water, soil, plants, animals, and microorganisms in a limited area that people have modified for agricultural production purposes.

The concepts and methodologies for agroecosystem analysis were developed at Chiang Mai University in Thailand by Conway (1986). This method is based on an ecological and

systematic approach to analyze agroecosystems in terms of their emerging properties such as productivity, stability, sustainability, and equity, and it was named Agroecosystem Analysis. Although agroecosystems are not exactly natural ecosystems, they can still be considered as ecosystems, and in general, all ecological concepts such as energy flow, material cycling, and others are applicable in their study. There are three types of agroecosystems: those with a crop subsystem (which can include annuals, perennials, forest trees, etc.), those with an animal subsystem, and those with both crops and animals (Krishna, 2013). Based on the above, the objective of the present research was to analyze the different currents of thought regarding the concept of agroecosystems, in order to describe the origin and evolution of the concept through various identified studies.

MATERIALS AND METHODS

Stage I. Information Search

In the first stage, a state-of-the-art analysis of the concept of agroecosystems was conducted through articles included in the platforms Google Scholar, Scielo, Web of Science, and Redalyc. A search was carried out using the word and concept of agroecosystem, which resulted in several articles being displayed. Those that contained the desired word in the title, abstract, and keywords were selected, resulting in a total of 137 articles. Subsequently, the Web of Science platform was used to conduct a more in-depth search and analysis, allowing the download of the document database. The search rubric TITLE “agroecosystem” AND + KEYWORDS “agroecosystem” was applied, resulting in a total of 225 articles.

Stage II. Information Analysis

A metrics analysis was conducted, which included the year, country of publication, Sustainable Development Goals (SDGs), language, DOE task, abstract, and conclusions. Additionally, a bibliometric analysis of the resulting articles was carried out using the VOSviewer software, a tool developed by the Center for Science and Technology Studies (CWTS) specifically designed to visualize bibliometric networks (Toro, 2017). This software generated graphs representing bibliographic connections between the documents obtained from the Web of Science database. These graphs included citations, keywords, years of publication, and other bibliometric elements (Guallar *et al.*, 2020). To represent the frequency of documents by country, the information was exported into a world map using Quantum GIS.

RESULTS AND DISCUSSION

Currently, there are a significant number of definitions of the concept of agroecosystems, and this is due to the fact that this term is a conceptual construction referring to physical spaces of various spatial dimensions that have been modified by humans to produce various social satisfiers, both material and non-material (Pérez, 1996). This concept has evolved over time and continues to undergo constant changes to this day, reflecting an increasing understanding of the complex interactions between the social, ecological, and economic components of agriculture. Below is a brief description of this ongoing evolution of the

concept of agroecosystems, clarifying that this evolution has not been linear, as different approaches coexist today, each with its own perspective: Early agronomic approaches (19th century - early 20th century): focus on production – agriculture was viewed as a simple system for food production, with an emphasis on maximizing efficiency and yield (Liebig, 1843 and King, 1911). Agricultural ecology (mid-20th century): recognition of ecological complexity – agriculture began to be understood as an ecosystem with interactions between crops, pests, natural enemies, and the environment (Odum, 1953 and Watt, 1968). Agroecology (1970s - present): holistic and social approach – agroecology became established as a discipline that seeks the sustainability of agricultural systems, integrating ecology, economics, and social dimensions (Altieri, 1987 and Gliessman, 2007). Agroecology for food sovereignty and social justice (current): focus on social justice and food – the importance of agroecology for the construction of just, equitable, and sovereign food systems is recognized (Holt *et al.*, 2013 and IPES-Food, 2016).

Some of the recent contributions to the concept of agroecosystems that have been identified are listed below. However, it is important to mention that this field of agroecosystems is in constant evolution, and it is highly likely that new contributions to this area of science will frequently emerge:

- **Agroecology as a Transdisciplinary Science:** This section acknowledges the need for a transdisciplinary approach that integrates different knowledge and perspectives to address the complexity of agroecosystems (Bacon and Koontz, 2015).
- **Focus on Resilience and Adaptation to Climate Change:** It highlights the importance of agroecology in building agricultural systems resilient to climate change and other environmental challenges (IPCC, 2019 and Gliessman, 2016).
- **Urban and Periurban Agroecology:** The fundamental role of urban and periurban agriculture in food security and the sustainability of cities is recognized (McClintock and Jabbour, 2014 and Orsini *et al.*, 2013).
- **Agroecology and the Sustainable Development Goals:** This approach recognizes the contribution of agroecology to achieving the Sustainable Development Goals (SDGs), such as food security, poverty, health, and climate change (FAO, 2018 and IPES-Food, 2016).
- **Agroecology and Social Justice:** The role of agroecology in building fair and equitable food systems that guarantee the right to food for all people is emphasized (Holt, 2017 and Desmarais and Wittman, 2019).

The emergent properties of agroecosystems are characteristics or behaviors that cannot be predicted from the properties of their individual components; they generally arise from the complex interaction between the different elements of the agroecosystem, such as crops, pests, natural enemies, soil, water, and climate, primarily. According to Conway (1987), Gliessman (2007), Altieri (1987), and Marten (1988), the main emergent properties—productivity, resilience, diversity, stability, and equity—are important for the overall sustainability of agriculture. Therefore, understanding these properties is crucial for designing and managing more resilient, productive, and equitable agroecosystems.

The health of agroecosystems is a relatively new concept, despite the fact that throughout history, agriculture has largely been based on the exploitation of natural resources, without much attention to the long-term sustainability of resources, leading to soil degradation, loss of biodiversity, water pollution, among other factors. In recent decades, there has been a growing interest in the health of agroecosystems due to various factors, such as increasing concerns about food security, climate change, environmental degradation, and others (Gliessman, 2007; Altieri, 1987; FAO, 2018). As a result, the health of agroecosystems is currently a major topic in most research areas, as new, more sustainable agricultural practices are constantly being developed, which in turn help protect the health of agroecosystems. It is worth mentioning that the indicators of agroecosystem health are variable and allow for the evaluation of an agroecosystem's status and its capacity to function sustainably. These indicators can be biological, physical, chemical, or socioeconomic in nature. The concept of agroecosystems has important methodological implications for agricultural research and management. These include the need for a holistic, multidisciplinary, participatory, long-term, systems-based, adaptive, and agroecological approach. According to Gliessman (2007), IFOAM (2008), and FAO (2018), the most relevant approaches include the holistic approach, multidisciplinary approach, participatory approach, long-term focus, participatory research methods, qualitative research methods, quantitative research methods, systems approach, adaptive approach, and agroecology approach.

It was found that, of the articles published between 1990 and 2023, 82% of the 225 articles found were published between 2018 and 2023. This indicates that most of them are recent, with an average of six years of age.

These articles present the latest research advancements focused on the study of agroecosystems worldwide, covering aspects such as soil, water, environment, society, and other disciplines.

On the other hand, the term Agroecosystems, implicit in the Sustainable Development Goals (SDGs), was found to be related as follows: 32% corresponds to the goal of life on land, followed by 25% for climate action, 21% for zero hunger, 7% for sustainable cities and communities, and finally, 6% for life below water. Regarding the language in which the 225 articles obtained from the Web of Science platform were written, it was found that 96% are published in English, followed by 3% in Spanish, and finally, the remaining 1% is written in Russian. These results show that the highest activity in research focused on and/or related to agroecosystem topics is in developed countries where the predominant language is English. However, it is worth mentioning that research on agroecosystems is also being conducted in Central American countries, but these studies are likely published in journals from other disciplines not included in the analyzed platform. The types of documents found in the Web of Science platform related to the word "agroecosystems" show that 88% of these documents are scientific articles, followed by 6% consisting of conference proceedings, and finally 3% being review articles, respectively. These data show a positive impact on the global scientific community, which is continually updating this type of information related to agroecosystems. In other words, science is in constant evolution, seeking new scientific contributions that will undoubtedly benefit society as a whole. Finally, in Figure 1, you can

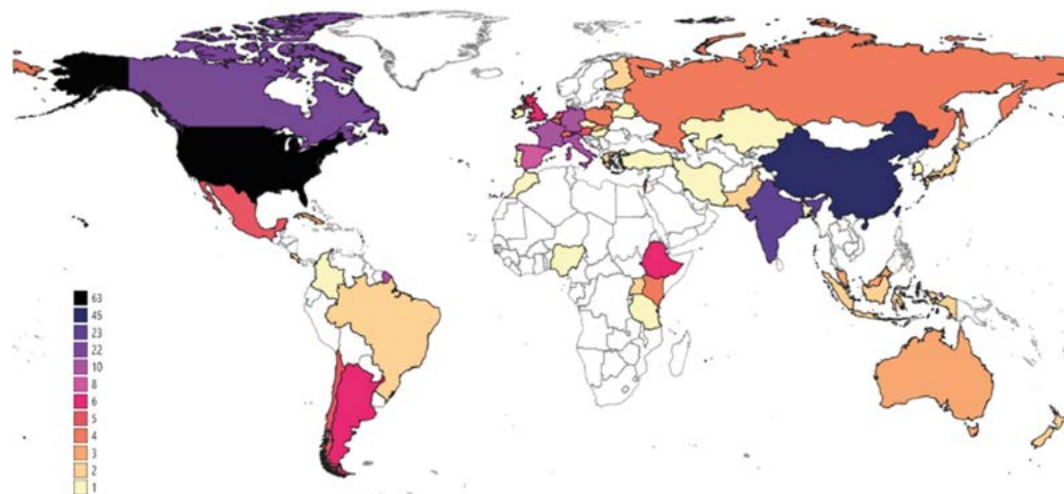


Figure 1. Number of documents published by countries worldwide, based on data obtained from the Web of Science platform for the 225 articles containing the term “Agroecosystems”.

observe the countries where these types of documents have been published. The highest number of articles published corresponds to countries in South America, with the color indicating the number of publications related to the term “agroecosystems”.

CONCLUSIONS

The activity carried out allowed for an expansion of the origin and concept of agroecosystems, which can generally be understood as the concept of AGS as an evolving conceptual construction in which reference is made to the use and application of geographic spaces of various sizes, where humans use them to select plants and animals to meet the constant demand for food and other requirements that society needs for survival on this planet. The concept of agroecosystems is implicit in the primarily social, cultural, political, and economic aspects and/or factors, as all these terms are directly related to agriculture, livestock, fishing, and other basic activities for human food production, which are developed through the agroecosystems managed by humans for commercial purposes or subsistence in meeting society’s basic demands for food, goods, services, and inputs.

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Soil application of sulfur in the production of chocolate habanero pepper (*Capsicum chinense* Jacq.)

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ABSTRACT

Objective: The cultivation of chocolate with habanero chili was established to determine the effect of the application of agricultural sulfur on its production.

Design/methodology/approach: The experiment was conducted from August to July, under field conditions and plastic mulch in Apatzingán, Michoacán, Mexico. Treatments consisted of wettable agricultural sulfur applied at doses of 15, 30, 45 and 60 kg ha⁻¹, plus a control without sulfur application. The sulfur was incorporated into the soil around the plants 10 days after transplanting.

Results: The number of fruits per plant was significantly lower in the control treatment compared to the 15 and 45 kg ha⁻¹ of sulfur doses. Similarly, fruit length and diameter were smaller in the control treatment compared to all sulfur doses. No significant differences in fruit weight were observed between the control and the sulfur treatments. The highest fruit yields were achieved with sulfur doses of 15, 30, and 45 kg ha⁻¹, yielding 22.5, 20.6, and 22 t ha⁻¹, respectively.

Limitations on study/implications: Sulfur fertilization constitutes an effective strategy to increase yields under the experimental conditions.

Findings/conclusions: Applying 15 kg ha⁻¹ of sulfur is sufficient to enhance fruit production in chocolate-type habanero peppers grown in alkaline soils.

Keywords: fruit diameter, fruit number, fruit yield.

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INTRODUCTION

From the Solanaceae family, the genus *Capsicum* is the most economically important, encompassing approximately 30 species. Its fruits have been used for about 7,000 years BC, initially as part of the diet of native Mexicans (Govindarajan, 1985; Dong *et al.*, 2014). Among the most significant species is the habanero chili (*Capsicum chinense* Jacq.), recognized as one of the chilies with the highest capsaicin content, reaching up to 131.8 μm g⁻¹ of dry fruit (Duelund and Mouritsen, 2017). In Mexico, 30,056 tons were produced



in 2023, representing a value of 670 million pesos (MXN) (SIAP, 2024). Approximately 80% of habanero chili production is marketed as fresh fruit, while the rest is used for the production of sauces, pastes, and dehydrated products (FIRCO, 2017). There is significant diversity in fruit colors, primarily orange, red, purple, yellow, and chocolate (López-Espinosa *et al.*, 2018). The latter is mainly produced in Michoacán and is highly valued for its pleasant flavor. However, the yield in this region is 6.05 t ha^{-1} , which is below the national average of 18.64 t ha^{-1} (SIAP, 2024).

An alternative to increasing agricultural crop yields is proper nutrition, which, under soil cultivation conditions, primarily relies on the supply of nitrogen, phosphorus, and potassium. Sulfur is the fourth or fifth most required macronutrient for plants; however, fertilization with this nutrient is not commonly recommended for crops grown in soil (Barros-Milhomens *et al.*, 2020). Sulfur in plant nutrition enhances the availability of phosphorus (P), potassium (K), zinc (Zn), manganese (Mn), and copper (Cu); it reduces soil pH and promotes the conversion of unavailable P into plant-available P. Sulfur is also required for the synthesis of essential amino acids such as cysteine and methionine (Hunde, 2020). Additionally, it is necessary for chlorophyll formation, which plays a critical role in physiological functions related to energy production (Aula *et al.*, 2019; Narayan *et al.*, 2023). Moreover, sulfur strengthens plant defenses against pest attacks, nutrient stress, and high temperatures (Hunde, 2020).

A significant effect has been demonstrated in increasing yield and improving the quality of agricultural crops such as onion (*Allium cepa* var. *cepa*), barley (*Hordeum sativa* var. *nutans*), and cabbage (*Brassica oleraceae* var. *capitata alba*) with the application of sulfur at doses ranging from 40 to 80 kg ha⁻¹ (Skwierawska *et al.*, 2008). Although in crops such as grasses, the response increases as the sulfur dose is raised up to 50 kg ha⁻¹, higher amounts result in yield reduction due to antagonistic effects (Aula *et al.*, 2019). In contrast, in other crops such as potato (*Solanum tuberosum* L.), even when grown in soils with very low sulfur levels, no response was observed to the addition of this element at doses of 10 and 20 kg ha⁻¹ (Giletto *et al.*, 2012). For this reason, it is essential to determine the response of each crop, as well as the most appropriate sulfur dose. In chocolate-type habanero chili, studies on this topic are scarce. Therefore, the objective of this study was to evaluate the effect of agricultural sulfur application on fresh fruit yield and yield components, as well as to identify the optimal sulfur dose for maximizing fruit yield in chocolate-type habanero chili.

MATERIALS AND METHODS

Study Area

The study was conducted at the experimental field of the Faculty of Agricultural Sciences of the Michoacan University of San Nicolás de Hidalgo in Apatzingán, Michoacán, located at the coordinates 19° 04' 56" N and -102° 22' 15" W, at an altitude of 325 meters above sea level. The climate is classified as BSh'g, corresponding to a very hot semi-arid steppe climate (García, 2004). During the crop cycle, maximum temperatures ranged from 37 to 43 °C, minimum temperatures ranged from 13 to 20 °C, and the total accumulated rainfall was 710.2 mm. These data were provided by

the Department of Surface Waters and River Engineering, Hydrometeorology Unit of Apatzingán, whose agrometeorological station is located at the study site.

Seedling Establishment and Crop Management

The research was conducted from August to July, beginning with the sowing of seedlings in 242-cell polystyrene trays, which were previously disinfected by immersion in a solution of water with sodium hypochlorite at a dose of 5 mL L^{-1} , and then washed with water to remove the chlorine. Subsequently, the trays were filled with peat moss, and one chocolate-type habanero chili seed was placed in each cavity. The trays were placed under shade netting. Irrigation was provided daily in the morning according to the crop's water needs. Prior to transplanting, the soil was prepared with a plowing, followed by a harrowing and furrowing at 80 cm between rows. The irrigation system was then installed using plastic drip tape and agricultural mulch 1.20 m wide, Calibre 90, silver/black color. Transplanting was done 40 days after sowing, with one plant placed every 40 cm, resulting in a density of 31,250 plants per hectare.

Prior to the establishment of the crop, at a depth of 0-30 cm, the soil was classified as loam with a slightly alkaline pH (7.8), medium levels of inorganic nitrogen (21.7 mg kg^{-1}) and phosphorus (18.6 mg kg^{-1}), high in potassium (976 mg kg^{-1}), high in calcium (5454 mg kg^{-1}), high in magnesium (1854 mg kg^{-1}), medium in sulfur (9.2 mg kg^{-1}), cation exchange capacity (CEC) of 45 cmol kg^{-1} , and an apparent density of 1.13 t m^{-3} ; according to the Mexican Official Standard NOM-021-SEMARNAT-2000 (DOF, 2002). The fertilization provided during the crop's production cycle was 130-120-160 kg ha^{-1} of N, P_2O_5 , and K_2O . The fertilizers used to supply the nutrients were: Urea (45% N), Potassium nitrate (13% N - 44% K_2O), and Monoammonium phosphate (12% N - 61% P_2O_5). The fertilizers were applied through a fertigation system. Irrigation was provided according to the crop's water needs, ensuring that the crop was always maintained at field capacity.

Treatments and Experimental Design

The treatments consisted of the application of 93% agricultural wettable sulfur at doses of 15, 30, 45, and 60 kg ha^{-1} , compared to a control without application. The treatments were applied as a base fertilizer, 10 days after transplanting, buried around the plant. The experimental design was a randomized complete block design, with each treatment repeated four times, resulting in 20 experimental units. Each experimental unit consisted of three rows, each 3 meters long. For the useful plot, five plants from the central part of the middle row were selected.

Response Variables

Several fruit harvests were made as they reached harvest maturity (coffee-colored fruits). The harvest started 120 days after transplanting and spanned a six-month period. In each harvest, five fruits were randomly selected per experimental unit, and their equatorial diameter (cm) and length (cm) were measured using a caliper. The total number of harvested fruits was counted, and their weight was recorded using a digital scale to

determine the number of fruits per plant and the average fruit weight. The fruit yield per hectare was estimated as follows:

$$Yield (t ha^{-1}) = (\text{number of plants per hectare}) \times (\text{fruit weight per plant})$$

Statistical Analysis

The response variable data were analyzed using the SAS statistical package, version 9.4 (SAS, 2017). Variables that showed statistically significant differences were subjected to Tukey's mean comparison test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The application of sulfur at all evaluated doses favored an increase in the number of fruits per plant, as well as the length and diameter of the fruits. With the application of 15 and 45 kg ha⁻¹ of sulfur, the habanero pepper plants showed the highest number of fruits per plant, with increases of 57.2% and 47.5%, respectively, compared to the control plants. Regarding fruit length and diameter, these were similar across all sulfur doses and statistically higher than those of the control. The plants with sulfur had fruits with lengths 22 to 36% greater than those from plants without sulfur application, and diameters 45 to 63% larger than those from plants without the nutrient. Similar fruit weight was observed between the control plants and the four sulfur doses (Table 1).

The higher number of fruits per plant with sulfur application can be attributed to this element, as it favors the fruit setting percentage and, consequently, the number of fruits per plant in *C. annuum* (Kumari *et al.*, 2017). This, along with the increase in fruit length and diameter, resulted in a significant increase in fruit yield in the plants with sulfur application in the present study. The highest increases were recorded with the doses of 15, 30, and 45 kg ha⁻¹ of sulfur (Figure 1).

The positive response to sulfur application in the habanero chili crop can be attributed to the fact that it is an essential element in plant nutrition, important for chlorophyll

Table 1. Tukey's mean comparison test of the evaluated variables in chocolate habanero pepper.

Sulfur doses kg ha ⁻¹	Number of fruits per plant	Fruit length (cm)	Fruit diameter (cm)	Average weight of fruits (g)
0 (Control)	110.9 b [¶]	2.36 b	1.79 b	4.38 a
15	174.3 a	2.89 a	2.82 a	4.14 a
30	142.8 ab	3.20 a	2.91 a	4.60 a
45	163.6 a	2.93 a	2.59 a	4.32 a
60	138.6 ab	3.01 a	2.87 a	4.19 a
General Mean	146.0	2.88	2.59	4.32
Prob. de F	**	**	**	NS
DMSH _{0.05}	41.2	0.50	0.52	0.75
CV%	12.53	7.69	8.93	7.71

[¶]Means with the same letters within the same column do not differ statistically from each other (Tukey, $p \leq 0.05$). ** $p \leq 0.01$; NS=not significant. DMSH_{0.05}=honest minimum significant difference. CV=coefficient of variation.

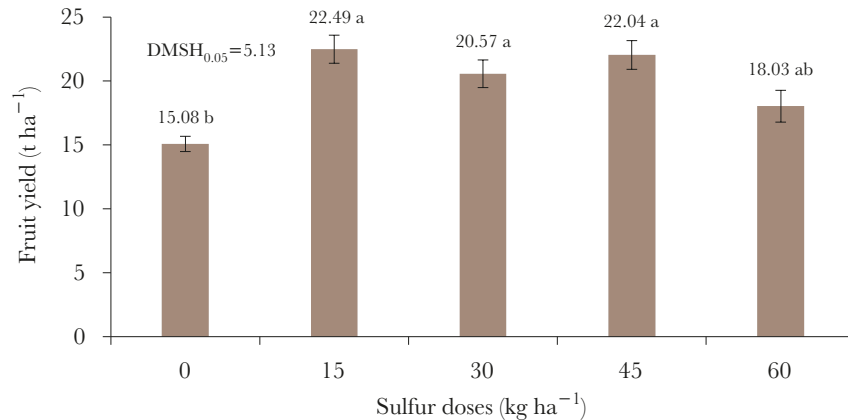


Figure 1. Habanero chili fruit yield as a function of sulfur dose. $DMSH_{0.05}$ =honest significant minimum difference.

formation, secondary metabolites, and necessary for physiological functions, growth, and plant development. Additionally, there are proteins containing Fe-S groups that are necessary for multiple biological processes, such as photosynthesis, energy generation, photoprotection, and metabolism (Aula *et al.*, 2019; Narayan *et al.*, 2023). Sulfur is also an element that, at appropriate levels in the plant, reduces damage caused by oxidative stress and high temperatures, such as those occurring during the growth cycle of the crop, which, on some days, reached 43 °C. In relation to its interaction with other nutrients, it has been found that sulfur increases the absorption of N, P, and K, which can improve the supply and enhance plant nutrition (Aula *et al.*, 2019; Narayan *et al.*, 2023).

It has been found that the application of sulfur increases crop yield as the application rate increases, when the sulfur content in the soil is low or medium and the pH is very acidic or alkaline. However, most crops show a decrease in yield at doses equal to or greater than 50 kg ha⁻¹ (Oliveira *et al.*, 2017; Nascente *et al.*, 2017), which may explain why habanero chili in our study showed a slight reduction in fruit yield at doses higher than 45 kg ha⁻¹ of sulfur (Aula *et al.*, 2019).

In the production site, according to the physical and chemical analysis, the soil has a medium sulfur content (9.2 mg kg⁻¹), so the addition of this element favored the production of habanero chili fruit. These results are similar to those found in *C. annuum* L. variety LCA-334, where the application of S at doses of 7.5, 12.5, 15, 20, and 25 kg ha⁻¹ significantly increased the fresh chili yield, with the dose of 7.5 kg ha⁻¹ showing the highest yield increase, which was 65.8% compared to the control treatment with no application (Kumari *et al.*, 2017).

Similarly, in wheat (*Triticum aestivum* L.) cultivation, with an initial sulfur content of 110.2 mg kg⁻¹ in the soil, the application of 40 kg ha⁻¹ of sulfur increased the grain yield by 27 and 22%, a result attributed to the stimulatory effect on chloroplast protein synthesis, which leads to greater photosynthetic efficiency, favoring agricultural yield (Singh *et al.*, 2014).

The addition of sulfur to soils with medium sulfur levels does not guarantee improved productive responses in crops, as occurred in potato (*Solanum tuberosum* L.) cultivation with

the supply of sulfur at doses of 10 and 20 kg ha⁻¹, which did not increase tuber yield (Giletto *et al.*, 2012). Similarly, no improvement was observed in canola with increasing doses of sulfur up to 60 kg ha⁻¹ as ammonium sulfate (Kaefer *et al.*, 2014). Therefore, the variation in the agricultural yield response to sulfur supply and the most appropriate dose, which differs for each crop, indicates variability in the requirements between plants (Narayan *et al.*, 2023). In the present study, where improvements in production were recorded with the addition of sulfur at doses of 15, 30, and 45 kg ha⁻¹, habanero chili yields exceeded the national average of 18.64 t ha⁻¹ (SIAP, 2024). Therefore, sulfur supply as a fertilizer is a viable option that enhances the yield of this crop under the given production conditions, with 15 kg ha⁻¹ of sulfur being sufficient to boost production, as it results in the highest increments. Although higher doses do not reduce yield (they remain stable), they can reduce profitability due to the cost of a larger amount of sulfur.

CONCLUSIONS

The application of agricultural sulfur as a base fertilizer affects the production and fruit dimensions of habanero chili. To obtain a higher number of fruits per plant, fruits with greater length and diameter, as well as higher yield per hectare, the most appropriate dose is 15 kg ha⁻¹ of wettable sulfur.

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Compost production through the aerated static pile method

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ABSTRACT

Objective: To evaluate and validate the method of compost production by aerated static piles (ASP), in comparison with a traditional method to optimize the composting process.

Design/methodology/approach: The static pile system (ASP) was designed and established for compost production at the Huimanguillo Experimental Field (Tabasco, Mexico), where composts of different manures were used in a completely randomized experimental design to evaluate nutritional and quality parameters of the composts to compare with the traditional method.

Results: It was found that the ASP method allows obtaining composts with higher nutritive values than those generated by the traditional method. In addition, it satisfies the required quality.

Limitations of the study/implications: It is suggested to study more doses of the composts generated by ASP in different crops and stages.

Findings/conclusions: The ASP method by injecting more air during the composting process, raises microbial activity, which in turn, raises compost temperatures, achieving higher fixation of nutrient compounds.

Keywords: manures, compost, quality, aeration, organic agriculture.

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INTRODUCTION

The More than 24 million hectares are organically cultivated in the world, the countries with the largest cultivated area are Australia, with 10 million hectares, followed by Argentina and Italy (Zink, 2022). Mexico occupies the 18th place in the world, with 216,000 hectares, located as a producer-exporter of organic food and the first producer of organic coffee (Forbes, 2018; Willer *et al.*, 2023).

In organic agriculture, organic matter (OM) is the fundamental basis, and many countries practice the agricultural use of residues (Ballesteros *et al.*, 2018). This agriculture contemplates the structural diversity and ecological management of the soil, which are not carried out in traditional agriculture (Oviedo-Ocaña *et al.*, 2017). Its short-term effect is



slow, due to the nutrient content of the inputs used, remaining available to the plant for a longer period of time.

Composting is an aerobic biochemical process, which degrades organic material, such as organic residues (OR), macro and microorganisms, resulting in compost. This is a replica of the natural system of decomposition of materials, but with anthropic intervention, shortening the decomposition time of materials, improving the final product (Labrador, 2001), obtaining direct and indirect benefits to the soil, increasing the OM, nutrient content, structure and edaphic mesofauna. In composting, a complex array of microbial populations develops as a function of temperature, nutrient availability, oxygen concentration, moisture content and pH (Ballesteros *et al.*, 2018). It is necessary to maintain adequate aeration for the process to take place under aerobic conditions, as well as a humidity of 50%, thus allowing the presence of decomposing microbes. Composting is a highly dynamic process, regulated by the interaction of multiple variables such as temperature, oxygen level, moisture content and accessibility to essential nutrients, all of which directly affect the microbial communities responsible for the biological decomposition process.

The active composting process goes through different phases to obtain the final product: mesophilic phase I (heating), in which the crushing of the materials by the mesofauna and the participation of mesophilic bacteria are intensified; in the thermophilic phase (sanitization), biochemical degradation by fungi and actinomycetes is intensified; and in mesophilic phase II (cooling), bacterial degradation of residual substances is pronounced and the activity of the mesofauna returns in the larger particles. Thus, composting contributes to the reduction of both weight and volume of the original materials by loss of gases and the synthesis of various substances such as water vapor, ammonia, nitrogen oxides or nitrates (according to oxide-reduction potential), sulfur dioxide, prehumified substances, assimilable elements, which results in a finished compost (García-Silva *et al.*, 2023). This method is essentially an aerobic activity; therefore, insufficient oxygen supply necessary for aerobic degradation by microorganisms leads to a significant slowdown in the decomposition of organic materials (Michel *et al.*, 2022).

Oxygenation, as a determinant of the environment, is crucial, as microorganisms require oxygen for metabolic energy generation, efficient growth and intensification of matter consumption. This oxygenation process consists of replacing the low-oxygen air present in the compost piles with a stream of fresh, oxygenated air. Under natural conditions, such gaseous exchange occurs when air heated by composting reactions rises through the pile, generating a suction effect that draws in fresh air from the surrounding environment. However, this process can be conditioned by several factors, such as wind intensity, the water content of the material and the degree of porosity present in the pile (Lim *et al.*, 2017). In this sense, the need arises to test and generate new methods to optimize the composting process in order to reduce processing times and increase the production of composts such as the composting in aerated static piles (ASP), consists of composting in elongated piles on a network of aeration pipes where air is supplied to provide an aerobic medium for composting (Longoria *et al.*, 2014).

The advantage of this method is that it does not require a periodic turning, in the initial phase an adequate mixture of materials should be given since, once the piles are

built, the same control system indicates the obtaining of a mature compost (DOF, 2002). In organic agriculture under tropical conditions in Tabasco, Mexico; the optimization of the composting process is still incipient, since manual turning is traditionally done as an alternative to oxygenate the compost piles, delaying the composting process, increasing the labor requirements and therefore the production costs of the composts. For this reason, the objective of the present research is to evaluate and validate the method of compost production by aerated static piles (ASP) in comparison with a traditional method and its application in indicator plants. Furthermore, it is the first time that this type of technology has been developed to improve the production and quality of compost.

MATERIALS AND METHODS

Study sites. The work was carried out from September to November 2022 in the organic module of the facilities of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Campo Experimental Huimanguillo, located at the geographic coordinates 17° 51' 07" N, 93° 23' 46" W. The climate of the area is warm and humid with abundant rainfall in summer, with an approximate annual range of 2,200 to 2,600 mm and absolute maximum and minimum temperatures of 14 °C and 45 °C (Salgado *et al.*, 2017). The organic module has a surface area of 300 m² in the open air.

Aerated Static Piles System (ASP). The structure of the system allows the establishment of three piles, it is composed of polyvinyl chloride pipe (PVC) sanitary or hydraulic type of 4" diameter, for each pile, consists of a central tube where the materials to be composted will be placed, in the final part a lid of the same material was installed to avoid air loss. The area of the pipe or main tube where the compost is placed, consists of rows of perforations in the upper middle part, each 1/2" in diameter, separated at a distance of 5 cm in length and 2 cm in width. These perforated tubes are connected to a 4" diameter ball valve of the same material (PVC), which allows the distribution of air as required, or can allow disabling the use of any tube if it is not necessary, which allows adapting the system to a certain production of compost, in smaller or larger quantities.

The keys are connected to a distributor pipe with T couplings and 90° elbows with the same dimensions and materials (PVC) which in turn is connected to the distributor pipe, which is connected with 45° elbows to the two outer piles. It also has a secondary pipe that allows directing the air to other compost piles if required, this also has a ball valve that regulates the air flow, which increases the possibility of staggered production.

The directional tubes are connected to a centrifugal extractor with a WEG[®] brand electric motor with a capacity of 1/3 HP, configured for nominal work of 127/220 volts, which couples a Francis type turbine, with a spiral or snail box of 26-gauge smooth sheet, with a distributor or impeller with galvanized sheet blades of 10 cm wide with a suction mouth of 8" and exhaust outlet adapted to a 4" pipe, providing a constant air supply of 7,600 m³/hour (Figure 1).

For the joints of the materials, it is recommended to use PVC glue, in order to avoid air loss in the joints of the pipes and faucets, and to maintain a correct flow towards the compost piles. Aeration of the pile is done twice a day, the interval is determined by the external

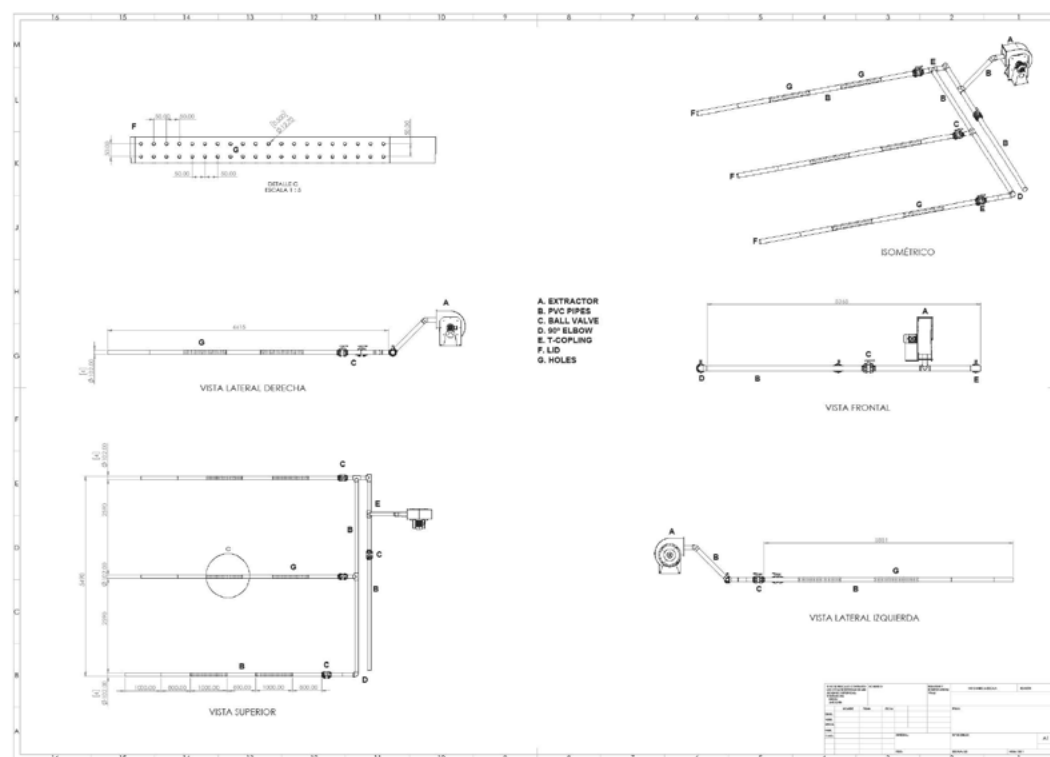


Figure 1. Design of the Static Pile System for compost production at the INIFAP-Huimanguillo Experimental Field.

temperatures, emphasizing that these are done during the hours of higher temperature, which can be from 30 minutes to 2 hours as required. The system can continuously supply air to the composite stack. This will be determined by size, type of materials, job site and environment.

Method validation. The validation method of the ASP system was compared with the traditional methods of compost production, which in the same way was established in the organic module of the INIFAP-Huimanguillo Experimental Field. Twelve beds measuring 1.40 m long and 1.40 m wide were used, with a 15 cm thick sand base, thus avoiding direct contact with the soil, and a plastic bag was placed on top of this base to avoid leaching of the composts (Figure 2).

Three types of manure were evaluated for a period of 72 days: bovine (treatment 1), horse (treatment 2) and sheep (treatment 3) in a completely randomized experimental design with three replicates each, and a control for each treatment, placed in the same way in the tubes of the aerated method. The useful bed was 1.20 m long by 0.80 m wide, with a space of 0.50 m between each bed. The treatments used in both methods had the same composition, modifying only the type of manure used. The formulation used was manure (bovine, sheep or horse) 50% of the wet weight, 20% of loam (forest soil), 10% of fresh weeds, 5% of ash, 5% of phosphoric rock, 5% of sawdust, 5% of cocoa husk, 1% of molasses. The methodology used for the composts consists of sieving, grinding, weighing, mixing and humidity of the mixtures.



Figure 2. Organic Module and evaluation beds in the INIFAP-Huimanguillo Experimental Field.

The variables taken during the evaluation period were: temperature inside the compost, temperature, environment and humidity, which were taken with a Brannan[®] brand alcohol thermometer with a capacity of up to 150 °C, introducing 15 cm inside the compost to obtain the humidity of the different piles. For relative humidity, a Sharp[®] Model 63-1032 digital thermohygrometer was used; the sensors were introduced in the center of the different piles to obtain this data. In addition, a physical-chemical analysis was performed to determine the nutritional contribution of the different composts (treatments) according to the Official Mexican Standard NOM-021- RECNAT-2000 and the Official Mexican Standard NMX-FF-109-SCFI-2008 (DOF, 2008), taking random samples for each treatment.

Evaluation of composts with habanero chile (*Capsicum chinense* Jacq.) as indicator plant

The composts obtained from the different treatments were evaluated by means of indicator plants, using habanero bell pepper (*Capsicum chinense* Jacq.) plants obtained from a commercial nursery with 45 days of germination. A completely randomized design with six treatments and four replications was used for the evaluation. The treatments consisted of compost percentages equivalent to 25%, 50%, 75% and 100%, plus an absolute control (100% sand) and a chemical treatment with application of fertilizer with an application formula of 180-180-150 (N, P, K). Sand was used as a complementary inert substrate, so that there was no external incorporation of other nutrients and the effect of the composts was reflected.

Two habanero bell pepper seedlings were used per 30×15 cm black polyethylene bag filled according to the treatments mentioned above, obtaining a total of 56 bags and 112 plants. The plants were established in the greenhouse of the Huimanguillo experimental field, in order to reduce the effect of pests and diseases. The agricultural practices used were daily irrigation with 500 ml of water per bag, control of fungi and mites with fungicide (Propamocarb+Fosetyl and Carbendazim).

Weekly data were collected for a period of five weeks to obtain the development of these plants. The variable evaluated was plant height. This data was obtained with a graduated ruler, starting from the base of the apical plant of the head. An analysis of variance and mean test (Tukey $p=0.05$) was performed with the SAS statistical package (SAS, 2004).

The composting process, both traditional and ASP, lasted 37 days, in which the ASP system was supplying air to the composts. The effect of the method was reflected in the variables evaluated during the active period, which allowed the elaboration of composts by both methods, with the variables described below.

RESULTS AND DISCUSSION

Behavior of environmental variables. Temperature, composts finalized using the aerated static pile system showed a maximum temperature of 49 °C and a minimum of 28 °C, with an overall average of 26.1 °C. During the thermophilic phase, internal compost temperatures can increase by up to 45% (Docampo, 2013). The highest temperature peaks occurred on days 13, 19, 22 and 30, representing 48% of the evaluation period.

Aeration reduced temperature peaks, contributing to the sterilization of pathogens, molasses seeds and other harmful microorganisms. For non-aerated composts, the maximum temperature was 48 °C and the minimum was 27 °C, with a mean of 32 °C, the maximum temperatures were reached in the period of 19 days, similar to the behavior of a traditional compost (García-Silva *et al.*, 2021).

Microbial activity increases the temperature, an exothermic process essential for rapid decomposition of organic matter, which must be maintained between 30 °C and 60 °C, since below this temperature the decomposition process slows down and above this temperature microorganism cannot survive (Ruíz, 2013).

Humidity, was monitored manually and maintained in an optimum range of 70-80% by applying water with molasses. Relative humidity presented a maximum of 90% before aeration and a minimum of 41%, with an overall average of 77.8%. During aeration, the maximum humidity was 83% and the minimum 41.3% with an overall average of 68.2%. Although the humidity decreased, it remained within the optimum range of 40-70%, suitable for the composting process (García-Silva *et al.*, 2021). Relative humidity is crucial for the availability of water in the compost, essential for microbial activity (Zhang *et al.*, 2021). This is relevant in the environmental conditions of the state of Tabasco, which is considered a with excellent humid tropical conditions, where relative humidity exceeds the national average due to its humid climate (Olvera-Rincón *et al.*, 2024).

Physico-chemical analysis. During decomposition, the pH of the composts increased, ranging from 7.5 to 8.5. The compost aerated with sheep manure presented the highest pH (8.04). These values are different from those found in previous studies in Figueroa (2014) and Ge *et al.* (2022), which reported a pH of 7.5 and 6.82, respectively. Higher pH in aerated composts indicates better substrate decomposition. The composts contain less exchangeable hydrogen ions, more calcium and magnesium (Meena *et al.*, 2021). Analyses showed higher amounts of these minerals in aerated composts compared to the traditional method Table 1.

Table 1. Physico-chemical analysis of bovine, sheep and horse manure composts with ASP aeration and traditional method without aeration.

Identification		EC	OM	N	P	K	Ca	Mg	Na	CEC	Fe	Cu	Zn	Mn	Humidity	Ashes	OC	C/N Rat.	AD
Description	pH	dS m ⁻¹	%		mg kg ⁻¹	cmol (+) kg ⁻¹					mg kg ⁻¹				%			G mL ⁻¹	
Bovine manure without aeration	7.52	2.33	60	2.10	35.64	2.00	5.56	1.43	0.30	43.82	95.61	43.77	10.05	10.9	24.54	22.10	34.80	16.57	0.52
Bovine manure with aeration	7.31	2.51	65	2.35	39.75	2.51	6.30	1.82	0.35	48.63	108.90	54.81	10.80	11.4	26.76	24.70	37.70	16.04	0.54
Horse manure without aeration	7.95	2.03	59	2.48	43.55	2.62	6.86	2.15	0.55	53.28	201.04	66.75	14.93	10.1	25.10	20.03	34.22	13.80	0.65
Horse manure with aeration	8.15	2.08	62	2.71	49.26	2.90	7.87	2.62	0.59	56.86	215.78	81.34	15.68	11.6	27.20	20.20	35.96	13.27	0.68
Sheep stool without aeration	8.05	2.12	63	2.52	51.82	2.45	7.82	2.61	0.68	58.37	248.20	78.10	17.41	14.1	33.58	24.78	36.54	14.50	0.72
Sheep stool with aeration	8.61	2.45	68	2.95	56.01	2.73	8.80	2.97	0.80	60.15	268.89	83.44	18.62	15.3	34.45	25.16	39.44	13.37	0.75

Electrical conductivity (EC), according to the Mexican standard NMX-FF109-SCFI-2008 (DOF, 2008), EC values in the composts were maintained within the optimal range (<4 dS m⁻¹), coinciding with the results reported in Vázquez *et al.* (2017). Higher values can reduce plant growth and cause soil salinization (Orden *et al.*, 2021).

The analyses showed that the amount of organic matter (OM) in the aerated and non-aerated treatments had an overall mean of 60%, exceeding the content established by NMX-FF109-SCFI-2008, which differs with the OM values (50.06% and 54.06%) in a study under similar conditions in Whang *et al.* (2022).

Macronutrients: nitrogen, phosphorus and potassium, in the composts were superior compared to the traditional method, all within the ranges established by NOM-021-RECENAT-2000. The nitrogen content was higher than those reported in Qiu *et al.* (2021).

Although nitrogen is lost in the thermophilic phase (García-Silva *et al.*, 2021), aeration reduces this loss. Phosphorus levels were lower than those reported in Liu *et al.* (2023), but not toxic. The composts under this method maintained high potassium levels.

The Cation Exchange Capacity (CEC) contained in aerated composts was found to be: Sheep 60.15 cmol (⁺) kg⁻¹, Horse 56.86 cmol (⁺) kg⁻¹, and Bovine 48.63 cmol (⁺) kg⁻¹, higher than those found in the traditional method: Sheep 58.37 cmol (⁺) kg⁻¹, Horse 53.28 cmol (⁺) kg⁻¹, and Bovine 43.82 cmol (⁺) kg⁻¹, the results obtained were similar in Vázquez *et al.* (2017).

Microelements: Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn). The composts with the ASP system showed higher amounts of these elements compared to the traditional method, all within the ranges established by NOM-021-RECNAT-2000.

Carbon to nitrogen ratio (C/N). The ratio obtained in this element in the different substrates with the ASP system was: Bovine 16.04, Sheep 13.37, Horse 13.27, similarly a minimal but greater difference was obtained in the treatments with the traditional method, which were: Bovine 16.57, Sheep 14.50, horse 13.80, unlike the sheep treatment which was presented higher in the traditional method to the aerated ASP treatment. According to NMX-FF-109-SCFI-2008 (DOF, 2008), the data obtained are within the indicated range. A C/N ratio between 12 and 20 is necessary for stability and maturity (Li *et al.*, 2022).

The Bulk Density (AD) obtained was within the indicated range (0.40 to 0.90 g/ml⁻¹), according to NMX-FF-109-SCFI-2008 (DOF, 2008), and higher than reported in Peña *et al.* (2020).

Aeration favors the activity of aerobic microorganisms, such as bacteria and fungi, which efficiently decompose organic matter. These microorganisms require oxygen to carry out their metabolism and oxidize complex organic compounds, such as carbohydrates, proteins and lipids, into simpler compounds (Nguyen *et al.*, 2020). This oxidation process produces carbon dioxide (CO₂), water (H₂O), and heat as subproducts. In aerobic composting, decomposition occurs primarily in the presence of oxygen, resulting in faster and more controlled decomposition than in anoxic conditions, where decomposition can be slower and less efficient, producing compounds such as methane (CH₄), which is a greenhouse gas (Peng *et al.*, 2023).

The oxidation of organic compounds was favored by ASP since the method presented the highest nutrient values of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn, which makes the ASP composted manure product valuable for its nutrient contributions (Elmrini *et al.*, 2022). CO₂ generated in the aerobic process is easily manageable and does not contribute significantly to climate change, unlike methane. In addition, aerobic decomposition also generates compounds such as volatile organic acids (acetic acid, formic acid) and essential nutrients (nitrogen, phosphorus and potassium), which enhance the compost and make it more beneficial to the soil (Michel *et al.*, 2022). Our findings are similar to those reported in other works where the composting process was optimized through ASP compared to traditional aeration methods, where nutritional values were higher in composts processed by ASP method (Table 1), than traditionally (Íñiguez-Covarrubias *et al.*, 2019; Abdoli *et al.*, 2019; Bondeson *et al.*, 2023). The control of aerobic conditions also prevents the formation of toxic compounds such as hydrogen sulfide (H₂S), which

is produced under anaerobic conditions and can be detrimental to both microorganisms and plants (Manga *et al.*, 2023).

Compost pH is a crucial factor affecting microbial activity and the quality of the final compost. During composting, aerobic microorganisms produce organic acids such as acetic acid, butyric acid and lactic acid, which can reduce the pH of the composted material (Bondeson *et al.*, 2023). However, aeration helps to regulate this acidification process by promoting the activity of oxygen utilizing microorganisms involved in the production of acidic compounds. At the same time, these aerobic microorganisms, as they break down organic matter, also generate basic compounds that act as buffers, preventing excessive pH drop. If the pH of the compost drops too low, it can become toxic to plants and negatively affect plant growth when the compost is used as an amendment. This was shown in the ASP treatments with the horse and lamb composts that ended up with a higher pH in contrast to the traditional aeration method (Table 1). Too low a pH can hinder the availability of nutrients and affect the balance of beneficial microorganisms in the soil (De los Santos *et al.*, 2022). Likewise, the microbial communities in the ASP system received more O₂, which theoretically increased microbial activity and compost temperature and more frequently, causing the specific heat to be high in water, which implies that drier compost mixtures can heat up and cool down faster than wetter ones.

Indicator plants. During the five weeks of evaluation of the indicator plants of habanero pepper, which were in the vegetable greenhouse at the Huimanguillo Experimental Field, the results of the behavior of the evaluated variable were obtained, according to each of the composts used Table 2.

In general, the chemical treatment obtained superior results in comparison with the treatments of the different types of compost (manure). The 25% and 50% treatments showed a similar response in the variable evaluated for the bovine compost treatment, which is similar to what was found in 2020 (Mendoza *et al.*, 2020), where similar percentages (25%-75% organic fertilization) were used in the plant height variables. In the case of sheep compost, there was no significant difference ($p=0.05$) between the different percentages used, but there was in comparison with the chemical treatment (Table 2). For the behavior

Table 2. Results of the height variable at five weeks of habanero bell pepper indicator plant under different concentrations of three types of compost.

Bovine compost	Mean	Sheep Compost	Mean	Horse Compost	Mean
CT	15.25* a	CT	15.25* a	CT	15.25* a
25%	11.50 ab	25%	9.25 b	25%	9.75 b
50%	11.25 ab	100%	9.00 b	50%	8.75 bc
75%	8.75 b	75%	9.00 b	T000	8.50 bc
100%	8.50 b	T000	8.50 b	75%	8.25 bc
T000	8.50 b	50%	8.25 b	100%	7.75 c
CV	20.24	CV		CV	7.96

*Means with the same letter are statistically equal (Tukey $p=0.05$). CT: Chemical treatment. CV: Coefficient of variation.

of the horse manure-based compost, a significant difference ($p=0.05$) was found between the chemical treatment and the 25% treatment; however, for the other percentages (50%, 75%) and the control (T000), there was no difference between the treatments, but there was a difference in comparison with the 100% percentage.

The tendency of greater vegetative growth was expressed in the chemical treatment, which may be due to the fact that, during the first phenological stages of the crop, it requires high amounts of macronutrients, including N, which could be supplied by chemical fertilization, where the availability of N is immediate (Chen *et al.*, 2021). In the case of the 100% manure treatments, the behavior of the indicator plants was similar to that of the control (Mendoza *et al.*, 2020), the behavior of the indicator plants was similar to the absolute control (T000), this may be due to the fact that manures are an important source of N in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Meena *et al.*, 2021), in addition to other nutrients available to the plant, which can cause a degree of intoxication by saturation in the substrate, which is expressed in the plant as slow growth, and can even cause plant death (Table 2). In addition, the composts generated by the ASP method are more nutritionally complete substrates, having more elements and in greater quantities, which is reflected in the electrical conductivity greater than 2 dS m^{-1} of the evaluated materials (Michel *et al.*, 2022). In addition, habanero bell pepper seedlings are sensitive to EC values higher than 1.5 dS m^{-1} (Luna-Fletes *et al.*, 2021; Liu *et al.*, 2023). Therefore, it is necessary to continue exploring different doses to obtain the optimum for this crop.

The only limitations in which the method may be compromised is the lack of electrical power for the operation of the electric motor and that does not allow the aeration to be carried out. Another limitation that can affect the results is excess rainfall. If the compost production is an outdoor area, and this is done in rainy seasons in an area with high rainfall, it can generate a problem of excess water and slow down the composting process.

CONCLUSIONS

Implementation of the ASP method to optimize the composting process showed that it is possible to generate composts from manures with good nutritional quality compared to those generated in the traditional way and with pH, OM and bulk density parameters within the standards established in the Official Mexican Norm (NOM). The increase in the nutritional values of the composts with the ASP method is due to the greater air input that increases microbial activity, raising the temperature of the composts and allowing the decomposition of the materials faster and in greater quantities than the traditional process. Further evaluation of different doses of composts generated by the ASP method for horticultural crops is needed.

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EcoCrop model approach for agroclimatic suitability of rice (*Oryza sativa* L.) cultivation

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ABSTRACT

Objective: To evaluate the suitability of commercial rice (*Oryza sativa* L.) cultivation in the state of Tabasco, Mexico; using the EcoCrop model to improve policy decisions on the crop.

Design/methodology/approach: A crop suitability analysis was carried out for the state of Tabasco using the EcoCrop model. The data for the variables of temperature, solar radiation, humidity and precipitation were taken from the WorldClim database (2023) for the period 1960-2010, with a spatial resolution of 1 km². The soil information was downloaded from INEGI (2023). The modeling was carried out in R studio with the terra, geodata and Recocrop libraries.

Results: The capacity of the area at a regional level to supply the national demand for rice was determined. The model predicted a high climatic suitability of rice in areas where it grows optimally.

Limitations on study/implications: It is suggested that simulation be explored for future situations of the areas currently identified in Tabasco for establishing rice cultivation.

Findings/conclusions: The considerable potential of this approach to assess global and regional food security problems and general climatic constraints is highlighted.

Keywords: Crop suitability, agroclimatic zones, ecocrop, *Oryza sativa*.

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INTRODUCTION

Rice is the world's most important staple food, feeding more people than any other crop (Fulkagawa, 2019; USDA, 2022). The International Rice Research Institute (IRRI) in the Philippines has reported that rice feeds 4 billion people, or 56% of the world's population (IRRI, 2024), making it the most important crop on the planet (Muthayya *et al.*, 2014; FAOSTAT, 2023). In Mexico, with the current agricultural policies, it has begun

to promote various basic grains such as rice cultivation to achieve food sovereignty. Under this context, it is important to define the potential areas where rice cultivation can grow and provide that information to decision makers and politicians (Tello and Corral, 2023; AGRICULTURE, 2024).

The state of Tabasco during the period 1980-1985, managed to have up to 15 thousand hectares of rice established (Jiménez-Chong *et al.*, 2017), however, due to a series of problems, mainly low profitability forced this crop to stop being planted and depend on imports in about 95% from USA (Hernández-Aragón *et al.*, 2016; Pureco *et al.*, 2017; SE, 2024). The soil and/or land of different geographical areas do not have the same suitability for crops; therefore, the development of spatial modeling to evaluate land suitability by integrating different techniques and diverse agroclimatic requirements, allows the development of strategic actions that protect water resources and land, and thus implement sustainable agricultural systems (García *et al.*, 2021). Therefore, the agricultural-environmental suitability analysis contributes to adequate land use planning and distribution, as well as to sustainable agriculture (Córdova-Sánchez *et al.*, 2023; Olvera-Rincón *et al.*, 2024).

The EcoCrop model uses the FAO EcoCrop database, which compiles the environmental requirements of an extensive list of plant species and can be used to identify potential crops in a specific environment (Dhanya *et al.*, 2023). This model allows zoning and spatial analysis of the agroecological suitability of particular crops. The EcoCrop model is used to predict the adaptation of a particular crop considering temperature and precipitation thresholds over a geographic region (Yarlequé *et al.*, 2024). The model calculates the suitability index for temperature and precipitation independently, and then integrates both to obtain a final suitability rating from very suitable to unsuitable zones. In this work, the climatic and edaphic layers were integrated to further delimit the analyses of the Ecocrop model as an alternative to agroecological zoning. The main objective of the research was to evaluate the suitability of commercial rice cultivation in the state of Tabasco, Mexico, using the EcoCrop model to improve policy decisions on rice cultivation.

MATERIALS AND METHOD

Study area. The state of Tabasco is located in the southeast of Mexico, south of the Gulf of Mexico, between the extreme latitudes 18° 39' 07" N to the north and 17° 20' 24" N to the south, and between longitudes 90° 59' 16" W to the east and 94° 07' 40" W to the west. It borders the Gulf of Mexico to the north, Campeche to the northeast, Guatemala to the east and southeast, Chiapas to the south, and Veracruz to the west (Salgado-Velázquez *et al.*, 2020). The continental surface of the state covers 24 694.60 km², which represents 1.3% of the national territory, placing it in 24th place with respect to the states with the greatest extension, however, it hosts on average 23% of the national biodiversity of vertebrates and 53% of the nation's freshwater wetlands (CONABIO, 2024). Tabasco's location in the tropical zone, its low elevation with respect to sea level and its proximity to the Gulf of Mexico along 191 kilometers (km) of coastline (Figure 1), determine the development of warm climates with maritime influence. The humid tropical climate is a very unique characteristic of the region. Temperatures range from 15 °C in the coldest months (January and December) to 44 °C in the hottest months; the average temperature



Figure 1. Geographic location of the State of Tabasco. Own elaboration with data from INEGI (2024).

is 26 °C, which due to the low altitude in relation to sea level remains constant. Annual precipitation ranges from 1800 to 3500 mm, with a dry season from April to May and a rainy season from August to October (CONAGUA, 2024).

Database. The EcoCrop model uses the FAO ecocrop database and requires mainly climatic data of minimum and maximum temperature (°C), precipitation (mm/month), wind speed (m/s) and solar radiation (W/m^2) for the region. For this purpose, the WorldClim2 database (Fick and Hijmans, 2017), in a 3-minute grid (1 km spatial resolution), was used in the R studio GIS environment for climate data. The edaphic layer was downloaded from INEGI for Tabasco with a resolution of 1 km, which was integrated in the model. FAO threshold values for growing season, maximum and minimum temperatures, and precipitation for rice cultivation (Hunter and Crespo, 2019) were considered in the EcoCrop model (Table 1).

Data extraction in R. Gridded (2-5 m) climate data from WorldClim were downloaded, extracted into a dedicated folder and also imported into the EcoCrop module. WorldClim is a global gridded dataset with monthly climatological means of maximum, minimum and mean temperatures, and total precipitation, developed by thin-plate spline interpolation from long-term (1950-2010) weather station records (Ramírez *et al.*, 2014). The R studio libraries geodata, ecocrop, terra were used. The geodata library allows the manipulation of climate variable files in raster format. The ecocrop library allows accessing the database of optimal growing conditions of crops and processing the modeling. The terra library was used to manipulate and edit the layers resulting from the modeling.

Table 1. EcoCrop parameters used for analysis of crop suitability for rice.

Parameters	Rice	Units
Gmin	110	Days of growth
Gmax	180	
Tkmp	40	°C×10
Tmin	110	
Topmin	210	
Topmax	280	
Tmax	340	
Rmin	950	mm
Ropmin	1500	
Ropmax	2000	
Rmax	3900	

Modeling of suitable zones with Ecocrop in R. Once the climatic and soil layers were processed, and the mask was applied to the state of Tabasco with the Recocrop function of the Ecocrop library, the raster files were loaded into the model to generate the suitable zones for rice cultivation. Finally, the generated map was visualized and saved as an image using the ggplot2 library. Likewise, the areas of each zone were quantified. Agroclimatological suitability zones were classified as not suitable, moderately suitable, suitable and very suitable (Chhogyel *et al.*, 2018). Therefore, a quantile class partitioning was performed by means of the break class quantile function to generate the classes based on the model score (Møller *et al.*, 2021).

RESULTS AND DISCUSSION

Climatic variables for Tabasco. Figure 2 shows the climatic variables used in the Ecocrop modeling, in monthly average values for the period 1950-2010.

It is observed that for the period 1950-2010 the average behavior of precipitation, temperatures and solar radiation show trends according to the geography of the state of Tabasco. The southern part of Tabasco has the highest precipitation (3500 mm), the lowest temperatures (<20 °C) and wind speed (<2 m/s). The highest wind speed occurs in the northern part close to the coast. The highest solar radiation values are present in the eastern part of the state, especially in the Los Ríos region (Navarro *et al.*, 2022).

EcoCrop model for Tabasco. Figure 3 shows the regionalization of suitable areas for rice cultivation according to the results of the EcoCrop model (FAO, 2023). Four classes are highlighted: not suitable, moderately suitable, suitable and very suitable (Heinz *et al.*, 2024).

The areas with the best conditions for the establishment of rice cultivation, according to their level of aptitude, are concentrated in the Chontalpa region, mainly in the municipalities of Cárdenas (66,235 ha), Huimanguillo (25,245 ha), Cunduacán (51,658 ha) and Comalcalco (38,875 ha); in the Los Ríos region, with Balancán (31, 611 ha) and Jonuta (34, 125 ha); and in Centro (10, 147 ha) (Figure 3), representing 10.3% (258,696 ha)

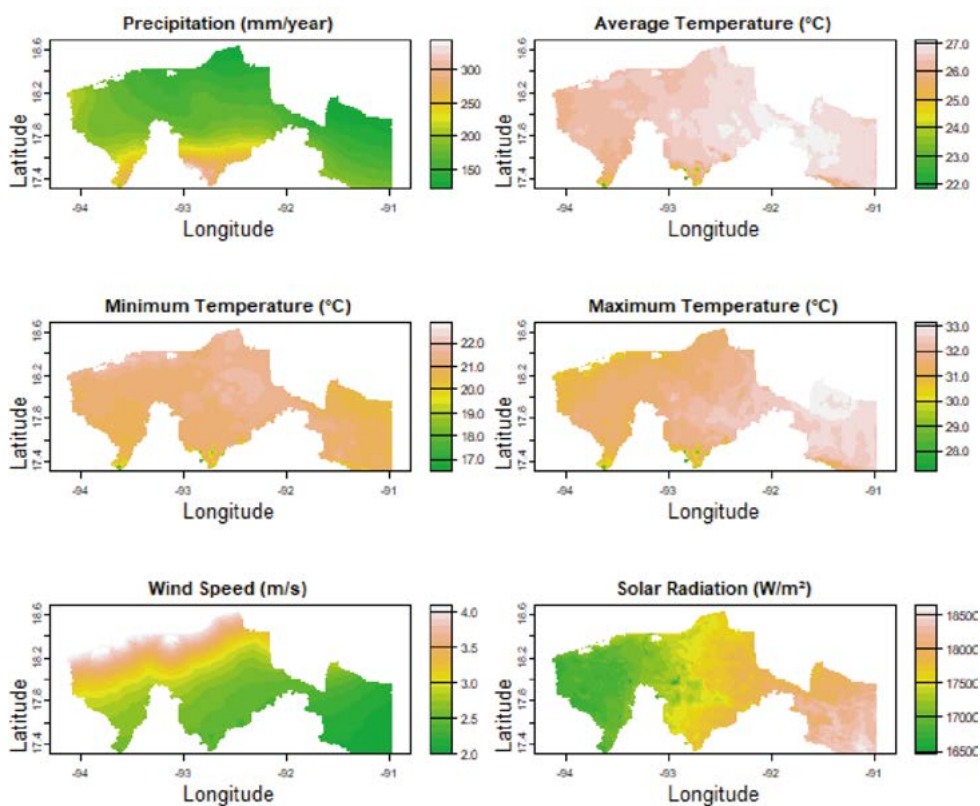


Figure 2. Monthly averages of climate variables for Tabasco for the period 1950-2010 from the WorldClim database.

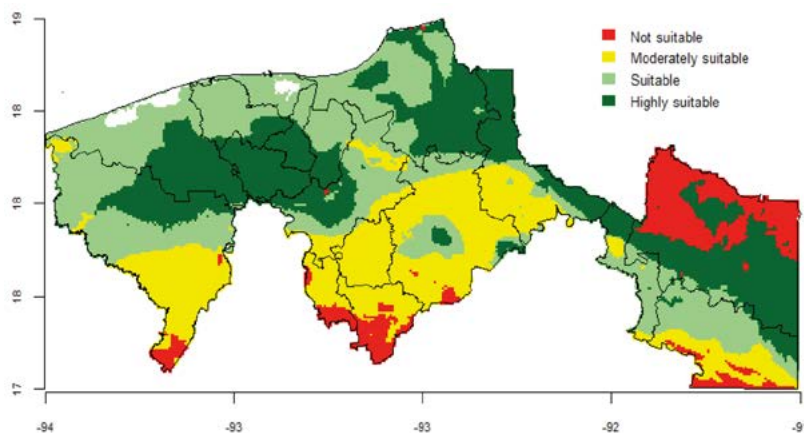


Figure 3. Suitable areas for rice cultivation in Tabasco using the EcoCrop model.

of the total area of the state, compared to the area currently planted, which is less than 0.2% (350 ha). The results obtained were similar to those reported by López *et al.* (2021), who identified similar municipalities with agroecological zoning for rice in Tabasco.

As shown in Figure 3, the state of Tabasco has different levels of suitability for rice cultivation. The 18.7% of the state does not present suitable conditions for cultivation, mainly in the northeastern part, since the minimum requirements for its development

are not met. In this case, it has low rainfall, high maximum temperatures and high solar radiation (Xu *et al.*, 2021). In 35% of the area has a conditioned suitability as moderately suitable due to the low annual rainfall, as well as the variation of temperatures and possible heat waves, which, although the crop could adapt in the early stages of development, its growth would be limited and the yield would be considerably reduced (Gao *et al.*, 2024). This is important given that the increase in temperatures leads to the more frequent appearance of insect pests that are transmitters of diseases, such as the rice leafhopper mite (*Steneotarsonemus spinki*), which carries the bacterium (*Burkholderia glumae*) that causes the disease and is considered the most destructive of rice (Salazar-Santiago *et al.*, 2022). For this reason, INIFAP is currently concentrating its efforts on genetic improvement with resistance to biotic and abiotic factors (Barrios-Gómez *et al.*, 2023; Hernández-Aragón *et al.*, 2023).

It is important to point out that the rice production systems in the areas identified as suitable are in accordance with the resources available in the areas and for the purposes required, so that the advantages offered by this crop can be exploited. Likewise, improvements must be made in agronomic management given that yields are low despite the fact that some rice plots are established in the very suitable areas (Jiménez-Chong *et al.*, 2017).

The model developed in this work represents an advance towards the automation of meteorological processes and the application of geographic information systems (GIS) in the R environment, facilitating the management of large amounts of data and their spatial analysis, as well as optimizing the time required to perform similar studies for different crops.

Import reduction forecast

With INEGI (2024) data for total population and FIRA (2024) data for *per capita* consumption, the total national rice requirement would be:

$$Cnal = Pobot \times cpc$$

Where: $Cnal$ =national consumption; $Pobot$ =total population and cpc =*per capita* consumption: $(130,232,645 \times 9.0) = 1,172.1$ thousand tons. The latter leads us to the fact that, when determining the national rice consumption in 2024 at 1,134.1 thousand tons, in the state of Tabasco, 258.7 thousand hectares susceptible to be planted were identified with medium and high productive potential. The main municipality with high productive potential was Cárdenas with 25% of the area, representing 66.23 thousand hectares. Under this scenario, 776.1 thousand tons of rice could be contributed (with an average yield of 3 t ha^{-1}), which would have represented 67% in the reduction of rice imports and would benefit the rice trade balance (López *et al.*, 2021).

CONCLUSIONS

The proposed land suitability assessment model considered agroclimatic variables determining the suitability of a crop in a specific area. The considerable potential of

this approach for assessing global and regional food security issues and general climatic constraints is highlighted. Site suitability analysis will be useful to optimize expenditures and increase the level of income in a region, and the EcoCrop model can be applied on a regional or global scale to assess the climatic suitability of a specific crop. Tabasco has the potential to produce rice crops and significantly reduce imports to achieve national food sovereignty.

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Effect of plant extracts on postharvest conservation of cut flowers

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ABSTRACT

Objective: To evaluate the effect of plant extracts on the postharvest preservation of cut flowers.

Methodology: Seven extraction methods were employed to obtain active compounds from fresh and dry plant material, using *Azadirachta indica* as a reference and rose as the model flower. With the extraction method defined, five plants with medicinal and antimicrobial properties (*Azadirachta indica*, *Piper auritum*, *Equisetum arvense*, *Hamelia patens*, and *Solanum nigrum*) were evaluated on five cut flowers species (rose, gerbera, carnation, gladiolus, and chrysanthemum) at three concentrations (25, 50, and 75 ppm), alongside a standard (Floralife[®]) and control. The experimental design was completely randomized in a factorial arrangement with three replications.

Results: Significant differences were observed (Tukey $P \leq 0.05$) among flower types, plant extracts, and concentrations, indicating a significant delay in floral senescence.

Limitations on study: Further studies are required to validate these findings, particularly regarding the metabolites present in the evaluated extracts.

Conclusions: Plant extracts are proposed as an effective and natural strategy to enhance cut flower preservation.

Keywords: Vase life, natural preservatives, floral senescence, flower quality.

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INTRODUCTION

Worldwide, floriculture is a highly relevant commercial activity for both local markets and exportation. In addition to its economic importance, flowers hold aesthetic, symbolic, and emotional value in many societies (Tejeda-Sartorius & Arévalo-Galarza, 2012). In Mexico, this agro-industrial activity is one of the most important, as it generates employment and foreign exchange, contributing significantly to the economic and social development of various regions. Among the most commonly traded crops internationally are roses (*Rosa*

spp.), carnations (*Dianthus caryophyllus*), chrysanthemums (*Chrysanthemum* spp.), gerberas (*Gerbera* spp.), and gladiolus (*Gladiolus* spp.), among other species (Meza-García *et al.*, 2017; Valencia-Sandoval & Guerrero-Morales, 2018).

To prolong the vase life of flowers and maximize product profitability, various preservation techniques are employed (Nguyen & Lim, 2021). These methods aim to extend the sales period, increase availability, facilitate long-distance transportation, and meet market demand (Thakur, 2020). These techniques include refrigeration, floral preservatives, pulsing, controlled atmospheres, UV radiation, and their combinations.

Refrigeration is the most widely used technique at the industrial level (Debnath *et al.*, 2019; Thakur, 2020), as it helps reduce the respiration rate and slows down senescence. However, the temperature and duration of cold storage may vary depending on the species and variety of the flower. Additionally, relative humidity and ventilation are critical factors that must be carefully controlled during refrigeration (Debnath *et al.*, 2019). Despite its effectiveness, refrigeration has drawbacks such as high initial investment costs, significant energy consumption, and condensation issues that can promote the proliferation of microorganisms (Juárez *et al.*, 2008). Similarly, storage conditions are specific to each type of flower (Debnath *et al.*, 2019); for instance, some flowers are cold-sensitive and cannot be stored below 10 to 13 °C, such as bird of paradise, heliconia, orchids, and anthuriums (Soleimani *et al.*, 2016; Nascimento *et al.*, 2018).

Similarly, the use of floral preservatives is particularly important for small-scale producers who lack adequate infrastructure and in situations where flowers need to be transported and stored for extended periods before their sale or use in floral arrangements. These preservatives are widely available on the market and are used by most florists and flower distributors, as they are easy to obtain, cost-effective, and do not require specialized infrastructure for their application. However, the use of commercial chemical preservatives may pose a risk to public health and have a negative environmental impact.

In this regard, sustainable alternatives based on bioactive agents, such as plant extracts, have the potential to improve the quality and extend the vase life of flowers. These agents may possess antimicrobial, antioxidant, and anti-ethylene properties (Cerna *et al.*, 2019; Nguyen & Lim, 2021). Some species with potential chemical defense properties that could replace fungicides and bactericides include neem (*Azadirachta indica*), hoja santa (*Piper* spp.), and moringa (*Moringa oleifera*), among others (Hassan & Fetouh, 2019). These plants contain natural metabolites that could provide an alternative in the search for new treatments to extend the shelf life of flowers, foliage, and fruits (Cerna *et al.*, 2019). Therefore, the objective of this research was to evaluate the effect of plant extracts on the postharvest preservation of cut flowers.

MATERIALS AND METHODS

Selection of Plant Material

Plants with medicinal, antiseptic, and antimicrobial properties were selected for the preparation of extracts. The species *Azadirachta indica*, *Piper auritum*, *Equisetum arvense*, *Hamelia patens*, and *Solanum nigrum* were chosen for their potential to produce secondary metabolites that act as chemical defenses and crop protection agents. The flowers evaluated

in the study were rose, gerbera, carnation, gladiolus, and chrysanthemum, due to their commercial importance in Mexico.

Method for the Extraction of Plant Extracts

Tests were conducted to determine the most efficient method for extracting plant compounds, using various techniques. The experiment focused on the senescence time of a model flower (rose), using *Azadirachta indica*, a plant known for its properties, as a reference. Seven extraction methods were tested: 1) Soxhlet with 100% ethanol (SE), 2) Soxhlet combined with 60% water and 40% ethanol (SWE), 3) reflux (RE), 4) infusion (IN), 5) hydrolysis (HY), 6) steam distillation (SD), and 7) pretreatment (PT).

The plant material used was collected fresh (fresh material) and previously dehydrated in an oven at 105 °C until a constant weight was achieved (dry material). A 250 g sample and 1250 mL of distilled water were used as the extraction solution, following the AOAC procedure 936.15 (1990). Once the extract was obtained, it was purified at 45 °C using a rotary evaporator for three hours and stored at 4 °C in amber bottles inside a kraft paper bag to prevent light exposure. Fourteen treatments were obtained (seven methods applied to two types of material), plus one standard (commercial preservative, in this case, citric acid salts and dextrose, known in the market as Floralife[®] or “oasis”) and one control (no treatment). Each treatment was applied at a concentration of 50 ppm in a 500 mL floral solution of water.

Evaluation of Senescence

Once the most efficient extraction method was identified, it was used to obtain the extracts from the five selected plants with phytochemical properties. The extracts were applied through absorption to five cut flowers of economic importance in Mexico (rose, gerbera, carnation, gladiolus, and chrysanthemum) to determine which extract was most effective in prolonging vase life (senescence). Three different concentrations were tested (25 ppm, 50 ppm, and 75 ppm), along with a control (no treatment) and a standard (Floralife[®]), with no change of the aqueous solution. The experimental design was completely randomized, with five replications. Senescence was evaluated based on bent neck symptoms or “drooping,” which occurs when the floral xylem is blocked by microorganisms, preventing water uptake and reducing the flower’s lifespan (Nguyen & Lim, 2021).

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) and a multiple comparison of means test (Tukey $P \leq 0.05$), using the STATISTICA[®] program.

RESULTS AND DISCUSSION

Method for Obtaining Plant Extracts

Plant metabolites possess unique properties and structures, which require the application of different extraction methods depending on their solubility, whether in water or in organic solvents such as ethanol or chloroform. To achieve optimal extraction, it is

necessary to evaluate several methods based on the type of metabolite (Puri & Chawla, 2012). In this study, significant differences were found in the days to senescence (Tukey $P \leq 0.05$) between the evaluated techniques: control (3.20b), standard (3.40b), PT (3.50b), RE (3.60b), HY (3.90b), SWE (4.00b), IN (4.30b), SE (4.70b), and SD (7.60a).

The substrates used (control=3.20a, standard=3.40a, dry material=4.17a, and fresh material=4.85a) did not show significant statistical differences (Tukey $P \leq 0.05$). Regarding the interactions, the best statistical group (Tukey $P \leq 0.05$) included: SE with dry material (4.60ab), SE with fresh material (4.80ab), IN with fresh material (5.00ab), SD with dry material (6.80ab), and SD with fresh material (8.40a) (Figure 1).

It is observed that the most efficient technique was SD, likely due to its ability to extract volatile and essential compounds, which preserve the antimicrobial and antioxidant properties of the extracts. Additionally, this method reduces the direct exposure of the compounds to high temperatures, minimizing their thermal degradation and preserving their biological activity (Bakkali *et al.*, 2008). As a result, it produces relatively pure extracts with fewer contaminants and residues, as the steam carries away the volatile components, leaving behind less volatile materials (Burt, 2004). Finally, this method is scalable and can be applied both in the laboratory and in industrial production, making it suitable for the floriculture industry and other commercial applications (Dutta, 2015).

Regarding the type of plant material used (fresh and dry material), although no statistically significant differences were observed, it is estimated that for the purposes of this experiment, fresh material is more suitable, as it is more representative of the plant in its natural state. This could better reflect the chemical composition and properties of the compounds of interest, which may be altered or lost during the drying process. In addition, fresh material does not require prior preparation, which considerably reduces the time and resources needed for processing. This aspect is reflected in the interactions between both

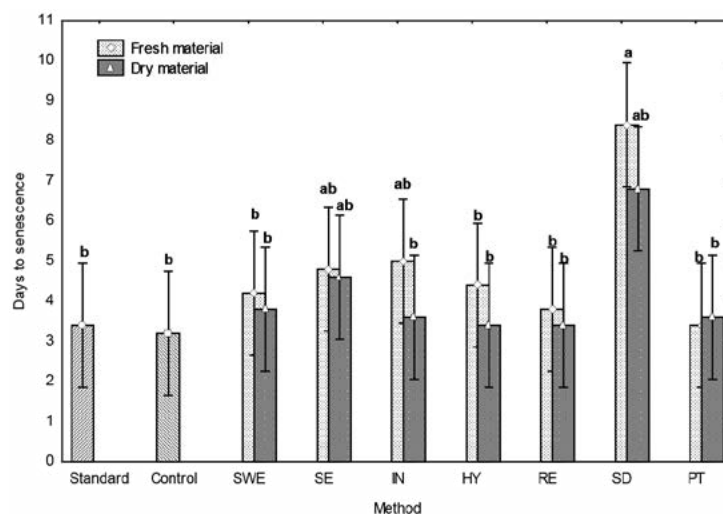


Figure 1. Days to senescence of rose according to different extraction techniques and substrates. Different letters indicate statistically significant differences (Tukey $P \leq 0.05$). SE=Soxhlet with 100% ethanol, SWE=Soxhlet combined with 60% water and 40% ethanol, RE=reflux, IN=infusion, HY=hydrolysis, SD=steam distillation, and PT=pre-treatment.

factors, highlighting the SD with fresh material group as the most outstanding (Figure 1), which aligns with the findings of Bakkali *et al.* (2008). Despite the results, future studies should consider the genetic variability of the plants and environmental conditions to validate and expand the current findings.

Senescence Evaluation

Significant differences (Tukey $P \leq 0.05$) were found between the different types of flowers: gladiolus (7.71d), gerbera (9.44c), rose (10.02b), carnation (16.26a), and chrysanthemum (16.44a). Differences were also observed between the treatments: control (4.67f), standard (5.35e), *Solanum nigrum* (11.62d), *Hamelia patens* (12.11c), *Equisetum arvense* (12.34c), *Azadirachta indica* (13.87b), and *Piper auritum* (14.59a), as well as between the concentrations: control (4.67e), standard (5.35d), 25 ppm (10.75c), 75 ppm (12.20b), and 50 ppm (15.77a).

Regarding the interactions, different statistical groups were identified (Tukey $P \leq 0.05$), with the best treatments being: carnation with *P. auritum* at 50 ppm (27.00a), chrysanthemum with *P. auritum* at 50 ppm (25.72a), and carnation with *A. indica* at 50 ppm (25.60a) (Figure 2a).

For the case of rose, the treatments showed significant differences in days to senescence (Tukey $P \leq 0.05$): control (2.80c), standard (3.40c), *H. patens* (9.85b), *S. nigrum* (10.16b), *E. arvense* (10.38b), *A. indica* (11.93a), and *P. auritum* (12.42a). The concentrations also presented differences: control (2.80d), standard (3.40d), 25 ppm (10.06c), 75 ppm (11.02b), and 50 ppm (11.76a), with the best results observed in the combinations of *P. auritum* at 75 ppm (12.92a), *P. auritum* at 50 ppm, and *A. indica* at 50 ppm (13.80a) (Figure 2b). These results are consistent with those reported by El-Naggar *et al.* (2019), who stated that the application of *A. indica* extracts prolongs the vase life of roses by inhibiting pathogenic microorganisms, reducing oxidative stress, and maintaining water balance.

Other studies have also highlighted the effectiveness of plant extracts, such as green tea, which extended the vase life of roses up to 15 days (Wu *et al.*, 2016), or the leaves and seeds of *Moringa oleifera*, which prolonged vase life to 13.7 and 11.9 days, respectively (Hassan and Fetouh, 2019). These findings emphasize the potential of plant extracts, particularly those from *P. auritum* and *A. indica*, as natural and effective alternatives to the synthetic preservatives commonly used.

In gerberas, significant differences (Tukey $P \leq 0.05$) were found between treatments: control (2.12d), standard (2.88d), *Solanum nigrum* (8.70c), *Equisetum arvense* (10.25b), *Hamelia patens* (10.56ab), *Azadirachta indica* (11.17a), and *Piper auritum* (11.18a), as well as between concentrations: control (2.12d), standard (2.88d), 25 ppm (8.75c), 75 ppm (10.38b), and 50 ppm (11.99a). The best combinations were *H. patens* (12.04abc), *A. indica* (12.80ab), and *P. auritum* (13.20a), all at 50 ppm, forming a statistically superior group (Figure 2c).

The treatments with *A. indica* and *P. auritum* are particularly noteworthy, as untreated gerberas typically have a vase life of about 5 to 7 days (Nahrabadi *et al.*, 2015; Combrink, 2018). In this experiment, the average duration was 11 days, which coincides with studies that used eucalyptus essences combined with sucrose, achieving a duration of 12.33 days. This is plausible, as sugars such as glucose have been investigated as a strategy to extend

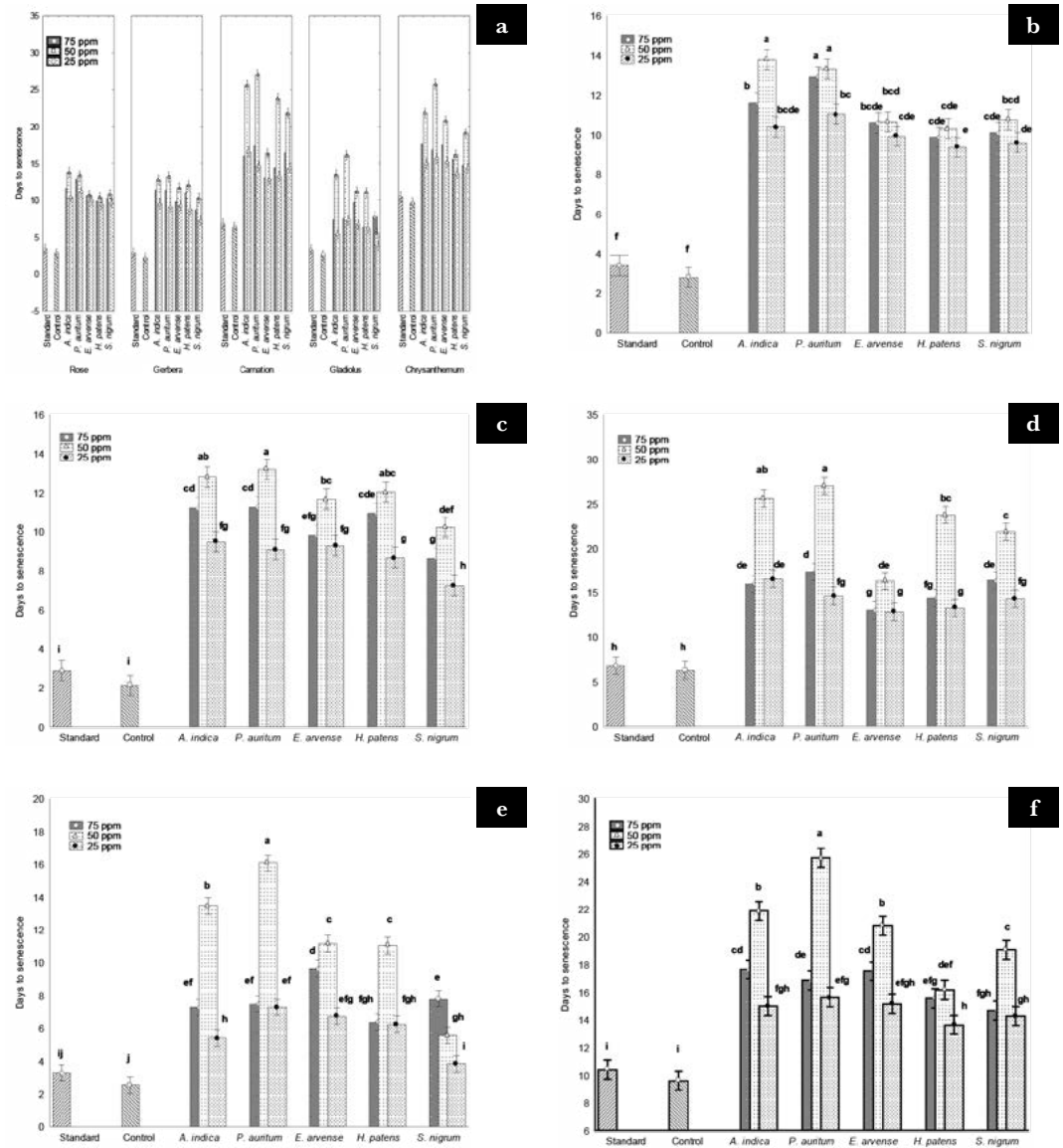


Figure 2. Days to senescence in different types of flowers treated with plant extracts. Different letters indicate statistically significant differences (Tukey $P \leq 0.05$). a) Interactions between flower types, treatments, and concentrations, b) interactions between treatments and concentrations in rose, c) interactions between treatments and concentrations in gerbera, d) interactions between treatments and concentrations in carnation, e) interactions between treatments and concentrations in gladiolus, and f) interactions between treatments and concentrations in chrysanthemum.

the vase life of cut flowers (Koyuncu *et al.*, 2017). Additionally, essential oils from thyme (*Thymus vulgaris*) and lavender (*Lavandula angustifolia*) have also been shown to extend the vase life of gerberas, reaching an average of 11 days (Nahrabadi *et al.*, 2015). Other studies have shown that vase life can be extended to 10-12 days with treatments not based on plant extracts, such as calcium chloride (CaCl_2), which improves flower longevity and quality by reducing scape curvature and increasing cell wall rigidity (Tonooka *et al.*, 2023). Similarly, silver nitrate and nano-silver have been shown to extend vase life

in gerberas and other flowers by reducing microbial attacks and inhibiting the ethylene hormone (Liu *et al.*, 2021).

Regarding carnations, significant differences (Tukey $P \leq 0.05$) were observed between the treatments: control (6.32d), standard (6.80d), *E. arvense* (14.08c), *H. patens* (17.14b), *S. nigrum* (17.52b), *A. indica* (19.37a), and *P. auritum* (19.66a), as well as between the concentrations: control (6.32d), standard (6.80d), 25 ppm (14.33c), 75 ppm (15.43b), and 50 ppm (22.90a). The most prominent interactions were *Azadirachta indica* (25.60ab) and *Piper auritum* (27.00a), both at 50 ppm (Figure 2d). These results align with those reported by El-Naggar *et al.* (2019), where *A. indica* extracts prolonged the vase life of carnations up to 22.73 days, compared to 11.33 days for untreated flowers. This extract, combined with glucose, extended the vase life to 15 days, improving flower quality in terms of turgidity and flower opening (Islam *et al.*, 2021). Similarly, other extracts, such as spearmint essential oils (*Mentha spicata*), prolonged vase life to 18.3 days (Dehestani-Ardakani *et al.*, 2022).

Despite the differences in senescence days between the various extracts used, it is clear that these extracts help prolong the vase life of carnations compared to their controls. This is particularly relevant in this study, as carnations showed the best results. However, it is also important to consider that, according to García-Sánchez *et al.* (2018), carnations have a longer vase life due to their lower respiratory rate and thicker petals, which contribute to their greater resistance to dehydration (Chidylo *et al.*, 2015).

Regarding gladiolus, significant differences were observed between treatments: control (2.52e), standard (3.28e), *Solanum nigrum* (5.73d), *H. patens* (7.88c), *A. indica* (8.72b), *E. arvense* (9.17b), and *P. auritum* (10.28a), and concentrations: control (2.52d), standard (3.28d), 25 ppm (5.89c), 75 ppm (7.71b), and 50 ppm (11.46a), with the best combination being *P. auritum* at 50 ppm (16.08a) (Figure 2e). Although no specific studies on gladiolus were found, the active principles of *A. indica* and *P. auritum* could have positive effects similar to those observed with other plant extracts. For example, the extract of *Calotropis procera* has been shown to extend vase life up to 14.5 days (Hassan and Fetouh, 2019).

Finally, in chrysanthemums, significant differences were observed between treatments (Tukey $P \leq 0.05$): control (9.60e), standard (10.40e), *H. patens* (15.12d), *S. nigrum* (16.01c), *E. arvense* (17.82b), *A. indica* (18.17b), and *P. auritum* (19.40a), and concentrations: control (9.60d), standard (10.40d), 25 ppm (14.74c), 75 ppm (16.44b), and 50 ppm (20.72a). The best result was observed with the *P. auritum* extract at 50 ppm (25.72a) (Figure 2f). There are few studies specifically addressing the use of *A. indica* and *P. auritum* extracts in chrysanthemums. However, research with other plant extracts suggests that these may extend the vase life of chrysanthemums and other flowers. For example, myrtle essential oil extended the vase life of chrysanthemums up to 15.73 days (Bidarigh, 2015), thyme (*Thymus vulgaris*) essential oil up to 14.71 days (Bazaz *et al.*, 2015), and geranium essential oil up to 18.41 days (Dashtbay *et al.*, 2015). These essential oils primarily work by inhibiting microbial growth and reducing ethylene production, which helps prolong the freshness and vase life of cut flowers.

In summary, extracts of *Azadirachta indica* and *Piper auritum* possess valuable properties for postharvest conservation, thanks to their bioactive compounds such as azadirachtin, nimbolide, nimbin, and nimbidin in *A. indica*, and piperine in *P. auritum*, all of which have antimicrobial and antioxidant properties (Singh *et al.*, 2017; Cárdenas-Coronel *et al.*, 2019).

Additionally, it was observed that the concentration of 50 ppm was particularly effective for the preservation of all flowers, maintaining an optimal osmotic balance without causing damage to the tissues or generating phytotoxicity. This allows the flowers to maintain their structural integrity and aesthetic appearance for a longer period. However, the senescence process in plant products is influenced by factors such as temperature, humidity, type of product, variety, maturity stage at the time of harvest, and storage and transportation methods (Cai-Zhong, 2012). Therefore, it is important to consider that the effectiveness of plant extracts may vary depending on these factors, as well as the dosage and method of application.

CONCLUSIONS

Piper auritum and *Azadirachta indica* stood out in nearly all the evaluated flowers, consistently showing the best results in extending vase life and forming part of the statistically superior groups. In particular, the concentration of 50 ppm proved to be the most effective in all cases, suggesting that it is optimal for postharvest handling of the different evaluated species. The combinations of *P. auritum* and *A. indica* at 50 ppm excelled in all flowers, confirming their high efficacy and significantly outperforming both commercial treatments and the control. The natural character of these extracts did not cause significant phytotoxicity at the concentrations used, making them a low-cost, effective, and safe option for postharvest preservation. The use of plant extracts represents a potentially effective and natural strategy to improve the preservation of economically important cut flowers in Mexico.

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Effectiveness of Fomesafen and Its Mixtures for Weed Control in Bean Crops

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ABSTRACT

Objective: To assess the effectiveness of the herbicide fomesafen both alone and in combination with other herbicides in controlling weeds and toxicity to beans, and to compare it with other chemical and manual treatments.

Design/methodology/approach: The experiment was established in Ignacio de la Llave, Veracruz, in January 2024. A randomized block experimental design with 10 treatments and four replications was employed: fomesafen at 125, 187.5, and 250 g ha⁻¹, fomesafen+fluazifop-p-butyl at 187.5+125 g ha⁻¹, fomesafen+clethodim at 187.5+118 g ha⁻¹, bentazone at 720 g ha⁻¹, imazethapyr at 100 g ha⁻¹, one hoe weeding, two hoe weedings, and a control with no application. Weed control and bean toxicity were evaluated at 10, 20, 30 and 42 days after the application of the treatments (DAT), and grain yield was quantified.

Results: At 42 DAT, the most effective treatments for controlling *Melampodium divaricatum* were bentazone at 720 g ha⁻¹ and fomesafen at 187.5 and 250 g ha⁻¹. Control of *Rottboellia cochinchinensis* was most efficient with fomesafen+fluazifop-p-butyl and fomesafen+clethodim. The best treatment for total weed control, was two hoe weedings. All herbicides caused slight toxicity to the bean, which disappeared after 10 DAT.

Limitations on study/implications: The recommendations can be applied by both small and large bean growers.

Findings/conclusions: The highest grain yields were obtained with two hoe weedings, the mixtures of fomesafen+fluazifop-p-butyl and fomesafen+clethodim and fomesafen at 187.5 and 250 g ha⁻¹.

Keywords: *Melampodium divaricatum*, *Rottboellia cochinchinensis*, herbicides, weeding, yield.

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INTRODUCTION

In the state of Veracruz, 36,130 ha of beans were cultivated in 2023, primarily black beans (*Phaseolus vulgaris* L.) under rainfed and residual moisture production systems (SIAP, 2024). The average bean yield in the region is around 800 kg ha⁻¹, due to various technical, biotic, and abiotic factors affecting the crop. Among the most notable factors are the scarce and erratic distribution of rainfall, pest and disease attacks, and the presence of high weed populations. It is considered that competition with weeds is the factor that most contributes to yield reduction (Esqueda *et al.*, 1997).

Weeds primarily originate from the soil seed bank, and thus appear from the emergence of the crops. If not controlled in a timely and proper manner, they compete with the beans for water, light, and nutrients, resulting in reduced growth and grain yield losses (Horvath *et al.*, 2023). The intensity of the competition is related to the genotype and growth habit of the beans (Teixeira *et al.*, 2009), as well as the type and density of the weeds present (Sankula *et al.*, 2024). In bean plantings in the central region of Veracruz, the butter daisy [*Melampodium divaricatum* (L. C. Rich.) D. C.] is considered the predominant weed species. The main methods for weed control in bean crops are hoe weeding, chisel plowing, and the application of selective herbicides (Hernández-Ríos *et al.*, 2022). In Veracruz, post-emergence application of herbicides formulated with fomesafen and bentazone is common (López-Salinas *et al.*, 2015). These herbicides primarily target broadleaf weeds (dicotyledons); however, when grassy weeds (monocotyledons) are also present, these herbicides are usually mixed with fluazifop-p-butyl, which exclusively controls grasses (Galon *et al.*, 2018). Although there are reports of bean toxicity caused by fomesafen, the damage is generally mild and temporary, with its intensity depending on the bean variety and weather conditions (Cieslik *et al.*, 2014; Marchioretto and Dal Magro, 2017).

Currently, the number of selective herbicides for post-emergence control of broadleaf weeds in beans is very limited, with fomesafen and bentazone being the most widely used. Imazethapyr can also be used in beans (Amini *et al.*, 2023), and unlike the previously mentioned herbicides, it is effective against both broadleaf and grassy weeds (Rhemana *et al.*, 2023). It is worth noting that bentazone, fomesafen, and imazethapyr were developed in 1968, 1977, and 1985, respectively. The last experiments to evaluate the biological effectiveness of fomesafen for weed control in beans in Veracruz were conducted over 30 years ago, highlighting the need to update its effectiveness. The objective of this research was to determine the effect of this herbicide, both alone and in combination with herbicides that exclusively control grassy weeds, on weed control and bean toxicity, and to compare it with the control provided by bentazone and imazethapyr, as well as one and two hoe weedings.

MATERIALS AND METHODS

On January 15, 2024, an experiment was established in the locality of Rincón del Tigre, municipality of Ignacio de la Llave, Veracruz. Sowing was carried out manually by dropping seeds continuously into the furrow lines with the Rubí bean variety at a density of 250,000 plants per hectare. Ten treatments were evaluated: Fomesafen at 125, 187.5, and 250 g ha⁻¹; Fomesafen + fluazifop-p-butyl at 187.5 + 125 g ha⁻¹; Fomesafen + clethodim at 187.5 + 118 g ha⁻¹; Bentazone at 720 g ha⁻¹; Imazethapyr at 100 g ha⁻¹; one hoe weeding; two hoe weedings; and a control without application. A randomized block experimental design was used with four replications and experimental plots consisting of five furrows, each 5 m in length and spaced 0.60 m apart.

To determine the weed population density, a 1 m × 1 m quadrat was randomly placed in the plots corresponding to the untreated controls immediately before the application of the treatments. The weed species within the quadrat were counted, and the height of five plants per replication was measured. Nineteen days after bean emergence (DAE),

the seven chemical treatments were applied using a motorized sprayer equipped with a boom containing four flat fan nozzles, calibrated to deliver 392 L ha⁻¹. A non-ionic adjuvant was added to these chemical treatments at a concentration of 250 mL per 100 L of water. The three central rows of each experimental unit were treated, leaving the two lateral rows untreated to serve as weedy controls for weed control evaluations. At the time of application, the height of the bean plants ranged from 11 to 26 cm (average 18.65 cm), and the number of trifoliolate leaves ranged from three to six (average 4.15). The first hoe weeding was also performed, while the second hoe weeding was conducted 39 DAE. Fertilization and pest control were carried out following INIFAP recommendations for bean cultivation in Veracruz (López-Salinas *et al.*, 2015). In addition to the germination irrigation, seven additional sprinkler irrigations were applied during the crop's development, with intervals of six to ten days between each irrigation, depending on soil moisture conditions.

At 10, 20, 30, and 42 days after application (DAA), visual evaluations were conducted to estimate the percentage of control for the dominant weed species, as well as the overall weed control percentage, including all species present. The evaluations involved observing the effect of each treatment on the weeds, comparing it to the weeds in the untreated lateral control plots flanking each experimental unit. A percentage scale (0-100%) was used, where 0 indicated no effect of the treatment on the weeds, and 100 indicated complete elimination of the weeds. At the same time the control evaluations were conducted, the toxicity of the treatments to the bean plants was assessed using a percentage scale, where 0 indicated that the treatment caused no toxic effect on the beans, and 100 indicated that the crop was completely eliminated.

The bean harvest was carried out at 91 DAE. The harvested grain was cleaned, its moisture content was determined, and it was weighed. Subsequently, the necessary conversions were made to obtain the grain yield in kilograms per hectare at 14% moisture content. To homogenize the variances, the weed control percentage data were transformed to their arcsine value, and the toxicity data to the square root equivalent (Dey and Pandit, 2020), while the grain yield data did not require transformation. Subsequently, analysis of variance was conducted for these three quantified variables, and in cases where significance was detected, the mean separation test based on the Least Significant Difference (LSD, $\alpha=0.05$) was applied. For clarity, in the Results and Discussion section, the field data are presented with the letters obtained in the mean separation tests of the transformed data.

RESULTS AND DISCUSSION

Five weed species were found at the experimental site: *Melampodium divaricatum* (L. Rich.) D.C. (Asteraceae), *Rottboellia cochinchinensis* (Lour.) Clayton (Poaceae), *Amaranthus hybridus* L. (Amaranthaceae), *Lagascea mollis* Cav. (Asteraceae), and *Cyperus rotundus* L. (Cyperaceae). At the time of treatment application, the average weed coverage was 42.88%, and the total population density was 1,452,500 plants per hectare, of which 1,160,000 (79.89%) were *M. divaricatum*. The height of *M. divaricatum* ranged from 5 to 24 cm, with an average of 15.5 cm.

Weed control and toxicity to beans

In the first evaluation period, bentazone at 720 g ha⁻¹ provided total control of *M. divaricatum*, being statistically superior to the other treatments (Table 1).

Control percentages of 90% or higher were obtained with fomesafen at 187.5 and 250 g ha⁻¹, the mixtures of this herbicide with fluazifop-p-butyl and clethodim, and with two hoe weedings. With the lowest dose of fomesafen (125 g ha⁻¹) and one hoe weeding, the control of this species ranged between 80 and 89%, while with imazethapyr at 100 g ha⁻¹, the control was slightly above 50%, surpassing only the untreated control. Fomesafen at its different doses and bentazone at 720 g ha⁻¹ had no effect on *Rottboellia cochinchinensis*, as these herbicides primarily control dicotyledonous weeds and occasionally cyperaceae, but not grasses. The latter species was controlled between 85 and 91.3% with fluazifop-p-butyl and clethodim in combination with fomesafen, as well as with one and two hoe weedings. Control with imazethapyr at 100 g ha⁻¹ was slightly below 50%. The total control of the present weeds was slightly over 90% with fomesafen at 250 g ha⁻¹ and two hoe weedings, being statistically similar to the treatments with fomesafen at 187.5 g ha⁻¹ and the mixtures of fomesafen with fluazifop-p-butyl and clethodim, whose controls ranged between 85 and 90%. Slightly higher than 80% control was achieved with fomesafen at 125 g ha⁻¹ and one hoe weeding, while with imazethapyr at 100 g ha⁻¹, the control was slightly above 50%. On the other hand, all herbicide treatments caused toxicity to the bean, which consisted of small chlorotic or necrotic areas on the leaves. The greatest damage was observed with imazethapyr at 100 g ha⁻¹ (6.5%) and fomesafen at 250 g ha⁻¹ (6%), while damage between 2.5% and 3.5% was observed in the plants of the treatments with fomesafen at 125 and 187.5 g ha⁻¹, the mixtures of fomesafen with fluazifop-p-butyl and clethodim, and bentazone at 720 g ha⁻¹ (Table 1). At 20 days after application (DAA), except for the treatment with imazethapyr at 100 g ha⁻¹, a slight reduction in the control of *M. divaricatum* was observed in all herbicide treatments. Bentazone at 720 g ha⁻¹, along

Table 1. Effect of treatments on weed control (%) and bean toxicity at 10 DAT. Rincón del Tigre, municipality of Ignacio de la Llave, Ver. Winter-Spring 2024.

Treatment (g ha ⁻¹)	<i>Md</i>	<i>Rc</i>	Total	Tox.
Fomesafen (125)	86.3de	0.0d	81.3b	2.5b
Fomesafen (187.5)	90.0cd	0.0d	87.5ab	3.0b
Fomesafen (250)	96.5b	0.0d	92.5a	6.0a
Fomesafen+fluazifop-p-butyl (187.5+125)	92.5bc	91.3a	88.8ab	3.5b
Fomesafen+clethodim (187.5+118)	95.0b	86.3ab	88.8ab	3.5b
Bentazone (720)	100a	0.0d	82.5b	3.0b
Imazethapyr (100)	55.0f	47.5c	52.5c	6.5a
One hoe weeding	83.8e	85.0b	82.5b	0.0c
Two hoe weedings	94.5b	91.3a	92.5a	0.0c
Control without application	0.0g	0.0d	0.0d	0.0c

The letters on the right represent the DMS test ($\alpha=0.05$). Quantities with the same letter are not statistically different. The comparison is between treatments for each variable. *Md*=*Melampodium divaricatum*, *Rc*=*Rottboellia cochinchinensis*, Tox.=Toxicity.

with two hoe weeding treatments, remained the most effective treatments with 99.5% and 100% control, respectively, and showed statistical superiority over the rest of the treatments (Table 2).

Fomesafen at 250 g ha⁻¹ and the mixtures of this herbicide with fluazifop-p-butyl and clethodim provided control of this species between 90 and 95%, while with the medium and low doses, the control values ranged between 80 and 90%. With one hoe weeding, the control was slightly over 70%, while with imazethapyr at 100 g ha⁻¹, although the control of *M. divaricatum* increased, it was slightly over 60%, making it the herbicide treatment with the lowest effectiveness.

Total control of *R. cochinchinensis* was achieved with two hoe weedings, a treatment that was significantly superior to the rest of the treatments. Controls above 90% were observed with the mixtures of fomesafen with fluazifop-p-butyl and clethodim, and slightly over 85% with imazethapyr at 100 g ha⁻¹, whose highest effectiveness was observed after 10 DAA (Esqueda *et al.*, 2006). With one hoe weeding, the control of this species was slightly below 80% (Table 2).

The highest control of the weed complex (100%) was achieved with two hoe weedings, a treatment that was statistically superior to the rest. With the treatments of fomesafen at 250 g ha⁻¹ and the mixtures of fomesafen with fluazifop-p-butyl and clethodim, the controls ranged between 85 and 90%. Controls between 70 and 80% were obtained with fomesafen at 125 and 187.5 g ha⁻¹, bentazone at 720 g ha⁻¹, and one hoe weeding, while the lowest controls, around 60%, were provided by imazethapyr at 100 g ha⁻¹. During this evaluation period, no toxicity damage was observed in the beans in any of the treatments (Table 2).

At 30 DAA, slight reductions in the control of *M. divaricatum* were observed in all treatments compared to the previous evaluation. Bentazone at 720 g ha⁻¹ provided control of this species close to 98%, which was statistically superior to the rest of the treatments (Table 3).

Table 2. Effect of the treatments on weed control (%) and bean toxicity at 20 DAA. Rincón del Tigre, municipality of Ignacio de la Llave, Veracruz. Winter-Spring 2024.

Treatment (g ha ⁻¹)	Md	Rc	Total	Tox.
Fomesafen (125)	82.5c	0.0f	72.5d	0.0
Fomesafen (187.5)	87.5bc	0.0f	78.8bcd	0.0
Fomesafen (250)	92.0b	0.0f	85.0bc	0.0
Fomesafen+fluazifop-p-butyl (187.5+125)	90.0bc	95.0b	84.5bc	0.0
Fomesafen+clethodim (187.5+118)	91.3b	91.3c	88.0b	0.0
Bentazone (720)	99.5a	0.0f	75.0cd	0.0
Imazethapyr (100)	61.3d	86.3d	58.8e	0.0
One hoe weeding	71.3d	78.8e	76.3cd	0.0
Two hoe weedings	100a	100a	100a	0.0
Control without application	0.0e	0.0f	0.0f	0.0

The letters on the right represent the DMS test ($\alpha=0.05$). Values with the same letter are not statistically different. The comparison is between treatments for each variable. Md=*Melampodium divaricatum*, Rc=*Rottboellia cochinchinensis*, Tox.=Toxicity.

Table 3. Effect of treatments on weed control (%) and bean toxicity at 30 DAA. Rincón del Tigre, municipality of Ignacio de la Llave, Ver. Winter-Spring 2024.

Treatment (g ha ⁻¹)	Md	Rc	Total	Tox.
Fomesafen (125)	78.8b	0.0e	71.3b	0.0
Fomesafen (187.5)	86.3b	0.0e	76.3ab	0.0
Fomesafen (250)	89.5b	0.0e	82.5ab	0.0
Fomesafen+fluazifop-p-butyl (187.5+125)	89.5b	95.0a	85.0a	0.0
Fomesafen+clethodim (187.5+118)	89.5b	95.0a	87.0a	0.0
Bentazone (720)	97.8a	0.0e	70.0b	0.0
Imazethapyr (100)	52.5c	90.0b	51.3c	0.0
One hoe weeding	47.5c	52.5d	40.0c	0.0
Two hoe weedings	83.8b	87.5c	81.3ab	0.0
Control without application	0.0d	0.0e	0.0d	0.0

The letters on the right represent the DMS test ($\alpha=0.05$). Values with the same letter are not statistically different. The comparison is between treatments for each variable. *Md*=*Melampodium divaricatum*, *Rc*=*Rottboellia cochinchinensis*, Tox.=Toxicity.

Controls between 80 and 90% were observed with fomesafen alone at 187.5 and 250 g ha⁻¹, and the treatments in which this herbicide was mixed with fluazifop-p-butyl or clethodim, as well as with two hoe weedings. All treatments showed statistical similarity among themselves and also with fomesafen at 125 g ha⁻¹, whose control of *M. divaricatum* was slightly below 80%. The lowest controls (around 50%) were provided by imazethapyr at 100 g ha⁻¹ and one hoe weeding, slightly above the untreated control.

In *R. cochinchinensis*, 95% control was observed with the mixtures of fomesafen with fluazifop-p-butyl and clethodim, which were significantly superior to the rest of the treatments. Among them, the control with imazethapyr at 100 g ha⁻¹ was 90%, 87.5% with two hoe weedings, and slightly above 50% with one hoe weeding. The highest overall weed control was obtained with the mixtures of fomesafen with fluazifop-p-butyl and clethodim, although these were statistically similar to the doses of 187.5 and 250 g ha⁻¹ of fomesafen and to two hoe weedings. At the same time, the control provided by the last three treatments was similar to the 125 g ha⁻¹ dose of fomesafen and bentazone at 720 g ha⁻¹. Although bentazone at 720 g ha⁻¹ provided the highest control of *M. divaricatum*, its effect on *A. hybridus* was null (data not shown), so its total control was only 70%. As observed with *M. divaricatum*, the lowest controls were seen with imazethapyr at 100 g ha⁻¹ and a hoe weeding. No toxicity was observed in the beans with any of the treatments (Table 3).

Finally, at 42 DAA, the control of *M. divaricatum* with bentazone at 720 g ha⁻¹ was over 90%, being statistically similar to fomesafen at 187.5 and 250 g ha⁻¹, whose controls ranged between 85 and 90%; these, in turn, had a control similar to the fomesafen+fluazifop-p-butyl mixture, which was 80% (Table 4).

Controls between 70 and 80% were achieved with two hoe weedings and the mixture of fomesafen+clethodim; fomesafen at 125 g ha⁻¹ only provided control of this species close to 60%, while with imazethapyr at 100 g ha⁻¹ and one hoe weeding, controls were only

between 30 and 35%. The mixtures of fomesafen with fluazifop-p-butyl and clethodim maintained controls of *R. cochinchinensis* at 95%, statistically surpassing the rest of the treatments. Controls between 80 and 85% were achieved with imazethapyr at 100 g ha⁻¹ and two hoe weedings, while with only one hoe weeding, control was close to 40%. The highest total control (75%) was achieved with two hoe weedings, which was statistically similar to the three doses of fomesafen and the mixtures of this herbicide with fluazifop-p-butyl or clethodim. Bentazone at 720 g ha⁻¹ provided control of less than 60%, while the lowest controls were obtained with imazethapyr and one hoe weeding. No toxicity to the bean was observed with any of the treatments (Table 4).

Grain Yield

The highest grain yields were close to 1,000 kg ha⁻¹, and were obtained with two hoe weedings; the yield of this treatment was statistically similar to that obtained with the mixture of fomesafen + fluazifop-p-butyl, as well as to those of the mixture of fomesafen + clethodim and fomesafen at 185.5 and 250 g ha⁻¹.

It is known that the critical period of competition between weeds and beans occurs between 24 and 40 days after emergence (Blanco and Leyva, 2011); for this reason, the treatments with the best controls during this period also achieved the highest grain yields. With the application of bentazone and fomesafen at 720 and 125 g ha⁻¹, respectively, yields between 750 and 800 kg ha⁻¹ were obtained, while with imazethapyr at 100 g ha⁻¹, a yield of less than 600 kg ha⁻¹ was obtained, mainly due to its limited effect on *M. divaricatum*, and with one hoe weeding, the yield was less than 500 kg ha⁻¹, although both treatments were statistically similar (Table 5).

Performing two hoe weedings resulted in a 54% higher grain yield compared to performing a single weeding. All treatments had significantly higher grain yields than the untreated control, which produced less than 50 kg ha⁻¹ (Table 5). The control of *M. divaricatum* showed significant variations with the herbicide treatments applied. Fomesafen,

Table 4. Effect of the treatments on weed control (%) and bean toxicity at 42 DAA. Rincón del Tigre, municipality of Ignacio de la Llave, Ver. Winter-Spring 2024.

Treatment (g ha ⁻¹)	<i>Md</i>	<i>Rc</i>	Total	Tox.
Fomesafen (125)	58.8d	0.0d	63.8ab	0.0
Fomesafen (187.5)	85.0abc	0.0d	66.3ab	0.0
Fomesafen (250)	88.8ab	0.0d	70.0ab	0.0
Fomesafen + fluazifop-p-butyl (187.5 + 125)	80.0bc	95.0a	70.0ab	0.0
Fomesafen + clethodim (187.5 + 118)	76.3c	95.0a	67.5ab	0.0
Bentazone (720)	92.0a	0.0d	56.3b	0.0
Imazethapyr (100)	32.3e	85.0b	32.5c	0.0
One hoe weeding	31.3e	37.5c	31.3c	0.0
Two hoe weedings	77.5c	83.8b	75.0a	0.0
Control without application	0.0f	0.0d	0.0d	0.0

The letters to the right represent the DMS test ($\alpha=0.05$). Quantities with the same letter are not statistically different. The comparison is between treatments for each variable. *Md*=*Melampodium divaricatum*, *Rc*=*Rottboellia cochinchinensis*, Tox.=Toxicity.

Table 5. Effect of the treatments on grain yield. Rincón del Tigre, municipality of Ignacio de la Llave, Ver. Winter-Spring 2024.

Treatment (g ha ⁻¹)	Grain yield (kg ha ⁻¹)
Fomesafen at 125	774.23b
Fomesafen at 187.5	886.61ab
Fomesafen at 250	915.75ab
Fomesafen+fluazifop-p-butyl at 187.5+125	957.38a
Fomesafen+clethodim at 187.5+118	932.40ab
Bentazone at 720	782.55b
Imazethapyr at 100	532.80c
One hoe weeding	457.88c
Two hoe weedings	994.84a
Control without application	41.63d

The letters to the right represent the DMS test ($\alpha=0.05$). Quantities with the same letter are not statistically different. The comparison is between treatments for each variable.

both at 187.5 g ha⁻¹ and 250 g ha⁻¹, either alone or in combination with fluazifop-p-butyl and clethodim, achieved control rates above 90%, which is also comparable to the findings of Cano and López (1996), who used fomesafen at 250 g ha⁻¹ in combination with fluazifop-p-butyl, although in this study similar results were achieved with a lower dose (187.5 g ha⁻¹).

There are reports of toxic effects on beans due to the application of fomesafen, bentazone, and imazethapyr (Procopio *et al.*, 2009; Galon *et al.*, 2018), but as observed in this study, the damage was mild and temporary. On the other hand, the results obtained with the fomesafen and clethodim mixture are consistent with those reported by Marchioretto and Dal Magro (2017). Fomesafen can remain residual in the soil, potentially harming crops planted after the crop where this herbicide was applied (Rector *et al.*, 2019). For this reason, if a crop that is not tolerant to fomesafen is to be planted after beans, the use of bentazone is preferable.

It is important to note that although both bentazone and fomesafen generally provided good to excellent control of *M. divaricatum*, this did not necessarily translate into grain yield, as the elimination of the dominant species allowed for greater development and competition from *R. cochinchinensis*, a species not affected by either of these herbicides. For this reason, the fomesafen mixtures with fluazifop-p-butyl and clethodim generally resulted in the highest yields, as they are effective at controlling both weed species.

Regarding the control of *R. cochinchinensis*, the mixtures of fomesafen with fluazifop-p-butyl and clethodim were the most effective, achieving control above 90%. This result is consistent with the findings reported by Galon *et al.* (2018) and Hernández-Ríos *et al.* (2022), who highlighted the high efficacy of these herbicide combinations for the management of grasses.

Some of the advantages of using herbicide mixtures compared to single herbicides are: a broader spectrum of weed control, reduced risk of the development of herbicide-

resistant weed biotypes, synergy, reduced application doses, time savings, and reduced costs (Diggle *et al.*, 2003; Barbieri *et al.*, 2022; Teymourinia *et al.*, 2023). Finally, although manual weeding provided efficient weed control, as also observed by Badgujar *et al.* (2003), this method has significant limitations due to high costs and labor demands.

CONCLUSIONS

Fomesafen at 187.5 g ha⁻¹ can efficiently control *M. divaricatum* up to 42 DAA, being similar to bentazone at 720 g ha⁻¹ and superior to imazethapyr at 100 g ha⁻¹, as well as to one and two hoe weedings.

To control both dicot and monocot weeds, fomesafen should be mixed with fluazifop-p-butyl or clethodim. The control of the weed complex with fomesafen at 250 g ha⁻¹ or in mixtures of fomesafen+fluazifop-p-butyl and fomesafen+clethodim was only efficient (>80%) up to 30 DAA, being comparable to two hoe weedings. Fomesafen caused slight toxicity to the bean, which disappeared between 10 and 20 DAA. The application of fomesafen at 250 g ha⁻¹, as well as its mixtures with fluazifop-p-butyl and clethodim, resulted in grain yields similar to those obtained with two hoe weedings.

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Performance of corn (*Zea mays* L.) with application of poultry manure and leachates as organic fertilizer

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ABSTRACT

Objective: To evaluate different sources and forms of organic fertilization on production parameters in *Zea mays* L..

Design/methodology/approach: A completely randomized design was used where the treatments were the different combinations of doses and sources of organic fertilizers from animal excreta.

Results: The foliar applied compost plus leachate treatments were found to be superior to the chemical treatments.

Limitations of the study/implications: Further evaluation of the virtues of organic fertilizers in different crops is needed.

Findings/conclusions: Organic fertilization either in soil form with composts or leachates in a phytophilic form are competitive to chemical fertilization.

Keywords: manures, compost, yield performance, organic agriculture, food sovereignty.

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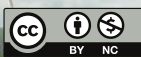
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INTRODUCTION

Corn (*Zea mays* L.) is a staple crop for Mexican food; 6,904,042 hectares are planted annually, with a production of 26,553,239 million tons in the 32 states of the country and average yields of 3.90 t ha⁻¹ (SIAP, 2023). The crop is grown under diverse agroecosystems and climatic conditions, which is why there are several problems due to different management practices, such as soil degradation, loss of biological biodiversity and, in general, negative effects on the environment and human health (SIAP, 2023). The crops that stand out within the Agrifood Chains of the Basic Food Basket are maize, which is a priority for INIFAP technology generation (Zamarripa *et al.* 2022). Among the factors affecting yield is agronomic management (Olvera-Rincón *et al.*, 2023), with fertilizer application being one of the most influential components in the expression of

crop production potential (Salgado-Velázquez *et al.*, 2020; Córdova-Sánchez *et al.*, 2023; Salgado-García *et al.*, 2023). However, the intensive use of agrochemicals has reduced to a minimum the living conditions of the diverse organisms and microorganisms that inhabit the soil.

In maize production, there is widespread application of chemical fertilizers, and sometimes manure is applied (Kato *et al.*, 2009). In this sense, the effect of alternative sources of fertilizers such as organic fertilizers has been studied as in Bravo (2016), where in the initial phase they showed a rapid response in the treatment with chemical fertilization, while with organic products they reach their greatest effect at 63 days since they are of slow release of their nutrients, evidencing their residual action in corn crop. Liquid organic fertilizers can be applied in the nutrition of agricultural crops, it has been proven that applied to the foliage in concentrations between 20 and 50% stimulates growth, improves the quality of the products and even has a certain repellent effect against plagues and can be applied to the soil in higher concentrations to promote root development (Bravo, 2016). In addition, beneficial effects have been observed on soil properties such as better structure, greater porosity, higher organic matter content, greater retention of exchangeable cations and less soil erosion, among others (Córdova-Sánchez *et al.*, 2023).

In the state of Tabasco, Mexico, is imperative the proper management of the manure produced in the livestock and poultry sector, in addition to the fact that it is a surplus input. The effect of corn fertilization with poultry manure on production has been studied by authors such as Sánchez *et al.* (2021), where the characteristic of organic fertilizers applied to the soil, act as reservoirs of nutrients and the organisms continue to work degrading the organic matter and releasing nutrients for the next cycle. In Velasco-Cruz *et al.* (2023), where height, stem diameter and number of leaves were significantly greater when irrigation with wastewater was applied, except for the variable number of leaves, which did not show significance, and forage biomass was significantly greater in the plots with wastewater, since it is an important source of nutrients.

Currently in Tabasco, Mexico, fertilization of corn crops is still predominantly from chemical sources, and given that local resources of residues from the livestock and poultry industries are available, there is a need to evaluate the effect of organic fertilizers on corn crops as fertilization alternatives, in addition to providing positive benefits to the soil. Therefore, the objective of this work was to evaluate the effect of composted pollination and animal excreta leachates applied to the soil and foliar application on corn crop and its effect on vegetative development and grain yield.

MATERIALS AND METHODS

Experimental Site. The experiment was carried out in the spring-summer cycle (2023), at the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, (INIFAP), in instalations of the experimental field of Huimanguillo, Tabasco, Mexico (17.847711, -93.396465 UTM). The soil type is Fluvisol where the crop was established, whose properties can be observed in Table 1. The analysis of the soil where the crop was established shows a slightly acid PH, with a good content of organic matter (OM), fertility

Table 1. Soil chemical properties of the experimental site.

pH H ₂ O	EC	OM	N t	P	K	Ca	Mg	Na	CIC	Fe	Cu	Zn	Mn	B
	dS m ⁻¹	%		mg kg ⁻¹	cmol kg ⁻¹				mg kg ⁻¹					
5.8	0.05	3.2	0.1	9.85	0.2	9.9	1.4	0.07	16	32	10.1	5.9	0.98	0.8

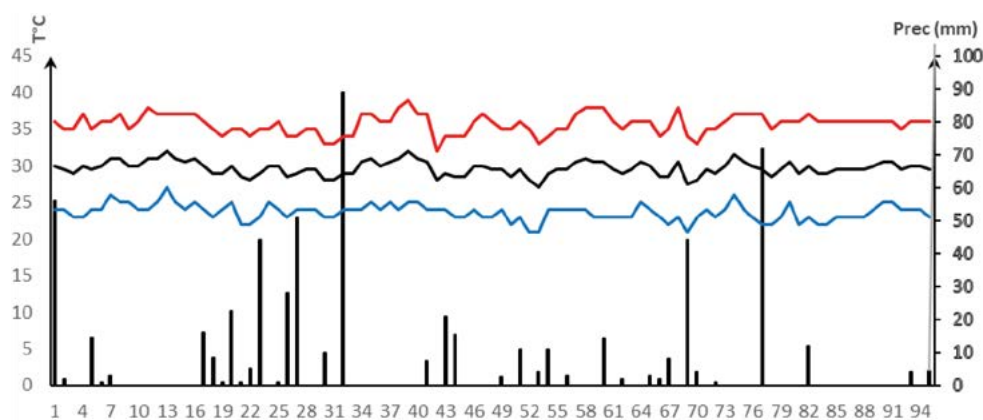
in the contents of nitrogen, phosphorus and potassium, in addition to the main minor elements required by the corn crop.

The main climatological conditions during the crop cycle are shown in Figure 1. Precipitation in the critical period of the crop at 60 days presented an accumulated rainfall of 386 mm, corresponding to 71.46% of the total 541.5 mm of the cycle. The maximum, minimum and average daily temperature, maintained approximately a regularity characteristic of this tropical season, within the 60 days and at the end of the crop, so it is considered that these factors did not influence the growth and yield of the crop and were not affected by climate change.

Previously, leachates derived from the compost were obtained in the organic module by the aerated static pile method (Sumano et al., 2020). The liquid ferments of the compost after 30 days were collected and stored for application. The physical and chemical properties of the composted poultry manure are presented in Table 1 and of the leachate in Table 2.

Methods: Analyses of pH, EC, OM, Nt, Ratio. C/N, Ash, Moisture, CO and Da (Mexican Official Standard NMX-FF-109-SCFI-2008) and K, Ca, Mg, Na, CIC, Fe, Cu, Zn, Mn (Mexican Official Standard NOM-021-RECNAT-2000).

Soil preparation was mechanized with three harrow passes; the maize H-520 hybrid was used for sowing. It should be noted that this is the third consecutive crop cycle sowing

**Figure 1.** Environmental conditions at the study site in Huimanguillo, Tabasco, Mexico. (Barrón, 2023).**Table 2.** Analysis of the physical and chemical properties of poultry manure.

pH*	CE	MO	N	P	K	Ca	Mg	Na	CIC	Fe	Cu	Zn	Mn
	dS m ⁻¹	%		mg kg ⁻¹	cmol (+) kg ⁻¹				mg kg ⁻¹				
7.29	2.87	70	2.69	48.80	2.40	5.66	1.68	0.31	49.98	145.03	60.07	12.13	11.45

Table 3. Chemical properties of leachates derived from compost.

Manure	K	Ca	Mg	Na	CIC	P	B	S	Fe	Zn	Mn	MO	N Tot
	Cmol(+) kg ⁻¹					mg kg ⁻¹		ppm	mg kg ⁻¹				
Bovine	37.9	21.6	26.8	4.1	80.5	BDL*	22.8	BDL*	42.0	46.7	11.1	39.1	0.35

*BDL: below the detectable limit.

the same variety in the same site under the same treatments, to observe the residual organic material composted pollinasse, hereinafter referred to as compost (C). It was sown manually on July 7, 2023 in strips with furrows separated at 80 cm and depositing three seeds every 40 cm to leave 2 plants per clump and ensure a density of 62,500 plants ha⁻¹. Pests such as codling moth were controlled with two applications of the insecticide cypermethrin (6 and 14 days after planting) and a third application (Palgus) after 22 days; weed control was carried out with two manual hoeing at 12 and 22 days and a third application of hilling with machinery.

Fertilization with corn leachates was carried out with a 50% concentration diluted in water. Three applications were made to the plant foliarly and to the soil in the first 40 days after establishment. The previously prepared leachate treatments were applied on three occasions, 16, 28 and 44 days to the soil and foliage as appropriate for each treatment, the application was done manually, with a manual spray pump, bathing the entire plant to the foliage and soil, opening the nozzle to throw a continuous flow in a band at the foot of the plant, equivalent to 200 l ha⁻¹. The chemical treatment (TQm) whit nitrogen, was applied in two parts (50%): the first at 12 days after sowing, and the second at 21 days and in a single application of phosphorus at plant emergence with the formula 120 40 00 of N, P, K respectively, in a band at 10 cm from the plant.

Treatments evaluated. The levels of composted pollinasse (C) were applied to the soil (S) at different doses: 2.5, 5.0, 7.5, and 10 t h⁻¹. Foliar application (F) was carried out with the leachates (L) diluted 50% in water in three applications (Table 4). The experimental unit was in strips of 6 furrows (4.80 m wide by 40 m long). In the poultry manure treatments, the plot was divided into three furrows where the leachate was applied to the soil and the other three furrows where foliar fertilization was applied.

Table 4. Treatments Compost (C) applied to the soil and leachates (L) at 50%.

T ₁ Compost (C) 10 t ha ⁻¹ + Leachate (L) to Soil (S)	T10CLS
T ₂ Compost (C) 10 t ha ⁻¹ + Leachate (L) to Foliar (F)	T10CLF
T ₃ Compost (C) 7.5 t ha ⁻¹ + Leachate (L) to Soil (S)	T7.5CLS
T ₄ Compost (C) 7.5 t ha ⁻¹ + Leachate (L) to Foliar (F)	T7.5CLF
T ₅ Compost (C) 5 t ha ⁻¹ + Leachate (L) to Soil (S)	T5CLS
T ₆ Compost (C) 5 t ha ⁻¹ + Leachate (L) to Foliar (F)	T5CLF
T ₇ Compost (C) 2.5 t ha ⁻¹ + Leachate (L) to Soil (S)	T2.5CLS
T ₈ Compost (C) 2.5 t ha ⁻¹ + Leachate (L) to Foliar (F)	T2.5CLF
T ₉ Chemical Fertilization (120 40 00), N, P, K respectively	TQm
T ₁₀ Absolute treatment (000), no fertilizer	T000

Experimental design. The statistical analysis was carried out with a completely randomized design (DCA), six experimental units were established in the field: chemical fertilization (TQm), the absolute control (T000), and the remaining four for the levels of compost (C) to the soil: 2.5, 5.0, 7, 5 and 10 t ha⁻¹ where each one was divided in two parts of three furrows to apply the leachates (L) to the soil (S) and foliar (F) treatments. 5 and 10 t ha⁻¹ where each of them was divided into two parts of three furrows to apply the leachate treatments (L) to the soil (S) and foliar (F): the central furrow of 40 m, as replicates 15 plants were taken at random by complete competition along the experimental unit.

Study variables. The variables evaluated were: the vegetative development of the plant from the 7th week after earling (constant height) and green matter yield. Plant height to spike (cm), leaf length (cm) taken under the ear along the midrib lamina, and the average leaf width of the same leaf (middle part of the leaf), all variables measured with a tape measure. Stem diameter at 10 cm from ground level (cm), by means of a vernier (Truper). Cob height (cm) from ground level to cob; cob length (cm), from the base of the stalk to the apex of the cob and cob weight (g); and cob diameter in (cm) in the middle part of the fruit; all by means of a vernier in (cm). Grain yield (g cob⁻¹) and bacal weight in (g), after shelling; weights with a digital balance.

Data analysis. Analysis of variance and multiple comparisons of means were performed with the minimum significant difference (LSD) test with an alpha less than or equal to 0.05, both tests by using the SAS statistical program (Statistical Analysis System, 2021).

RESULTS AND DISCUSSION

Plant height. Figure 2 and Table 5 show the results obtained and the effects of the different treatments. The application of the highest doses of composts to the soil+foliar fertilization corresponded to treatments T7.5CF, T10CF, T10CS and T7.5CS t ha⁻¹ in descending order, with 282.67±10.2, 282.27±12.9, 278.00±7.94 and 272.67±10.8, respectively. Each experimental treatment surpassing TQm with an intermediate value (249.67±3.69 cm) for plant height, although results were statistically equal to T5CF, T5CS and T2.5CF, and higher according to the DMS test (p≤0.05). T7.5CS shows 19.1% greater

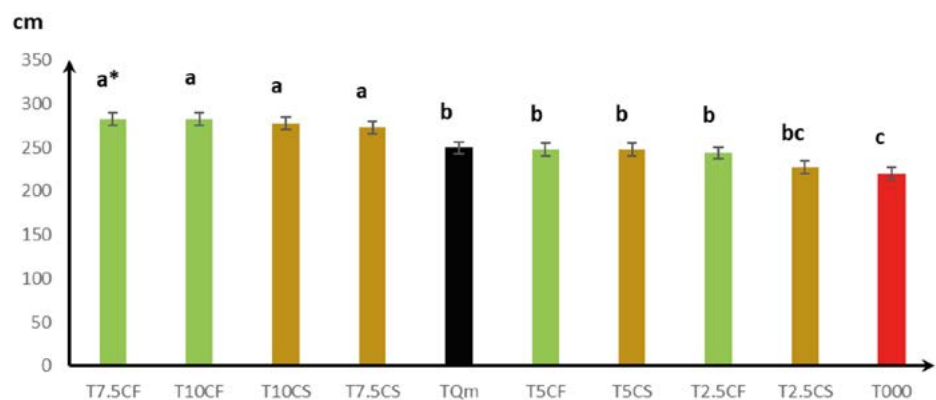


Figure 2. Height (cm) of corn plant with different applications of Compost to the Soil and Leachates to the Foliage (E.F. Huimanguillo Cycle P-V 2023). *Treatments with the same letter are not significant ($\alpha=0.05$ p.), C.V. Coefficient of Variation **SE=standard error of the mean.

Table 5. Vegetative growth of corn plant at 60 days, in height, leaf length and width, and stalk diameter.

Treatment	Height (cm)	Length (cm)	Width (cm)	Stem (mm)
T7.5CLF	262.67±5.2 EE**a*	102.93±1.5 EE ab	8.80±0.2 EE ab	22.40±0.7 EE abc
T10CLF	255.60±4.9 EE a	100.26±1.7 EE ab	8.47±0.2 EE bc	22.20±0.6 EE bcd
T10CLS	273.33±4.2 EE a	102.06±1.6 EE ab	8.92±0.1 EE ab	23.53±0.4 EE a
T7.5CLS	256.00±4.9 EE a	99.40±1.6 EE ab	8.73±0.2 EE ab	22.80±0.9 EE ab
TQm	249.67±3.6 EE b	104.13±1.4 EE a	8.97±0.1 EE ab	23.06±0.6 EE abc
T5CLF	248.06±4.2 EE b	103.46±1.6 EE ab	8.80±0.2 EE ab	21.86±0.6 EE bcd
T5CLS	246.73±3.9 EE b	100.80±1.8 EE ab	9.32±0.2 EE a	22.73±0.8 EE abc
T2.5LCF	224.40±4.3 EE b	92.73±1.3 EE c	8.62±0.2 EE b	19.93±0.7 EE d
T2.5CLS	227.20±4.7 EE bc	95.80±1.7 EE b	9.02±0.2 EE ab	21.66±0.7 EE bcd
T000	221.86±4.3 EE c	87.26±1.6 EE c	7.77±0.2 EE c	18.33±0.5 EE cd
C.V.	11.88	9.34	9.83	15.27

*Treatments with the same letter are not significant ($\alpha=0.05$ p.), C.V. Coefficient of Variation **SE=standard error of the mean.

height compared to T000 220.53±3.92 cm and with respect to the latter treatment, the other values showed greater plant height.

The vegetative variables are important to obtain the response of the crop treatment effects, also in the morphological characterization of the plants, for their possible use as fodder. Mariscal-Nava (2019), mentions that corn needs nitrogen, phosphorus and potassium, highlighting in the early stages of development is very sensitive to the lack of water and nutrients and in soils poorly fertile, do not have the ability to provide nutrients for efficient plant growth, to the detriment of yield, so it is necessary to resort to the use of chemical or inorganic fertilizers. In Cerón (2019), the effect of liquid fertilizers on the growth and yield of corn was evaluated, its response in growth curves is very similar in vegetative growth obtained, also mentions that they are useful to observe the effect of treatments and further explain the growth process. In other crops, the application of leachates in corn at 55 days, at a dose of 500 L ha⁻¹ was the maximum biomass yield with 37.5 t ha⁻¹ and in the sorghum crop of 4.8 t ha⁻¹ of grain (Patishtan *et al.* 2023). It has been reported that the application of 20 t ha⁻¹ of poultry manure, the highest yields in plant height were obtained (Perez, 2012).

Regarding the evaluation of organic leachates in corn plant height, the treatment leached with vermicompost obtained 205.67 cm, and commercial leachate with 218.50 cm, without statistical difference compared to T000 (221.86±4.3 cm), these were below in this work (Lopez, 2021). Organic leachates were evaluated in corn, plant height found no significant differences between the control 198 cm, vermicompost leachate 280 cm and plantain rachis leachate 286 cm (Villalobos-Gonzalez *et al.*, 2019). In evaluation with different corn genotypes under rainfed conditions and chemical fertilization in Campeche, in plant height the tallest material was purple creole maize with 370 cm, and the shortest was white cock maize with 274 cm height. Values higher than the highest of the treatment above T7.5CF (262.67±5.2 EE cm), this may be due to chemical fertilization and the potential of the genetic material.

Stem diameter. The results of this variable and their respective values are presented, in which the effects of the treatments can be observed (Table 5). Similar tendencies to the previous variable of the corn crop can be observed in the variable stem diameter at the base of the plant at 10 cm from the soil. The application of the highest doses of compost to the soil plus foliar fertilization corresponded to T10CS and T7.5CS $t\ ha^{-1}$ with values of 24.93 ± 1.10 EE and 24.13 ± 1.40 EE mm of stalk respectively, as those with the highest stalk diameter surpassing TQm with 22.72 mm the rest of the treatments were below these values statistically surpassing T000 according to the DMS test ($\alpha \leq 0.05$). Arnesto and Benavides (2003), reported the application of 20 and 10 $t\ ha^{-1}$ of poultry manure, obtained the largest stem diameters at 61 days with 2.54 and 2.59 cm by 2.13 cm (control) respectively without statistical difference between doses, but different from the control. And compared to the chemical control with the dose of 150 kg N ha^{-1} (2.42 cm) stem diameter. Quintanilla *et al.* (2013) for their part, coincided with identical values in corn stalk thickness between the poultry manure and chemical treatments with 1.65 cm. The application of organic fertilizers increases the availability of nutrients in the soil, more completely than with chemical fertilizers, due to the fact that organic fertilizers contain nutrients that can be assimilated by the plant.

Leaf length. The results for leaf length (cm) are shown in Table 5. The values of the treatments can be appreciated, although in this cycle it differs from the tendency of the previous variables because TQm 104.13 ± 1.4 cm was the one that presented the best result, although statistically equal to T5CF, T10CS, T7.5CF, T10CF, T7.5CS although inferior according to the DMS test ($\alpha \leq 0.05$). A greater leaf length development was obtained compared to (T000), exceeding 16.4%. The rest of the treatments resulted with intermediate values within this range, with the exception of the lowest dose T2.5CF which was outside the above mentioned range with the lowest value (87.03 ± 5.70 cm height).

A particular physiological characteristic of corn that favors its adaptation to tropical zones where evapotranspiration is sometimes high, is the anatomical structure of its leaves, in which there are two types of cells (epidermal and stomatal) with different biochemical and structural organization, which during the photosynthetic process allows them to fix CO_2 (Ramirez and Goyes 2004). The leaves are generally lanceolate, long and thin, reaching 120 cm in length, it should be noted that the treatments T7.5CS and the lowest in compost T2.5CS and T2.5CF along with T000 were below 100 cm. Lopez (2024), when evaluating leachates, reported leaf length values under the corn in treatments TL50% and TL75% with very similar values 104.00 ± 3.9 and 104.3 ± 2.3 cm, respectively. T7.5CF, T10CF T10CS TQm T5CF and T5CS were able to equal or exceed those reported by this author, which may suggest a better plant utilization of leachates. Golik *et al.* (2018), reported that the lamina is 60 to 90 cm long, ribbed, with wavy edges longer than the midrib, yellowish and prominent. In comparison to this author's report, all treatments exceeded this range with the exception of T000 87.26 ± 1.6 cm, which can be attributed to the lack of nutrients.

Leaf width. The results obtained for this variable are shown in Table 5. Their corresponding values, the effects of the different treatments show the same tendency as the other variables mentioned for the average leaf width variable of the corn crop. The

application of compost and foliar doses that obtained the best results corresponded to T5CS, T2.5CS, TQm, T10CS, T5CS, T7.5CF, T7.5CS, in descending order respectively, with 9.32 ± 0.22 , 9.02 ± 0.26 , 8.97 ± 0.16 , 8.93 ± 0.17 , 8.92 ± 0.19 , 8.83 ± 0.24 , 8.72 ± 0.27 cm of corn leaf mean width, statistically equal, although T5CS is superior according to the DMS test ($\alpha \leq 0.05$) where the T5CS treatment was 15.2% wider in leaf mean width compared to T000, while the other treatments showed greater leaf width. In the case of the compost (C), leachate (L) and chemical (TQm) treatments, the organics compete with each other and there is a marked tendency to surpass the control (T000) in leaf width value. Casarrubias-Castillo *et al.* (2019), mentions that in maize leaves stomata are located at the plant-atmosphere interface, regulate photosynthesis and water use efficiency, which translates into these cells can be determining factors to increase plant productivity, therefore stomata can absorb part of the applied foliar nutrients.

Weight of plant in fresh. The results of the variable fresh weight (kg) and their respective values are presented in Table 6. The applications of the highest doses of compost to the soil plus foliar fertilization corresponded to T10CS T7.5CS, T7.5CF, T10CF, and t ha^{-1} in descending order respectively, with 0.80 ± 0.02 , 0.78 ± 0.04 , 0.73 ± 0.04 , 0.73 ± 0.04 EE kg of maize plant wet matter. Each treatment outperforming TQm with a lower value (0.63 ± 0.04 kg) and statistically with respect to T000 (0.43 ± 0.03 EE) according to the DMS test ($\alpha \leq 0.05$). The T10CS showed 46.71% higher green matter compared to T000 and with respect to T000 the other values showed higher plant fresh weight. Matheus *et al.* (2007), mentions that the growth of corn plants was at 63 days the highest amount of biomass was registered; from there on, production decreased in a progressive and very accentuated way. The greatest effect of the treatment with chemical fertilization reached its maximum level in the initial phase of the trial, while the treatments with organic products reached their greatest effect at 63 days, evidencing their greatest residual action. Lopez

Table 6. Characteristics in fresh corn plant at 60 days, in fresh weight, corn kernel weight, soil-ear height and ear length.

Treatment	Fresh weight (kg plant ⁻¹)	Weight of corn (g)	Soil-corn cob height (cm)	Cob length (cm)
T10CLS	0.80 ± 0.02 EE a*	305.18 ± 15.25 EE abc	134.54 ± 2.97 EE ab	15.77 ± 0.61 EE c
T7.5CLS	0.78 ± 0.04 EE a	332.27 ± 23.59 EE ab	141.36 ± 5.22 EE a	17.72 ± 0.68 EE a
T7.5CLF	0.73 ± 0.04 EE ab	338.55 ± 17.28 EE a	128.81 ± 3.90 EE bcd	17.54 ± 0.45 EE ab
T10CLF	0.73 ± 0.04 EE ab	340.91 ± 9.90 EE a	141.63 ± 5.35 EE a	17.18 ± 0.46 EE abc
T5CLF	0.71 ± 0.04 EE ab	316.36 ± 18.69 EE abc	127.72 ± 4.40 EE bcde	17.45 ± 0.69 EE ab
T2.5CLS	0.65 ± 0.05 EE bc	284.18 ± 22.95 EE bcd	116.36 ± 2.78 EE e	16.59 ± 0.60 EE abc
TQm	0.63 ± 0.04 EE bcd	292.36 ± 22.11 EE abc	133.36 ± 4.29 EE abc	15.77 ± 0.52 EE c
T5CLS	0.58 ± 0.03 EE cd	279.64 ± 17.76 EE cd	120.54 ± 3.06 EE of	16 ± 0.43 EE bc
T2.5CLF	0.52 ± 0.03 EE of	239.55 ± 11.49 EE of	122.81 ± 4.33 EE cde	15.59 ± 0.53 EE c
T000	0.43 ± 0.03 EE e	198.51 ± 19.51 EE e	104.18 ± 3.88 EE f	13.72 ± 0.83 EE d
C.V.	12.31	20.82	10.71	12.12

*Treatments with the same letter are not significant ($\alpha = 0.05$ p.), C.V. Coefficient of Variation **SE=standard error of the mean.

(2024), evaluating the application of leachates, found that the best treatment obtained values within a range of 0.831 and 0.658 kg plant⁻¹ corresponding to the treatments and TL25% respectively; compared to those obtained in this research, it exceeds the highest value of T10CLS 0.80±0.02 with respect to TL50%, the latter being slightly higher.

Corn weight. The results obtained in this variable are shown in Table 6. In the first place, T10CF was where the best results were observed in the variable fresh weight of corn with a weight of 340.91±9.90 g, the treatments that were in between T10CLS, TQm in descending order respectively 305.18±15.25 EE, 292.36±22.11 g are statistically equal to T10CF although inferior according to the DMS test ($\alpha \leq 0.05$). T10CF presented a higher fresh weight of corn in comparison with T000, exceeding it by 41.6%, and the other treatments had better weight gain in this variable. With respect to other works in this variable, López (2024), reported that when applying TL75% leachates, 279.20±21.30 g had the highest weight compared to the highest obtained in this work (T10CLF 340.91±9.90 g), which was 81.89% higher.

Soil-corn cob height. The data acquired in this variable are presented in Table 6. And their corresponding values, the effects of the different treatments can be appreciated in the fourth cycle of corn cultivation in the variable soil-corn cob height of maize. The application of compost and foliar doses with the best results corresponding to T10CF, T7.5CS, T10CS, followed by TQm, T7.5CF, in descending order respectively. With 141.63±5.35, 141.36±5.22, 134.54±2.97, 133.36±4.29, 128.81±3.90, soil-corn cob height (cm) of maize, statistically equal DMS ($\alpha \leq 0.05$), although the T10CF was 26.4% higher in soil-corn cob height compared to the control T000 104.18±3.88 EE cm, while the other treatments showed greater height in this variable. Córdova (2005) in corn, reports a range of 147.0 to 111.7 cm for the highest application 30 t ha⁻¹ of poultry manure (plus 20 t ha⁻¹ of worm castings) and control respectively in average ear height. The evaluation with different corn genotypes under rainfed conditions and chemical fertilization 110N-46P-00K at 30 days in Campeche, the cob height cm resulted higher in purple creole maize (218 cm), and the lowest was white cock maize with 274 cm height (Villalobos-González *et al.*, 2019)

Cob length. Table 6 shows the results of the variable cob length (cm), where the greatest length is distinguished by the treatments T7.5CS, 17.72±0.68 EE and T7.5CF 17.54 ±0.45 EE cm, respectively were those that presented better results in this variable and in other variables surpassing the T10CF, T10CS level, in this variable T7.5CS surpassed TQm 15.77±0.52 with 11% and 22.5% to T000 13.72±0.83 statistically inferior to T7.5CS according to the DMS test ($\alpha \leq 0.05$) since T000 was the lowest in this variable the other treatments had better fruit development. In this regard, Larios and Garcia (1999), reported the application of 15, 5 and 10 t ha⁻¹ of poultry manure in the corn crop in the variety NB-6, where they obtained ear length values: 14.35, 13.46 and 14.75 (cm), respectively, these data are lower than those of the present work, even using less amount of poultry manure T7.5CS, T7.5CF, T5CF, greater lengths were obtained 17.72±0.68, 17.54±0.45, 17.45±0.69, being T7.5CS superior in 19% to the best treatment. Another work by Córdova (2005) on the application of poultry manure 20 t ha⁻¹ plus earthworm humus, showed a range of 17.97 cm *vs.* 15.27 cm with respect to the control.

Cob diameter. The results obtained in cob diameter (mm) can be observed more clearly in the fourth cycle of corn cultivation (Table 7), that the TQm and T000, were the ones that presented lower results 44.72 ± 3.60 , 44.36 ± 1.24 mm therefore the other treatments were more efficient in comparison to these. T10CF was the one that obtained the highest values surpassing TQm and T000 by 14.1% and in the variable diameter (mm) of corn cob, T7.5CF, T10CS, T5CF, T7.5CS, T2.5CS, are statistically equal although inferior to T10CF, according to the DMS test ($\alpha \leq 0.05$). The results obtained by Lopez (2021), reports that the evaluation of vermicompost leachates and plantain rachis, in the variable corn cob thickness values of 45 and 47 mm, without statistical difference, these values are very similar to those obtained in this variable. In another work, Lopez (2024), reported a statistical response in the application of leachates at 25% (TL25%), the best ear thickness in the variety H520 with 19.30 ± 0.50 cm, with statistical difference with respect to treatment TL00%, with 16.55 ± 1.01 .

Cob weight. The results obtained for cob weight (g) of corn, the effects of treatments of the third crop cycle are observed (Table 7). The highest compost application of T10CLF in turn, obtained the best response with 286.00 ± 8.92 , surpassing the control T000 (163.27 ± 16.04 g) by 42.90% and by 12.4% to chemical fertilization (TQm), with 250.45 ± 18.89 g, as the best treatment effect, leaving the TQm with an intermediate value with respect to other treatments based on compost resulting in significant differences ($\alpha \leq 0.05$). López (2024), suggest that the range of cob weight was found between application of leachate TL75% 144.80 ± 8.48 g and TQm with 105.30 ± 9.71 g respectively, a difference of 27.27%, and with an intermediate value the control control (T000 134.00 ± 14.53 g), exceeding 50.62% of T10CLF this difference is attributed to the greater nutritional contribution of the compost. Salinas-Vargas *et al.* (2022), reported when evaluating different corn genotypes, that the highest cob weight was with the white maize (308 g) and the lowest was the blue variety (111 g), with chemical fertilization (180N 80P 00K) and irrigation, exceeding the highest value obtained from T10CLF by

Table 7. Corn plant cob characteristics: ear diameter, ear weight, kernel weight and ear weight.

Treatment	Cob diameter (mm)	Cob weight (g)	Grain weight (g)	Weight of cod (g)
T10CLF	52.09 ± 1.23 EE a	286 ± 8.92 EE a	159.54 ± 4.26 EE a*	26.09 ± 0.53 EE a*
T7.5CLF	49.18 ± 0.93 EE ab	282.27 ± 12.92 EE ab	159 ± 6.64 EE a	25.36 ± 1.25 EE ab
T10CLS	48.72 ± 0.64 EE abc	244.36 ± 11.64 EE bcd	140.54 ± 7.60 EE ab	24.72 ± 1.48 EE abc
T5CLF	48.72 ± 0.54 EE abc	257.91 ± 12.04 EE abcd	143.63 ± 5.60 EE ab	21.27 ± 0.98 EE bcde
T7.5CLS	49.63 ± 0.66 EE abc	272.09 ± 17.51 EE abc	144.91 ± 9.55 EE ab	24.18 ± 1.96 EE abcd
T2.5CLS	48.54 ± 1.26 EE abc	233.18 ± 17.0 EE cde	123.27 ± 8.86 EE bc	20.18 ± 1.62 EE def
T5CLS	45.63 ± 0.76 EE bcd	228.09 ± 14.06 EE of	127 ± 7.95 EE bc	20.9 ± 0.896 EE cde
T2.5CLF	44.90 ± 0.99 EE cd	201 ± 8.58 EE ef	111 ± 5.14 EE c	18.09 ± 1.17 EE ef
TQm	44.72 ± 3.60 EE cd	250.45 ± 18.89 EE abcd	141.36 ± 11.20 EE ab	20.54 ± 2.02 EE of
T000	44.36 ± 1.24 EE d	163.27 ± 16.04 EE f	83.82 ± 9.14 EE d	16.27 ± 1.94 EE f
C.V.	10.18	19.43	19.58	22.40

*Treatments with the same letter are not significant ($\alpha=0.05$ p.), C.V. 10.18, SE=standard error of the mean.

92.85%. This difference is attributed to chemical fertilization and irrigation under the conditions of Guasave, Sinaloa. In cob weight, Escobar *et al.* (2013), determined that the organic mixture: coffee pulp+litter+chicken manure+cattle manure was the highest with 611.2 g, a value 46.79% higher than the highest value of T10CLF (286 ± 8.92 g), which can be attributed to the maize genetic material, with greater potential.

Grain weight. The effects of treatments were more evident in the fourth cycle of corn cultivation in the variable grain weight (Table 7). The application of 10 and 7.5 (t ha⁻¹) compost to the soil plus foliar fertilization surpassed the absolute control (T000) by 52.53% and 47.47% to the chemical application (TQm), as the best responses, leaving the TQm with an intermediate value (250.45 ± 18.89), with respect to other treatments based on compost resulting with statistical difference ($\alpha \leq 0.05$). The above results coincide with those reported by Vásquez *et al.* (2015), who found response to the application of raw and composted manures to supply the nutritional requirements of maize in the tropical region of Veracruz in grain (kg ha⁻¹) with the maize variety H-520. Wood *et al.* (1996), found that with the application of poultry manure to corn crops at a dose of 8 t ha⁻¹ optimum yields were obtained, while at higher doses of 18 t ha⁻¹ yields decreased. In another work, Microbial consortia were applied in liquid form, with doses of 10 ml at the foot of each corn plant at 20, 40 and 60 days after planting. The results were 11.59 t ha⁻¹, statistically superior to other treatments (Rosabal-Ayan *et al.* 2022). In this research, with lower doses of compost applied, the yield was acceptable, possibly due to the combination with leachates, in addition to the organisms that contribute and the residual nature of the compost, as mentioned by Sánchez (2021).

Weight of rachis (olote, bacal). The results obtained for the variable weight (g) of corn stool are presented in Table 7. The applications of the highest doses of composts to the soil plus foliar fertilization corresponded to T10CF T7.5CF, T10CS, T7.5CS, and t ha⁻¹ in descending order respectively, with 26.09 ± 0.53 , 25.36 ± 1.25 , 24.72 ± 1.48 , 24.18 ± 1.96 , of weight (g) of bacal, for each treatment surpassing TQm with a lower value (20.54 ± 2.02 g) and statistically with respect to the control T000 (16.27 ± 1.94) according to the DMS test ($\alpha \leq 0.05$). The T10CF showed a 37.6% higher weight compared to the T000 and with respect to this the other values showed higher bacal weight. The results obtained indicate that there are favorable trends to the application of composts and leachates in the

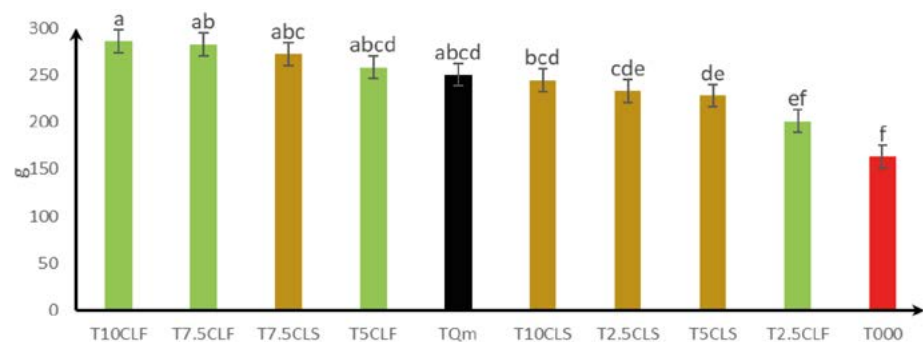


Figure 3. Cob weight (g) of corn with different applications of compost to the soil and leachates (E.F. Huimanguillo Cycle P-V 2023).

vegetative variables of corn growth and dry matter. Lopez (2024), in an investigation with organic fertilization with leachates of corn crop variety H520, found significant differences between treatments T25% with 30.20 and TQm 28.10 g respectively with a range between higher and lower values corresponding to 32.4%. Compared with the values obtained with respect to T10CLF (26.09 ± 0.53 g) against the highest T25% (30.20 g), it resulted 86.39%, higher the application of only leachate, while for the lowest level T000 (16.27 ± 1.94 g) against the TQm (28.10 g), this resulted in 57.90%, which may be related to the amount of grain per cob⁻¹, as a possible effect of treatments, which was reflected in higher cob weight to the detriment of grain.

CONCLUSIONS

The effects of the treatments in the third cycle of corn production were consistently manifested in the variables: height, stem diameter, average width and length of the leaf, length and average diameter of the corncob, grain weight and basal; being superior with respect to the absolute control (T000) and the lowest treatment based on compost (T2.5CL), and compared to the chemical fertilization (TQm). On the other hand, the fertilization treatments T5CL, T7.5CL and T10CL were competitive in the different variables analyzed, and even superior in their values with the exception of the leaf length variable. It is evidently concluded that this type of fertilization is beneficial and can be an alternative to improve and practice agriculture with less application of chemical fertilizers to contribute to a cleaner and safer environment for humans.

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Assessment of *Hemileia vastatrix* Severity in *Coffea arabica* L., using Image Analysis (Pliman)

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ABSTRACT

Objective: To determine the severity index of *Hemileia vastatrix* on coffee leaves using the Pliman package in R Studio, aiming to optimize quantitative evaluations and improving disease monitoring.

Methodology: Sampling was conducted at Los Barreales farm located in Teocelo, Veracruz, where leaves from the Catuaí amarillo coffee variety, both infected by *Hemileia vastatrix* and healthy, were selected. The leaves were photographed to capture the leaf surface, and the images were subsequently processed in Photoshop to calculate the healthy and rust-affected areas. Subsequently, the RGB color index of the images was analyzed using the Pliman package in R Studio. Various indices were evaluated, and the NGRDI was selected for the automatic quantification of affected and healthy areas. Finally, an analysis of variance (ANOVA), followed by Tukey's test, was performed to compare significant differences among the samples.

Results: Indices based on specific combinations of RGB colors effectively highlighted subtle differences in leaf reflectance, facilitating the detection of disease symptoms, particularly with the NGRDI index.

Study Limitations: While reflectance spectroscopy is highly accurate, it can be expensive and requires specialized equipment.

Conclusions: Computational tools offer precise and rapid disease detection, providing critical support for integrated pest and disease management strategies in agriculture.

Keywords: Leaf disease, rust, image processing.

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INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important beverages worldwide and a crop of significant commercial, economic, and social value (Gichuru *et al.*, 2012). However, coffee production has been severely affected by coffee leaf rust (*Hemileia vastatrix* Berkeley and Broome), which is considered the primary disease causing substantial economic losses (Cabral *et al.*, 2009; Gichuru *et al.*, 2012; Alvarado-Castillo *et al.*, 2017). The main symptoms include small yellow spots on the underside of the leaves, whose appearance is influenced by climatic conditions, facilitating the sporulation and dispersal of uredospores, eventually leading to plant death (Huaman, 2021; Muttappagol *et al.*, 2022).

For early disease detection, standard area diagrams based on visual estimations have been developed, allowing for the assessment of disease severity and serving as crucial tools for decision-making to identify the most effective treatments (Barbedo, 2014). However, these visual estimations can be subjective and depend on the observer's experience, potentially leading to inaccuracies (Avelino *et al.*, 2015).

In an effort to standardize visual estimations, the National Coffee Research Center (CENICAFÉ) developed diagrams of rust-affected areas for technical assistants and coffee growers in Colombia (López-Vásquez *et al.*, 2018). In Mexico, the National Service for Agrifood Health, Safety, and Quality (SENASICA) established a seven-level evaluation scale ranging from 0 to 6, with damage percentages varying between 0% and 70% (Calderón, 2016). Recently, plant disease assessment has advanced toward the use of digital image analysis, enabling faster processes and more accurate quantitative evaluations (Mutka & Bart, 2015; Bock *et al.*, 2020; Gallego-Sánchez *et al.*, 2020).

Reliable and accurate estimations are essential for predicting yield loss, forecasting epidemics, evaluating crop resistance to diseases, and understanding the biological processes of pathogens. Some of the software tools used for these evaluations include ImageJ with the rust script (Schneider *et al.*, 2012), Quant (Bock *et al.*, 2022), and R Studio with the Pliman package, which estimates rust severity in crops such as wheat and soybean (Olivoto *et al.*, 2022). These programs are based on the analysis of RGB (red, green, and blue) values from digital photographs to distinguish plant diseases by quantitatively differentiating symptomatic areas from healthy ones (Bock *et al.*, 2020). However, there is no literature reporting the severity analysis of coffee rust.

The objective of this study was to determine the severity index of *H. vastatrix* on coffee leaves using the Pliman package in R Studio, with the aim of improving quantitative evaluations and enhancing disease monitoring in susceptible varieties.

MATERIALS AND METHODS

Study Area

The sampling was conducted at Los Barreales farm, located in the municipality of Teocelo, Veracruz (19° 23' 37.3" N, 96° 59' 01.0" W; 19° 23' 39.7" N, 96° 59' 12.4" W and 19° 23' 34.0" N, 96° 59' 14.9" W; 19° 23' 29.5" N, 96° 58' 59.0" W).

Leaf Sampling and Photography

A total of 144 coffee leaves from the Catuaí amarillo variety were collected, consisting of 72 leaves infected by *H. vastatrix* and 72 healthy leaves. Photographs of the leaf surface were taken using a white sheet as a neutral background. A Sony A6400 camera was positioned 15 cm away vertically with the help of a tripod. The integrated flash was used, and a Sigma 30 mm lens was employed with the following settings: aperture f/9, exposure time 1/60 s, ISO 500, and focal length 30 mm.

Image Processing

An initial manual classification was performed using Adobe Photoshop to delineate the areas of healthy tissue (S), infected tissue (I), and background (F). Four validation stages

were conducted with increasingly precise samples, aiming to determine the best estimation of tissue infected by *H. vastatrix* on coffee leaves.

First Validation (p1): A tissue sample of S, I, and F areas was selected from the image (Figure 1A). Second Validation (p2): 90 samples of healthy tissue, 54 samples of infected tissue, and 64 samples of the background were selected (Figure 1B). Third Validation (p3): 122 samples of healthy tissue, 74 samples of infected tissue, and 187 samples of the background were obtained (Figure 1C). Each sample set was combined into an independent image using Photoshop to calculate the healthy area and the asymptomatic area affected by rust on the coffee leaves (Figure 1).

The fourth stage (p4) involved the analysis of the RGB indices performed by the Pliman script, which makes estimations using the red, green, and blue colors with the following equation $(G-R)/(G+R)$ ($green-red)/(green+red)$ to highlight the infected tissue from the healthy tissue on coffee leaves (Olivoto *et al.*, 2022). It was observed that the HI indices reveal healthy tissue, NGRDI detects pustules, and NDGBI neither shows pustules nor healthy tissue (Figure 1D). Twenty-five indices are shown (Figure 2A) with different estimations for detecting *H. vastatrix* pustules, using different codes, from which NGRDI was selected due to being the most precise (Figure 2B). The infected leaf by *H. vastatrix* is shown (Figure 2C). Then, the selected code was analyzed with Pliman, which detected all pustules, as seen in Figure 2D in black. A threshold graph was created to define the accuracy of the NGRDI index in which the pustules of rust were best detected, and it was established at 0.045 (Figure 3).

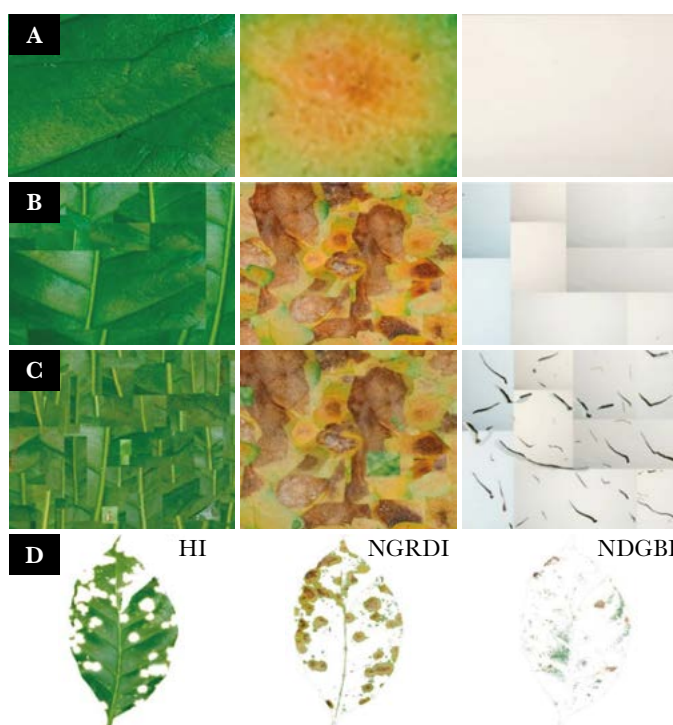


Figure 1. Sample images of healthy tissue, infected tissue, and background for analysis in Pliman.

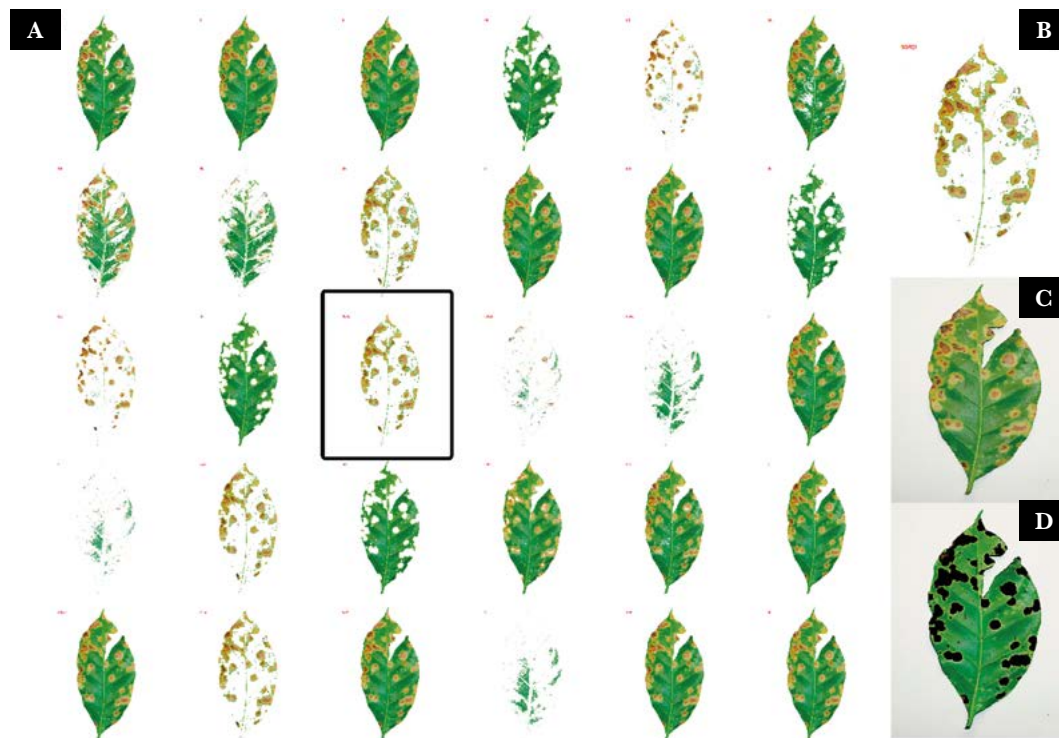


Figure 2. A) Comparison of different RGB indices, with the selected one for detecting *H. vastatrix* pustules on coffee leaves shown in the black box. B) The index that best detected the pustules was NGRDI. C) Original photograph of coffee pustules. D) Detection of the pustules with the selected code.

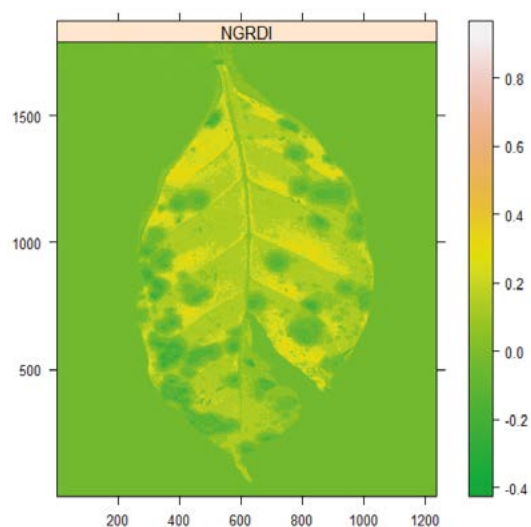


Figure 3. Color threshold based on RGB colors (Red, Green, and Blue) that allows the identification of *H. vastatrix* pustules with the highest accuracy.

The Pliman R package

This package is designed to perform various analyses on plant leaf photographs, including measuring area, counting objects in an image, calculating the symptomatic area

of the disease, extracting RGB values for each object in an image using different color indices, and calculating object measurements (Olivoto *et al.*, 2022).

Pliman script (R Studio software) for *H. vastatrix*

Once the samples for healthy tissue (S), infected tissue (I), and background (F) were established, the selection of leaf areas was carried out automatically. The current stable version of the package (0.3.0) requires R 4.1.0 and can be installed directly through the R console using: `install.packages`, following the methodology of Olivoto *et al.* (2022).

Statistical analysis

An analysis of variance (ANOVA) and a Tukey's test were performed to compare significant differences between tests with a p-value <0.05 in R Studio to compare the different validation stages and precision of the results.

RESULTS AND DISCUSSION

Effectiveness of color indices in plant disease detection

The use of color indices for detecting the severity of *H. vastatrix* is relatively new. In this study, 25 indices based on RGB (Red, Green, and Blue) colors were obtained, and the NGRDI code was selected, as it detected the coffee rust pustules most effectively. According to Olivoto *et al.* (2022), indices based on specific combinations of RGB colors can highlight subtle differences in leaf reflectance, facilitating the detection of disease symptoms. In particular, the NGRDI index has proven to be effective in this study for highlighting infected tissue, which is consistent with other studies that have used vegetation indices to detect stress and diseases in plants (Rivadeneira and Huamán, 2021). In other studies, NGRDI is used as part of a method to improve the detection of grassy weeds in rice fields (Barrero and Perdomo, 2018).

It is important to note that the severity analysis of *H. vastatrix* was carried out using the ImageJ program with the Rust script through the free Fiji image software, which is one of the most widely used programs for measuring rust pustules in wheat. It is also recognized in the scientific community because it allows the evaluation of diseases by transforming color using pixel-based threshold measurements to calculate leaf area in certain cereal crops (Gallego-Sánchez *et al.*, 2020). However, for this study, it was not effective, as it failed to detect small coffee rust pustules and also encountered difficulties in distinguishing leaves from their background when using a single threshold (Easlon and Bloom, 2014; Alheeti *et al.*, 2021). It is worth mentioning that each leaf (healthy and infected) was analyzed for infected tissue (Figure 4A; Figure 5A) and healthy tissue (Figure 4B; Figure 5B) to determine if the tests correctly detected the tissue. Both healthy leaves (Figure 4) and infected ones (Figure 5) showed that the p4 test was significantly different from the p1 test ($p < 0.05$).

This could be due to the image crop captures of healthy tissue (S), infected tissue (I), and background (F). In the first stage (p1), only one sample was selected, compared to the third stage (p3), where 122, 74, and 187 image captures were obtained, respectively. It is worth noting that, although no significant differences were observed between p3 and p4, the latter does not require image samples of the different tissues, since the indices

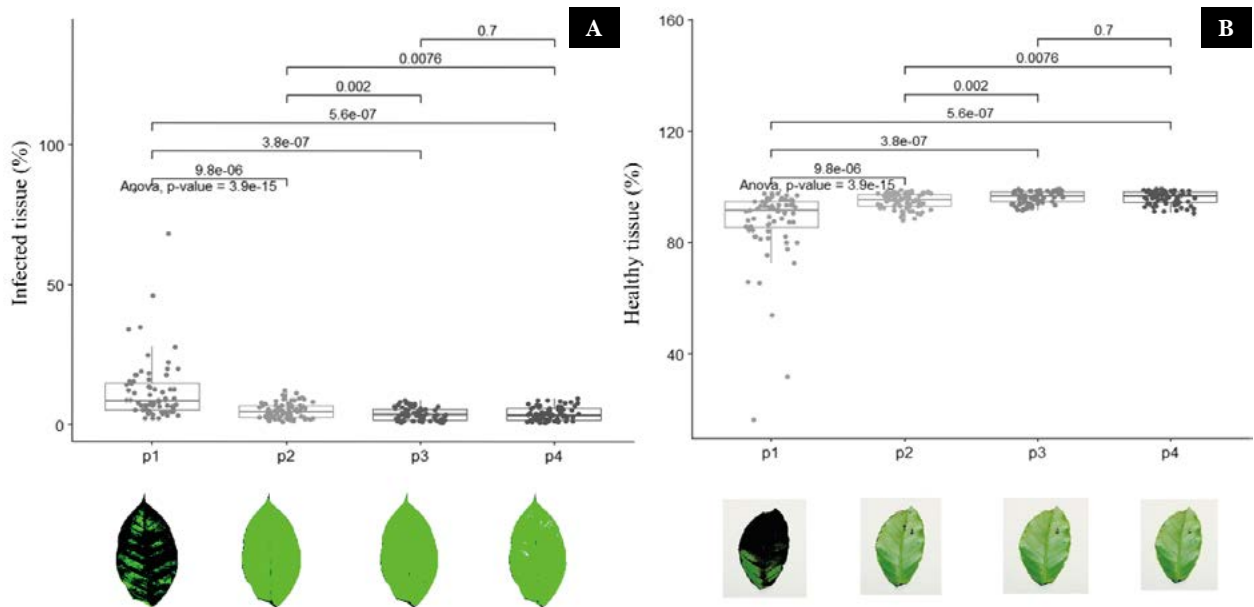


Figure 4. In healthy leaves, infected and healthy tissue are observed from the different training tests of Pliman (p1-p2-p3-p4). Significant differences between the tests are indicated at the top ($p < 0.05$).

used by Pliman only consider the RGB values of a photograph to distinguish healthy and infected tissue. This allows for reduced working time, as the image processing can be done more quickly.

In the second test, p1 and p2 detected the veins as symptomatic, because *H. vastatrix* has a yellow coloration very similar to that of this tissue (Figure 5A), so this type of error is likely the reason for quantifying false positives, according to Barbedo (2014), who removed the petiole and veins of some coffee leaves using a two-pixel radius to prevent the algorithm from incorrectly counting the veins as symptomatic tissue. Therefore, it was necessary to take precise digital captures and include them in the sample image of healthy tissue, so that Pliman could perform a better analysis and the program could detect the symptoms of the coffee leaves more accurately. Similar phenomena have also been reported in the accuracy of manual disease severity assessments performed by individual evaluators, such as Leaf Doctor (Pethybridge and Nelson, 2015; Bock *et al.*, 2020). Thus, as the samples increase and parameters are optimized, it is consistent with the literature that suggests that a greater number of samples and parameter refinement improve the accuracy in disease detection.

The use of the Pliman package in R to automate image analysis is becoming increasingly common in plant pathology. Pliman allows for complex image analysis, such as measuring areas, counting objects, and extracting RGB values, in an efficient and precise manner. The methodology applied in this study demonstrates how computational tools can facilitate the accurate and rapid detection of diseases, which is essential for integrated pest and disease management in agriculture.

In p1, the affected area percentage ranges from 26.61% to 78.38%. This suggests that these leaves are severely affected by *H. vastatrix*. However, this result is inaccurate as

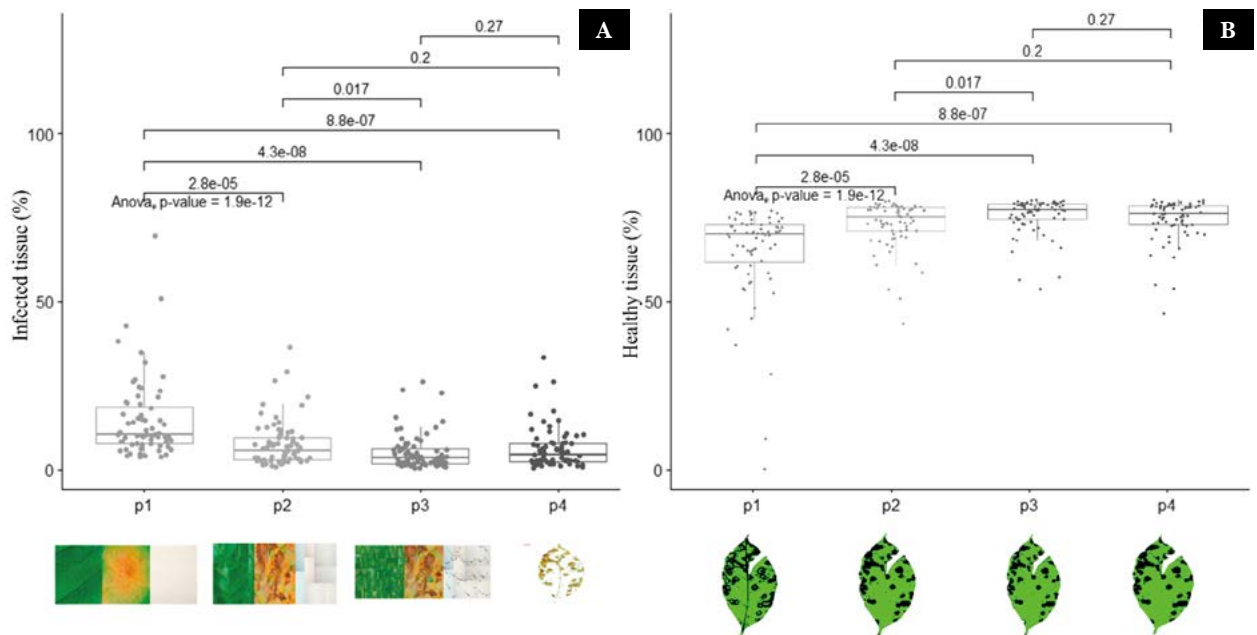


Figure 5. In coffee leaves infected by *H. vastatrix*, infected tissue and healthy tissue are observed from the different training tests of Pliman (p1-p2-p3-p4). Significant differences between the tests ($p < 0.05$) are indicated at the top.

healthy tissue is detected as infected. In p2, the leaves show a lower percentage of infected tissue compared to the first test, with percentages ranging from 0.89% to 36.36%. In p3, the affected areas are smaller, with percentages ranging from 0.53% to 25.84%. In p4, the range is from 0.48% to 33.44% (Figure 6).

In the research, these classifications are generally derived from visual assessments and the use of infected area diagrams, which are used as evaluation tools; however, they lack accuracy, precision, and reliability. In coffee, visual estimation has been used (Melo *et al.*, 2020), and in Mexico, SAGARPA proposes seven classes in its diagrammatic scale for severity in leaves with a percentage less than 0% and greater than 70% (Calderón, 2016). In Colombia, CENICAFE has a series of 15 images for visual estimation, which ranges between 0.05% and 80.0%, assigning a class mark of midpoint points at 1.0%, 2.0%, 4.0%, 8.0%, 16.0%, 32.0%, and 64.0% severity (López-Vásquez *et al.*, 2018). In this study, six evaluations of the affected area were carried out in the four tests to detect the symptoms of *H. vastatrix*, and the most accurate evaluation was p4, with the highest percentage of 33.44% and the lowest at 0.48%.

Therefore, with this test, a reliable, quantitative, and precise evaluation is being conducted to detect the presence of *H. vastatrix* in coffee leaves, which can be reproducible for continued monitoring of rust in the field (Malooof *et al.*, 2013), determining the loss of leaves, fruit, and branch death, as well as plant death, improving the understanding of the disease's epidemiology, and evaluating the effect of treatments (Bock *et al.*, 2022). The method proposed by Pliman is ideal for situations where speed is important or when a large number of images must be processed. This package can improve the accuracy of *H. vastatrix* detection quantitatively and strengthen the disease monitoring in susceptible varieties.

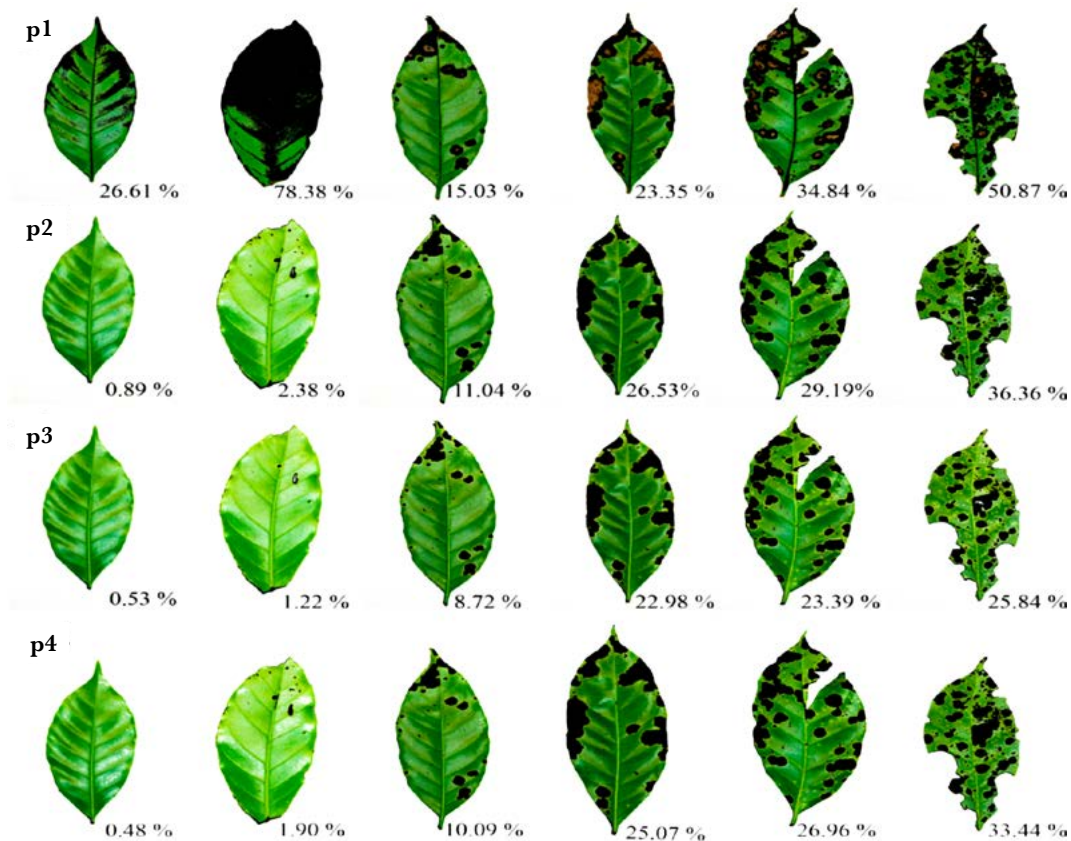


Figure 6. Diagram of the severity percentage of *H. vastatrix* in the different validation tests (p1-p4), comparing the accuracy of pustule detection.

Comparing this approach with traditional disease detection methods, such as visual inspection and reflectance spectroscopy, the methodology based on NGRDI and image analysis presents clear advantages in terms of speed, accuracy, and the ability to process large volumes of data. Visual inspection can be subjective and dependent on the observer's experience, while reflectance spectroscopy, although precise, can be expensive and requires specialized equipment.

Despite the positive results, it is important to consider some limitations. The methodology may be influenced by variations in lighting and environmental conditions during the image capture process. Additionally, while NGRDI was accurate in this study, its effectiveness may vary with other coffee varieties or plant species. Future research could focus on validating this index under different conditions and with other plant species to generalize its applicability.

CONCLUSIONS

The results obtained demonstrate that the NGRDI index is an effective tool for the detection of coffee rust, providing superior and objective precision in identifying infected areas. With the Pliman package, the severity of *H. vastatrix* in coffee leaves was assessed, and it was determined that the best validations were p3 and p4.

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Identification of the Causal Agent of Dieback in Blueberry (*Vaccinium corymbosum*) and Its *In Vitro* Control

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ABSTRACT

Objective: The objective of this study was to isolate, identify, and characterize the causal agent of blueberry (*Vaccinium corymbosum*) dieback and its *in vitro* control.

Design/methodology/approach: Samples were collected from three municipalities in Michoacán with different edaphoclimatic conditions. Using the Agrios protocol, three strains identified as *Pestalotiopsis* sp. were isolated and labeled as CAZr01, CAZh02, and CATg03. The isolates' morphological characteristics were determined. For *in vitro* sensitivity bioassays, the fungicides Robust R[®], Tacora 25 Ew[®], Cabrio[®] C, Programic[®] Mega, and Aliette[®] Wdg were tested at three doses (low, medium, and high) according to the manufacturer's recommendations, with sterile water serving as the control treatment. The virulence and severity of the isolates were determined through pathogenicity tests on healthy plants. The experimental design was completely randomized, and a Tukey mean comparison test was applied with a 5% probability of error.

Results: The most effective fungicide for controlling mycelial growth was Tacora 25 Ew[®], which showed significant inhibition at all tested doses. Of the isolated strains, CAZh02 was the most virulent, causing plant death in the shortest time.

Limitations/implications: This study provides information on the pathogen affecting blueberry and proposes an effective method for its control.

Findings/conclusions: It was found that blueberry dryer is caused by *Pestalotiopsis* sp., which is most efficiently controlled *in vitro* with Tacora 25 Ew[®]. Among the three isolated strains, CAZh02 was found to be the most virulent.

Keywords: isolates, fungicide effectiveness, *Pestalotiopsis*.

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INTRODUCTION

Mexico has specialized in the production and export of predominantly blueberries, thanks to the diverse climates and favorable conditions that enhance their growth and yield



[1]. In 2023, blueberry production in Mexico reached 80,133 tons, cultivated across 5,836 hectares, with an average yield of 13.7 tons per hectare and a production value of 5.212 billion pesos [2].

At the national level, Michoacán ranks third with 209 tons of blueberries but generates the highest production value. Within the state, the municipality of Salvador Escalante leads, accounting for 24% of the state's production, followed by Tangancícuaro with 16%, and Los Reyes in third place with 12% of the total production. Ziracuaretiro ranks tenth, contributing 4% of the state's production [2].

In areas where blueberries are cultivated, one of the main production limitations is the prevalence of various diseases, particularly fungal diseases. These significantly impact fruit quality and cause considerable production losses due to the massive death of plants and the decline of orchards. The main fungal pathogens include *Colletotrichum* sp., *Fusarium* sp., and *Alternaria* sp. [3], which cause damage to roots, stems, and leaves.

However, there are other microorganisms of significance that produce similar symptoms and have yet to be identified to establish appropriate control measures. This is the case with blueberries, where symptoms of dieback have been observed, significantly reducing yield and even causing plant death at any age. The objective of this study was to isolate, identify, and characterize the causal agent of blueberry dieback and its *in vitro* control.

MATERIALS AND METHODS

Study Area and Biological Material

Samples were collected from blueberry plants showing symptoms of leaf chlorosis, defoliation, complete branch dieback, and reddish necrosis at the basal part of the stem. These samples were placed in Ziploc[®] bags labeled with the following information: date, collection order, orchard name, and GPS coordinates from three municipalities in Michoacán, Mexico.

The municipality of Salvador Escalante is located at 19° 24' 23" N and 101° 38' 24" W at an altitude of 2,239 meters; Ziracuaretiro is situated at 19° 21' 31" N and 101° 48' 00" W at an altitude of 2,400 meters; and Tangancícuaro is located at 19° 53' 17" N and 102° 17' 30" W at an altitude of 3,400 meters.

The isolations were performed from stems showing disease symptoms, following the protocol described by [4]. The technique involved washing and disinfecting the stems with 33% sodium hypochlorite, followed by two rinses with sterile distilled water. Five disinfected stem fragments were placed equidistantly in a Petri dish containing Bioxon[®] PDA culture medium.

For the purification of the isolated strains, hyphal tips were collected using a previously sterilized scalpel. These were then individually transferred to new Petri dishes containing PDA culture medium. Once fungal colonies emerged from the explants and grew on the culture medium, they were transferred again, using hyphal tips, to new dishes with SNA medium for conidia production. After three weeks of incubation, 5 mm diameter discs of SNA medium were taken and placed in test tubes containing 5 mL of sterile distilled water.

The tubes were shaken to obtain a conidial suspension, and 25 μ L were taken with a micropipette and deposited at the center of a Petri dish with PDA medium. The suspension

was spread using a previously sterilized inoculating loop and incubated at 25 °C for 24 h. After incubation, 4 mL of sterile 25% glycerol was added, and the suspension was shaken with the micropipette to release the conidia. The solution was then recovered into sterile cryogenic vials and stored at -80 °C until use. From each isolate, 20 µL of conidia suspension in 25% glycerol was inoculated in Petri dishes with PDA, to determine the microscopic and macroscopic characteristics that were obtained with the support of the results obtained by [5] and [6].

***In vitro* Sensitivity Evaluation to Fungicides**

The three isolates were cultured in Petri dishes with PDA medium to obtain young strains, which were then evaluated for *in vitro* sensitivity to the fungicides Robust R[®] (Benomyl), Tacora 25Ew[®] (Tebuconazole), Cabrio C[®] (Boscalid+Pyraclostrobin), Programic Mega[®] (*Larrea tridentata* extract), and Aliette Wdg[®] (Fosetil-Al) at three doses: low, medium, and high, along with a control treatment of distilled water. The fungicide doses were determined according to the manufacturer's recommendations. For each strain, a completely randomized experimental design was used with eight repetitions, and two Petri dishes were used for each repetition.

The sensitivity response of the strains to the fungicides was determined based on the final average growth diameter of each strain, measured with a 30 cm long transparent plastic ruler. Measurements were taken every 24 hours, and they were suspended when the mycelium of any treatment reached the filter paper discs.

Pathogenicity Tests

Pathogenicity tests were conducted on healthy blueberry plants of the Biloxi variety with each of the obtained isolates. Three repetitions were performed for each strain, along with a control treatment using distilled water. The substrate used was peat moss mixed with oak soil, sterilized in an autoclave at 121 °C for 60 min. Once the temperature for handling was reached, it was placed in plastic pots that had been previously disinfected with 33% sodium hypochlorite. For inoculation, a mixture was made using the entire Petri dish fully colonized by each of the obtained isolates in 40 mL of sterile distilled water. The mixture was stirred with a mechanical rotor until a homogeneous solution was achieved. Once the inoculum was ready, 5 mL was taken and applied to the root of each plant without causing any damage. For the pathogenicity tests, the variables of severity and virulence were evaluated for each of the isolates. Severity was determined based on the visual symptoms of the foliage, which were scored as follows: 0=healthy plant, 1=plant with yellow leaves, 2=foliar yellowing, 3=apical necrosis, and 4=plant death. Observations were made every 24 hours, starting from the inoculation until the death of the first plant.

RESULTS AND DISCUSSION

From the isolates obtained from material with symptoms of dieback in blueberry plants, three isolates with characteristics matching those reported for the genus *Pestalotiopsis* sp. were obtained. These isolates were assigned the strain codes: CAZr01, CAZh02, and CATg03 (Figure 1).

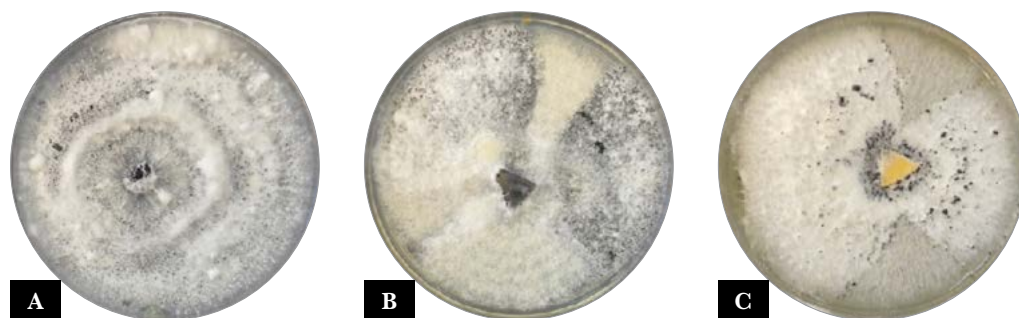


Figure 1. Strains obtained from the samples collected. A) CAZr01 from the municipality of Ziracuaretiro, B) CAZh02 from the municipality of Salvador Escalante, C) CATg03 from the municipality of Tangancicuaro, all from the state of Michoacán.

The strains were identified based on both cultural and microscopic characteristics. Abundant conidia were observed in the different strains from the three municipalities. The conidia had five cells, two hyaline at the ends and three verruculose in the center, as well as the presence of three apical appendages and one basal appendage, except for strain CAZh02, which had two apical appendages and one basal appendage (Figures 2, 3, 4). In terms of macroscopic characteristics, the strains exhibited abundant and cottony mycelial growth that covered the entire Petri dish. The coloration was white-yellowish with the presence of acervuli (black-colored spots). The results of the microscopic and macroscopic characterization of the pathogen align with those reported for the genus *Pestalotiopsis* [7, 8].

Pestalotiopsis species show notable phenotypic diversity and are grouped based on similarities in conidial morphology [5, 8, and 9]. Characteristics such as length, width, pigmentation, and the presence of appendages on the conidia appear to be consistent and useful for the identification of *Pestalotiopsis* [5 and 10]. The conidia of *Pestalotiopsis clavispora*, according to Steyaert [11], are fusiform and straight, with three central versicolor cells, the lower and upper cells being hyaline, and having two to three apical ornamentations, which coincides with the three isolates CAZr01, CAZh02, and CATg03. The conidia showed five cells, three versicolor cells in the center, and two hyaline cells at the ends; with two to three apical ornamentations and one basal ornamentation.

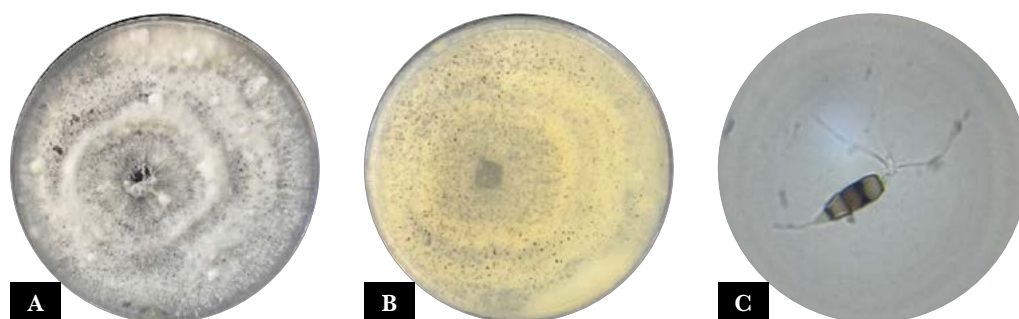


Figure 2. Strain CAZr01 identified as *Pestalotiopsis* sp. grown on PDA nutrient medium: A) Front of the strain, presence of acervuli, B) Back of the strain, C) Conidium of *Pestalotiopsis* sp., 5 cells, 2 hyaline and 3 versicolor, with three apical appendages and one basal appendage.

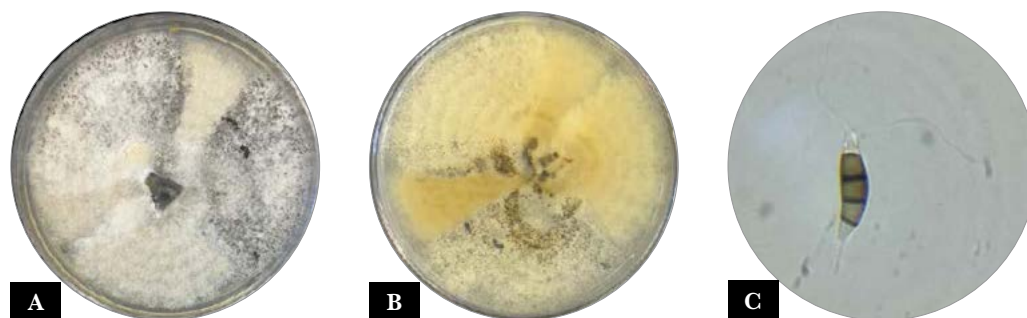


Figure 3. Strain CAZh02 identified as *Pestalotiopsis* sp. grown on PDA nutrient medium: A) Front of the strain, showing the presence of acervuli, B) Back of the strain, C) Conidium of *Pestalotiopsis* sp., with 5 cells: 2 hyaline and 3 versicolor, with two apical appendages and one basal appendage.



Figure 4. Strain CATg03 identified as *Pestalotiopsis* sp. grown on PDA nutrient medium: A) Front of the strain, presence of acervuli, B) Back of the strain, C) Conidium of *Pestalotiopsis* sp., with 5 cells: 2 hyaline and 3 versicolor, with three apical appendages and one basal appendage.

***In vitro* Sensitivity Bioassay to Fungicides**

The *in vitro* sensitivity study of the *Pestalotiopsis* sp. pathogens showed variation. The analysis of variance indicated that there is a significant difference between the treatments of the evaluated fungicides, as indicated below:

In the analysis of variance for the CAZr01 strain, a highly significant difference ($P < 0.0001$) was detected for these fungicides. The mean comparison revealed the formation of five groups (Tukey $\alpha = 0.05$). The inhibition of mycelial growth of *Pestalotiopsis* sp. with these fungicides ranged from 0.856 to 0.160 cm. The product with the lowest sensitivity to the fungus was Aliette Wdg[®] at its low dose, as well as Reva Gobex[®] at its low dose. The highest sensitivity was observed with the fungicide Tacora 25 EW[®] at its low, medium, and high doses (Figure 5).

For the analysis of variance of the CAZh02 strain, also identified as *Pestalotiopsis* sp., a highly significant difference was detected ($P < 0.0001$) among these fungicides. In the mean comparison, six groups were formed (Tukey $\alpha = 0.05$), and the inhibition of mycelial growth of *Pestalotiopsis* sp. fluctuated between 1.089 and 0.311 cm. Robust R[®] presented the least sensitivity at its high, medium, and low doses. In contrast, the fungicide Tacora 25 EW[®] at its high and low doses was the product that showed the best inhibition of mycelial growth of *Pestalotiopsis* sp. (Figure 6).

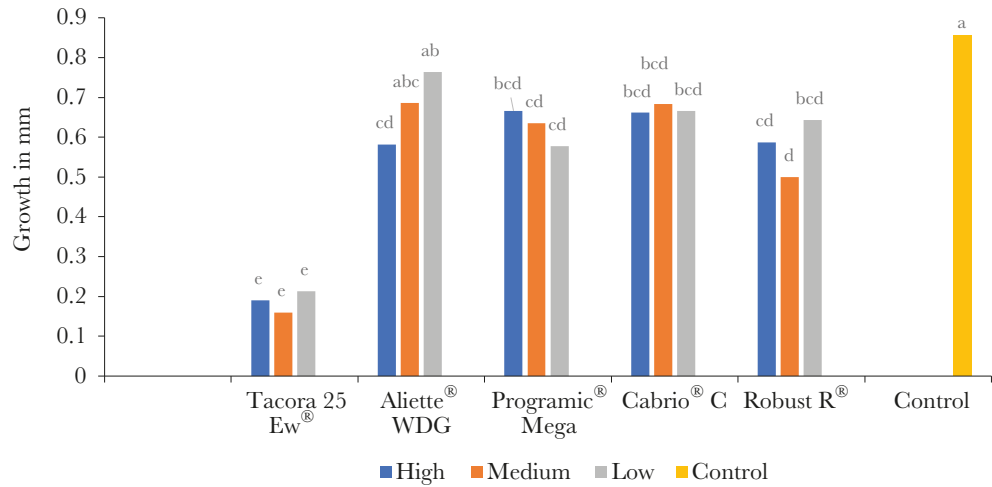


Figure 5. Effect of fungicides on the inhibition of mycelial growth of the CAZr01 strain (*Pestalotiopsis* sp.).

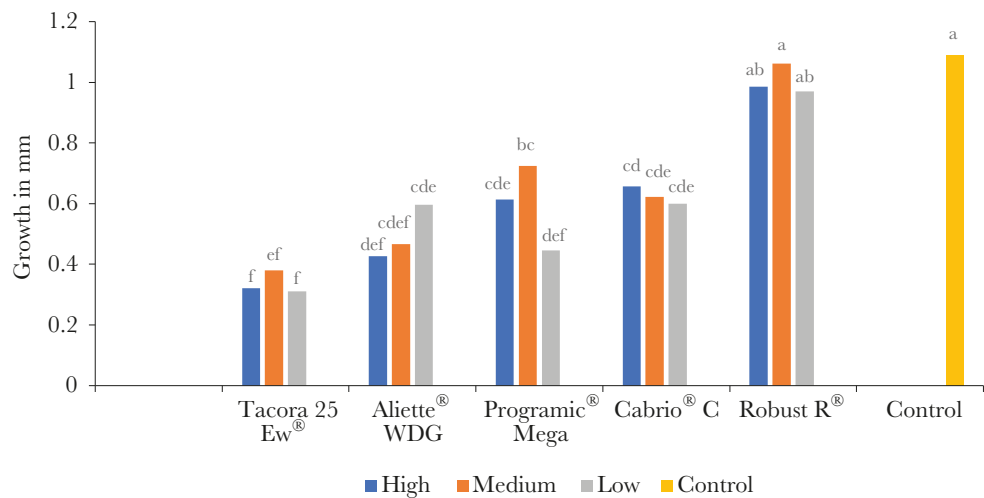


Figure 6. Effect of fungicides on the inhibition of mycelial growth of the CAZh02 strain (*Pestalotiopsis* sp.).

The analysis of variance for the CATg03 strain identified as *Pestalotiopsis* sp. showed a highly significant difference ($P < 0.0001$) between these fungicides. In the mean comparison, the formation of six groups was observed (Tukey $\alpha = 0.05$), with the mycelial growth inhibition of *Pestalotiopsis* sp. fluctuating between 0.234 and 0.846 cm. Robust R® showed the lowest sensitivity at low, medium, and high doses, while the highest inhibition was obtained with the fungicide Tacora 25 EW® at all three doses (Figure 7).

[12] evaluated fungicides for the control of radial growth in vitro of *P. clavispora*, using active ingredients such as Boscalid, Chlorothalonil, Ciprodinil, Fludioxonil, Iprodione, and Pyraclostrobin at doses of 0.025, 0.05, 0.1, 1.0, and 1.5 g mL⁻¹. They determined that increasing fungicide doses significantly reduced the mycelial growth of the phytopathogen. These results are similar to those reported in this study with the product Tacora 25 Ew® at its high and medium doses, which showed the highest inhibition of growth in all three *Pestalotiopsis* sp. strains.

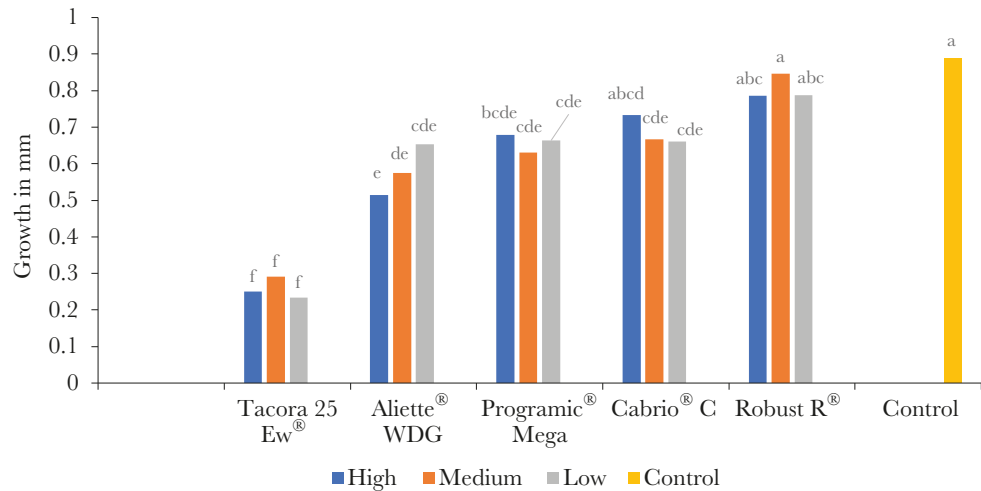


Figure 7. Effect of fungicides on mycelial growth inhibition of the CAZh02 strain (*Pestalotiopsis* sp.).

Pathogenicity Tests in Blueberry Plants

The appearance of symptoms was influenced by temperature and environmental humidity conditions, which directly affected the virulence and severity of the disease caused by the pathogens, allowing the symptoms to manifest in the blueberry plants.

In the block of Biloxi variety blueberry seedlings inoculated with the CAZr01 strain of the *Pestalotiopsis* sp. pathogen, eight days after inoculation, symptoms corresponding to grade II were observed, characterized by apical wilting. After 10 days, the plants reached grade III, showing apical necrosis and defoliation.

The seedlings inoculated with the CAZh02 strain of the *Pestalotiopsis* sp. pathogen, eight days after inoculation, showed symptoms corresponding to grade II, characterized by apical wilting. After 10 days, the plants reached grade III, with brown lesions observed on the branches and apical necrosis. At 26 days post-inoculation, grade IV (plant death) was observed (Figure 8).

Blueberry seedlings inoculated with the strain CATg03 of the *Pestalotiopsis* sp. pathogen, eight days after inoculation, exhibited symptoms corresponding to grade II, characterized

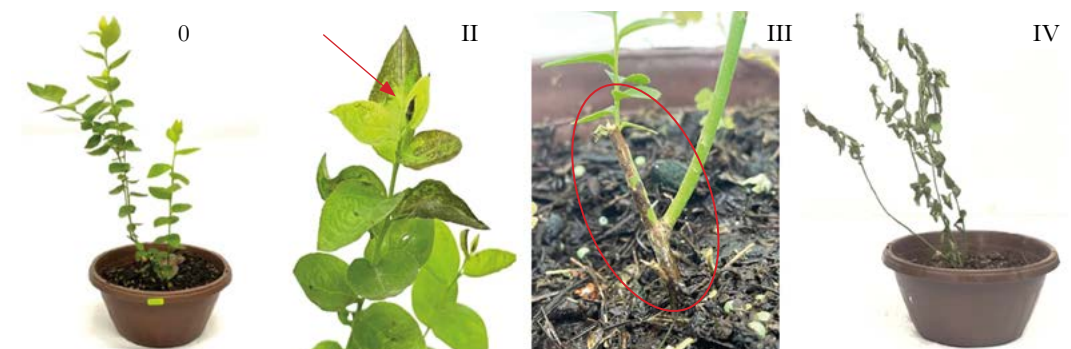


Figure 8. Foliar symptoms of *Pestalotiopsis* sp., strain CAZh02 in blueberry seedlings: Healthy plant (0), apical wilting (II), Brown lesions on the branches and apical necrosis (III), Plant death (IV).

by apical wilting. After 12 days, the plants reached grade III, showing drying of lateral branches, apical necrosis, and defoliation.

The symptoms observed in these pathogenicity tests are very similar to those reported by Espinoza *et al.* (2008), who observed lesions on branches with brown discoloration and necrosis on leaves caused by *P. clavispora*.

CONCLUSIONS

1. In the present study, it was found that the causal agent of the dieback in blueberry was *Pestalotiopsis* sp. 2. In the bioassay performed for the inhibition of mycelial growth of the strains CAZr01, CAZh02, and CATg03, the fungicide Tacora 25 Ew[®] at all three evaluated doses was the one that achieved the greatest inhibition of fungal growth. 3. In the pathogenicity tests, the first symptoms appeared eight days after inoculation, and at 26 days, the first plant inoculated with strain CAZh02 died, making it the most virulent strain.

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Bokashi: An Alternative for Pitahaya Cutting Production

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ABSTRACT

Bokashi organic fertilizer is a potential alternative substrate for pitahaya cutting production. This study aimed to evaluate root and shoot development in pitahaya cuttings grown with different substrates composed of soil, bokashi, sand, and the inorganic fertilizer ammonium diphosphate (DAP) applied in liquid form. In order to identify the best alternatives for cutting production in the central coastal region of Veracruz, Mexico. River sand and soil were mixed with bokashi in the proportion 1/1 and 1/0.5 (volume/volume) (1Sand/1Bokashi, 1Sand/0.5Bokashi, 1Soil/1Bokashi and 1Soil/0.5Bokashi). Additionally, treatments included only river sand, only soil, and soil with weekly applications of DAP through irrigation water at concentrations of 1% and 5% (Soil+DAP1 and Soil+DAP5). A randomized block experimental design was used; with land slope as the blocking factor. One plant constituted the experimental unit. 54 plants per treatment were considered. Statistical differences were detected in root area (cm²), number of roots, average root length (cm), fresh root weight (g), number of shoots per cutting, length and central diameter of the shoot (g), as well as fresh shoot weight (g). It is concluded that the mixture of silt-loam soil or river sand with bokashi organic fertilizer in the proportion 1:1 (volume/volume) is a viable alternative for obtaining pitahaya cuttings with 13 to 16 roots over a period of 50 days, weighing between 6 and 7 grams of fresh weight and that this is reflected in stems that emit between 2.4 and 3.0 shoots.

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INTRODUCTION

The pitahaya (*Hylocereus undatus*) is a species native to Mexico; however, despite being an indigenous species, it still occupies a relatively small cultivated area. At the national level, a total of 2,538 hectares are reported, distributed across the states of Aguascalientes, Guerrero, Nuevo León, Puebla, Quintana Roo, Sinaloa, Sonora, Tabasco, and Yucatán. Currently, Quintana Roo stands out as the leading producer, accounting for 73% of the total cultivated area (SIAP, 2023). Due to the limited fruit supply in the country and its high demand in the export market, pitahaya reaches attractive prices for producers. At the national level, the average price is \$18,242 per ton, with an average yield of 6.35 t/ha (SIAP, 2023). Pitahaya is a crop with significant growth potential due to its characteristics,



such as its acceptance in both local and international markets. It can be consumed as fresh fruit, as a vegetable, and even as forage for animals. As a cactus species, it adapts to various environments, including low-fertility soils, rainfed conditions, and areas with low precipitation (Montesinos Cruz *et al.*, 2015). Currently, one limitation to increasing the cultivated area is the availability of planting material. Due to the limited number of production areas, it is essential to have adequate technology that allows for the rapid production of high-quality cuttings using low-cost and easily accessible inputs.

For mass seedling production, *in vitro* propagation techniques can be used (Martínez Arroyo, 2023). However, this method requires trained personnel, a higher investment, and a longer period for the plant to reach the productive stage. Traditionally, producers multiply their own cuttings. For proper cutting production, gladioles or stems should be obtained from productive plants to ensure fruit production begins within two years after planting. Cutting production can take place after the fruit harvest, at the time of stem pruning. Traditionally, the substrate used by producers consists of the soil from the production unit. However, it has been shown that some agroecological inputs, such as efficient microorganisms, can promote greater root and shoot production (Carrera Abanto, 2023). Similarly, the use of inorganic fertilizers applied in liquid form can help produce vigorous cuttings (Navarrete Torres, 2023). Another agroecological input that has shown potential for seedling production in other plant species is the organic fertilizer bokashi, mixed with soil in 1:1 or 1:2 (volume/volume) proportions (Gashua *et al.*, 2022). Bokashi has the advantage of being an input that can be prepared by the producer using family labor. The preparation process allows for the use of a variety of regional organic residues, such as manure from intensive livestock farming, local weeds, harvest residues, agro-industrial waste, mine waste, river minerals, etc. (Pérez *et al.*, 2008). The objective of the research was to determine the characteristics of root and shoot production in pitahaya cuttings managed with different substrates consisting of soil, bokashi, sand, and the inorganic fertilizer ammonium diphosphate (DAP) applied in liquid form, with the aim of identifying the best alternatives for cutting production in the central coastal region of Veracruz, Mexico.

MATERIALS AND METHODS

Study Area

The research was conducted from January to April 2024 at the Cotaxtla Experimental Field, belonging to the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), located at km 34.5 on the Veracruz-Córdoba highway, in the municipality of Medellín de Bravo, Veracruz (18° 49' 59" N; 96° 22' 59" W). The soil used had a pH of 6.63, with 2.41% organic matter, and a loam texture (23, 17, and 60% clay, sand, and silt). The concentrations of nitrate nitrogen (N), phosphate (P-PO₄⁻), sulfate (S-SO₄⁻²), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), iron (Fe²⁺), zinc (Zn²⁺), copper (Cu²⁺), manganese (Mn⁺), and boron (B³⁺) were 12.8, 96.0, 6.67, 400.0, 1940.0, 320, 72, 8.6, 3.3, 5.5, and 0.2 ppm, respectively. Temperature and precipitation were recorded during the experiment period (Figure 1).

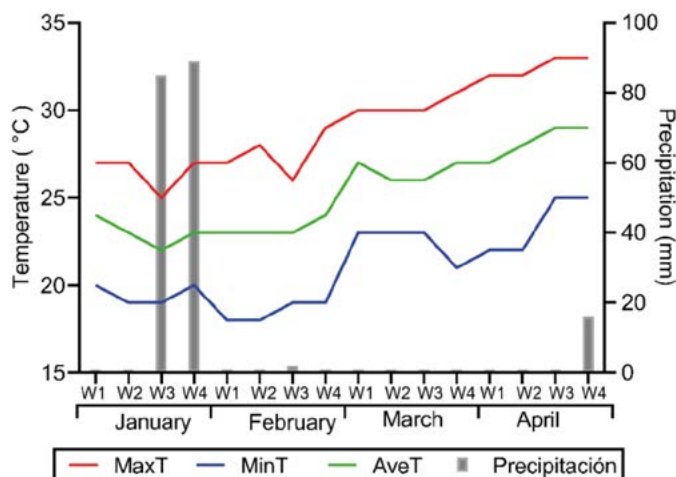


Figure 1. Behavior of maximum temperature (MaxT), minimum temperature (MinT), average temperature (AvgT), and precipitation from January to April 2024, in Medellín, Veracruz.

Pitahaya Cuttings

The cuttings were obtained from productive pitahaya plants (pink skin and white flesh) established at the Cotaxtla Experimental Field. Vigorous and healthy plants were selected. Stems that had reached at least 90% of their final length were cut; these stems were identified by their dark green color, with areoles and fully matured spines (Yadira Vargas Tierras *et al.*, 2024). Stems with a thickness of 5 to 7 cm were selected, and the length of the stems was 25 cm.

Experimental Design and Treatments

A randomized block experimental design was used, with the slope of the land as the blocking factor. The treatments consisted of different substrates made up of soil, river sand (1 to 3 mm in diameter), bokashi organic fertilizer, and the inorganic fertilizer Ammonium Diphosphate (DAP), which contains 18-46% N-P₂O₅. The soil was mixed with bokashi in the ratio 1/1 and 1/0.5 (volume/volume) (1Soil/1Bokashi and 1Soil/0.5Bokashi). Soil was also combined with weekly applications of DAP applied in irrigation water at concentrations of 1 and 5% (Soil+DAP1 and Soil+DAP5), and the exclusive use of soil. Additionally, a mixture of river sand with bokashi in the ratio 1/1 and 1/0.5 (volume/volume) (1Sand/1Bokashi and 1Sand/0.5Bokashi) was used, as well as the exclusive use of sand. One plant constituted the experimental unit. 54 plants per treatment were considered.

The bokashi used was prepared with bovine manure, local grassy weed residues, soil, sand, wheat bran, and ash in a 4:2:1:1:0.7:0.7 (volume/volume ratio). The materials were humidified with a mixture of molasses and water (10% molasses). When the bokashi was used, it had a moisture content of 30%.

The characteristics of the bokashi were as follows: 19.52% and 11.32% organic matter and carbon, respectively; pH of 6.61 and electrical conductivity of 0.43 mS/cm; density of 0.83 g/cm³; C/N ratio of 12.58; 0.9, 1.17, 0.84, 2.67, 0.68, 0.95, 0.03, 0.00, 0.05, and 0.03% total N, P₂O₅, K₂O, CaO, MgO, Fe²⁺, Zn²⁺, Cu²⁺, Mn²⁺, and B³⁺, respectively.

Establishment of Cuttings

To establish the cuttings, excavations were made in the soil. An area of $2 \times 1 \times 0.2$ m was dug. The excavated areas were filled with each of the substrates, and then the pitahaya cuttings were planted. Prior to planting, a cut was made at the base of the cuttings to remove the succulent part of the stem, leaving only the woody part. Immediately after, the cut area was submerged for 5 minutes in a solution made of 800 ml of calcium sulfate solution in 20 L of water. In each planting area, the cuttings were arranged in three rows, with 18 plants placed in each row. The space between rows was 30 cm, leaving a 20 cm margin. The distance between cuttings was 10 cm.

To hydrate the cuttings, weekly irrigations were carried out. On each irrigation date, an equivalent of 1.35 L of water was applied per cutting. For the treatments that included DAP, 67.5 or 13.5 g of this fertilizer were applied on each irrigation date. In total, six irrigations were performed, representing a total application of 405 and 81 g for the Soil+DAP5 and Soil+DAP1 treatments, respectively.

Production of roots and shoots of pitahaya

The effect of the treatments was evaluated 50 days after planting. In five randomly selected experimental units, the area occupied by the roots (cm^2), the number, average length, and fresh weight of roots were recorded. The roots of each shoot were collected, washed, and placed in a forced-air oven at a temperature of 70 °C until constant weight was achieved. Additionally, the number of shoots per stem, as well as the length, central diameter, and fresh weight of the shoots, were recorded.

Data analysis

To compare the treatments, an analysis of variance and a Scott-Knott mean comparison test were conducted, both with a 95% probability ($p \leq 0.05$). For each treatment, a Pearson correlation analysis ($p < 0.05$) was performed to determine the degree of association between the root variables and the characteristics of the shoots obtained from the pitahaya cuttings.

RESULTS AND DISCUSSION

Similar root area was detected with 1Soil/0.5Bokashi, 1Sand/0.5Bokashi, and Sand, all of which outperformed the other treatments (Figure 2a). The highest number of roots was found with 1Soil/1Bokashi, 1Soil/0.5Bokashi, and Soil (Figure 2b). The greatest root length was detected with Sand (Figure 2c). The lowest fresh root weight was found with Soil+DAP1, 1Sand/1Bokashi, and Sand (Figure 2d). No statistical differences were detected in the dry root weight (Figure 2e).

The highest number of shoots was achieved with 1Soil/1Bokashi, 1Soil/0.5Bokashi, Soil+DAP5, and Soil+DAP1 (Figure 3a). The largest shoot diameter was detected with 1Sand/1Bokashi (Figure 3b). The length and fresh weight of shoots obtained with 1Soil/1Bokashi, 1Sand/1Bokashi, and 1Sand/0.5Bokashi were statistically equal, and all three outperformed the other treatments (Figure 3c, d).

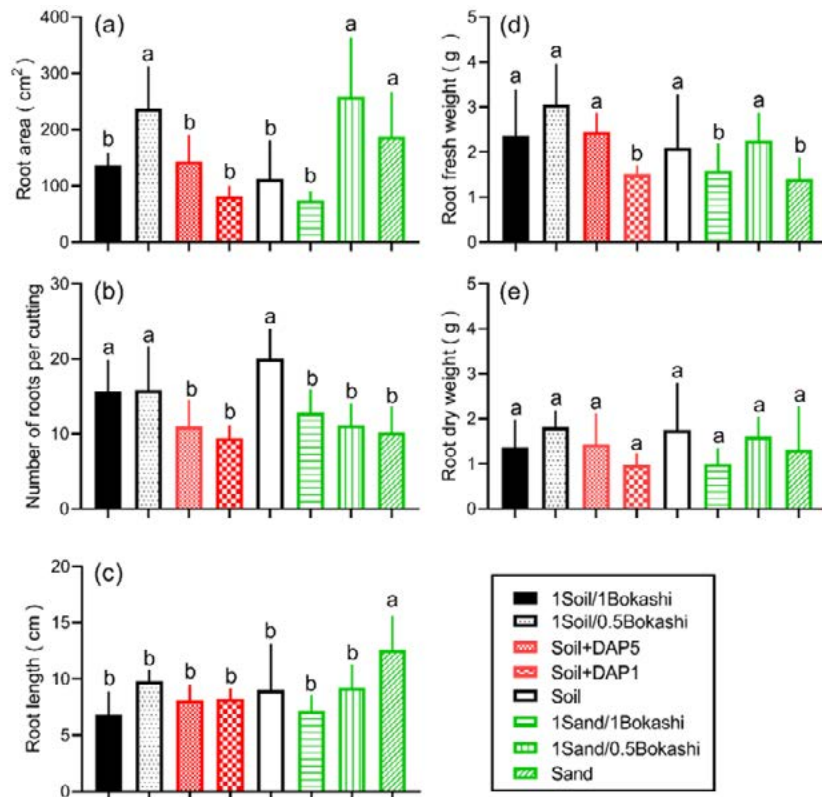


Figure 2. Characteristics of pitahaya root cuttings based on different substrates, consisting of the exclusive application of soil or sand, soil or sand mixed with bokashi fertilizer in the ratio 1/1 and 1/0.5 (1Soil/1Bokashi, 1Soil/0.5Bokashi, 1Sand/1Bokashi, and 1Sand/0.5Bokashi), as well as soil with applications of DAP at concentrations of 1% and 5% (Soil+DAP1 and Soil+DAP5), in Medellín, Veracruz.

With the 1Soil/1Bokashi treatment, a high negative correlation was detected between root area and fresh shoot weight, but a positive correlation was found between fresh shoot weight and both fresh and dry root weight, as well as with shoot length and diameter (Table 1). With the 1Soil/0.5Bokashi treatment, a negative correlation was detected between the number of shoots and both root area and dry root weight. Fresh shoot weight was positively correlated with dry root weight and shoot dimensions; however, a negative correlation was observed between the number of shoots and fresh shoot weight (Table 1). With the Soil+DAP5 treatment, only positive correlations were detected. The number of shoots was correlated with root area and length, and fresh shoot weight was correlated with the number, length, and fresh weight of roots, as well as with the shoot dimensions (Table 1). For the Soil+DAP1 treatment, only a correlation between fresh root weight and shoot dimensions was detected (Table 1). In the case of the Soil treatment, a negative correlation was detected between root area and fresh shoot weight. A positive correlation was also found between fresh root weight and both the number and length of roots, as well as with the shoot dimensions (Table 1). For the 1Sand/1Bokashi treatment, a negative correlation was detected between the number of shoots and the number of roots, while a positive correlation was found between fresh shoot weight and the number and length of roots, as well as with the shoot dimensions (Table 1). In the 1Sand/0.5Bokashi treatment, a negative

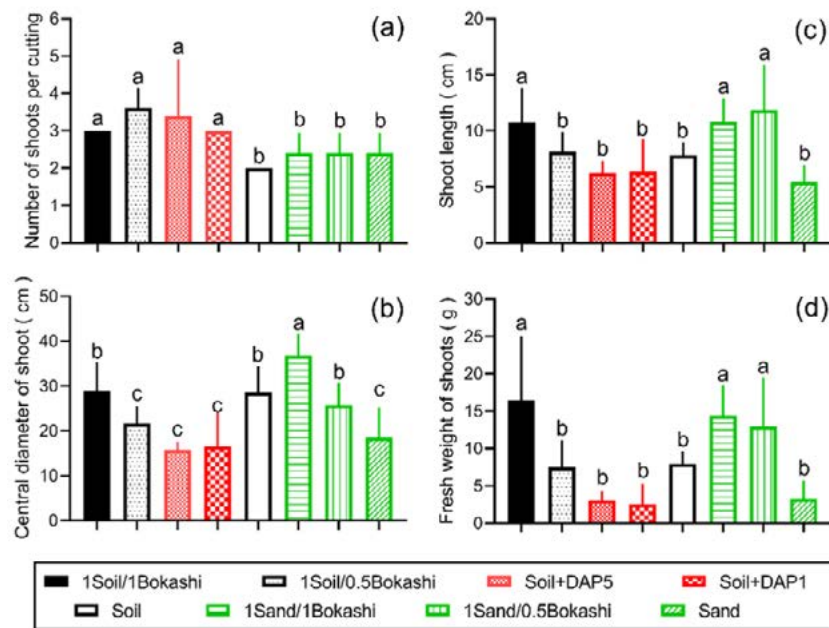


Figure 3. Characteristics of pitahaya cuttings' shoots based on different substrates, consisting of exclusive applications of soil or sand, soil or sand mixed with bokashi fertilizer in the ratios of 1/1 and 1/0.5 (1Soil/1Bokashi, 1Soil/0.5Bokashi, 1Sand/1Bokashi, and 1Sand/0.5Bokashi), as well as soil with DAP applications at concentrations of 1% and 5% (Soil+DAP1 and Soil+DAP5), in Medellín, Veracruz.

correlation was detected between the number of shoots and the dry weight of roots, but a positive correlation was found between fresh shoot weight and the number of shoots, dry root weight, and the shoot dimensions (Table 1). In the treatment that only included sand, a positive correlation was detected between the number of shoots and the number and dry weight of roots; however, fresh root weight was negatively correlated with the number and dry weight of roots, as well as with the number of shoots (Table 1). For this study, a silty-loam soil was used, which, according to its chemical characteristics, is classified as a neutral soil of medium fertility (Salgado-García *et al.*, 2006). The exclusive use of soil promoted the maximum number of roots (20 roots per cutting); however, this higher number of roots did not translate into positive effects for shoot production. On the other hand, the exclusive use of river sand favored both the lateral and vertical growth of roots, as it excelled in root area (187 cm²) and was the treatment that achieved the maximum root length (12.6 cm). However, these benefits were not reflected in shoot production and size. Both soil and sand were the treatments with the lowest nutrient availability; therefore, it is speculated that the plant's physiological response was to prioritize root production over shoot production as a survival strategy (Gruber *et al.*, 2013). This would explain the negative correlation detected with these treatments, specifically between the fresh weight of shoots and root area and number. On the other hand, the inorganic fertilizer DAP did not show a difference compared to the exclusive use of soil or sand, as it only promoted positive effects on root fresh weight and the number of shoots produced, specifically when the 5% concentration was applied. Therefore, it is not considered a viable alternative for cutting production.

Table 1. Pearson correlation coefficient between shoot characteristics and pitahaya root characteristics based on different substrates, consisting of the exclusive application of soil or sand, soil or sand mixed with bokashi fertilizer in a 1/1 and 1/0.5 ratio (1Soil/1Bokashi, 1Soil/0.5Bokashi, 1Sand/1Bokashi, and 1Sand/0.5Bokashi), as well as soil with DAP applications at concentrations of 1% and 5% (Soil+DAP1 and Soil+DAP5), in Medellín, Veracruz.

	Area of the root	Number of roots	Length of root	Fresh weight of root	Dry weight of root	Number of shoots	Length of shoots	Central diameter of shoots
1Soil/1Bokashi								
Number of shoots	-	-	-	-	-	1.00	-	-
Fresh weight of shoots	-0.61	0.27	0.58	0.91	0.80	-	0.95	0.86
1Soil/0.5Bokashi								
Number of shoots	-0.67	0.28	-0.30	-0.34	-0.81	1.00	-	-
Fresh weight of shoots	0.42	-0.25	-0.04	0.24	0.65	-0.86	0.92	0.99
Soil+DAP5								
Number of shoots	0.64	0.42	0.71	-0.32	-0.31	1.00	-	-
Fresh weight of shoots	-0.03	0.55	0.63	0.54	-0.07	0.21	0.88	0.62
Soil+DAP1								
Number of shoots	-0.22	-0.41	-0.08	-0.11	-0.10	1.00	-	-
Fresh weight of shoots	0.20	0.14	-0.15	0.27	0.08	-0.38	0.91	0.80
Soil								
Number of shoots	-	-	-	-	-	1.00	-	-
Fresh weight of shoots	-0.57	0.67	0.58	0.23	0.14	-	0.81	0.63
1Sand/1Bokashi								
Number of shoots	0.08	-0.53	0.20	0.40	0.39	1.00	-	-
Fresh weight of shoots	0.25	0.92	0.63	0.34	0.05	-0.39	1.00	0.95
1Sand/0.5Bokashi								
Number of shoots	-0.34	-0.22	0.26	-0.45	-0.50	1.00	-	-
Fresh weight of shoots	0.64	-0.36	0.45	0.30	0.54	-0.28	0.92	0.81
Sand								
Number of shoots	-0.33	0.73	-0.36	0.47	0.60	1.00	-	-
Fresh weight of shoots	-0.12	-0.85	-0.13	-0.40	-0.54	-0.78	0.80	0.48

According to the results, bokashi is a good alternative for the production of cuttings. A greater root area achieved by mixing soil or sand with bokashi in a 1:0.5 ratio (volume/volume) indicates that a higher lateral root growth was promoted (237 and 259 cm² with 1Soil/0.5Bokashi and 1Sand/0.5Bokashi). This characteristic is desirable in commercial plantations since it allows for better utilization of nutrients and water distributed in the soil. The positive correlation between the weight of the shoots and the fresh or dry weight of the roots indicates that the positive effects on root production favored the production and characteristics of the shoots, particularly when soil and sand were used in the 1:1 ratio, as these treatments stood out with the highest number (2.4 to 3.4 shoots/cutting), length (10.7-11.9 cm), central diameter (2.7 to 3.7 cm), and fresh weight of shoots (14.4 to 16.5 g). This better response achieved with bokashi is attributed to the nutrients provided and the

increase in organic matter in the substrate. Considering the volume of the planting area (0.4 m^3), the density of bokashi (0.8 g/cm^3), and an apparent soil density of 1 g/cm^3 , each plant received 6.7 kg of substrate, of which 3 kg were bokashi. According to the chemical characteristics of bokashi, these 3 kg supplied each plant with 578, 27, 35, 25, 79, and 20 g of organic matter, total N, P_2O_5 , K_2O , CaO, and MgO, respectively. It is speculated that a temperature between 20 and 30 °C, along with weekly irrigation, favored nutrient mineralization; furthermore, the increase in organic matter in the substrate promoted water retention and root aeration (Olle *et al.*, 2020). All these benefits were reflected in cuttings with a better root system and consequently greater shoot production.

CONCLUSIONS

The mixture of silty-loam soil or river sand with the organic fertilizer bokashi in a 1:1 ratio (volume/volume) is a viable alternative to obtain pitahaya cuttings with 13 to 16 roots, weighing between 6 and 7 grams of fresh weight, over a period of 50 days. This also results in stems emitting between 2.4 and 3.0 shoots, with a fresh weight of 14 to 17 grams. These values are higher than those obtained with the exclusive use of river sand, soil, or soil combined with weekly applications of the inorganic fertilizer DAP.

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Potential of the native bamboo *Guadua aculeata* Rupr. for the carbon credit market

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ABSTRACT

Objective: To estimate biomass production, quantify carbon sequestration in natural stands of the native bamboo *Guadua aculeata* Rupr. at the “Las Margaritas” Experimental Station, and assess the economic value in a voluntary carbon credit market.

Design/methodology/approach: A forest biomass and carbon inventory was conducted in stands of *G. aculeata* in Puebla, Mexico. Using a simple random sampling design, 12 square plots of 100 m² each were established, and the diameter at breast height (Dbh) was measured in each bamboo individual. Aboveground dry biomass for structural components (culm, branches and leaves) and total biomass per individual were estimated with a system of additive equations; the carbon (C) concentration was obtained with C fractions of 0.46, 0.44 and 0.42 for culm, branches and leaves, respectively.

Results: On average, the stands contained 263.14 Mg·ha⁻¹ of biomass; distributed in 91% for culm, 5% in branches and 4% in the leaves. The average carbon stock was 120.34 Mg·ha⁻¹. At the farm level, the carbon inventory and storage amounted to 7,701.73 Mg in 64 ha of bamboo stands forest. Annual carbon sequestration was estimated at 23.72 Mg·ha⁻¹ per year, equivalent to mitigating 87.05 Mg·ha⁻¹ of CO_{2e} per year, representing an economic value of \$3,133.8 MXN.

Limitations on study/implications: Biomass, carbon, and CO₂ in mulch and soil were not quantified.

Findings/conclusions: *G. aculeata* demonstrates high potential for inclusion in the carbon credit market.

Keywords: Giant native bamboo, carbon storage, biomass, voluntary market, carbon offsets.

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INTRODUCTION

In recent years, significant changes in climatic conditions have been recorded in Mexico, primarily associated with global climate change caused by the increase in greenhouse gases (GHG). Some of its adverse effects have been reported by Flores-Campaña *et al.* (2012) and Cuervo-Robayo *et al.* (2020). Environmental changes are of the highest interest to governments, as they directly affect agricultural, livestock, and forestry production (Gómez-Pineda *et al.*, 2020). To address this critical issue, the United Nations signed the Kyoto Protocol in 1997, which directly tackled the problem of climate

change. A total of 192 countries committed to adopting policies aimed at reducing GHG emissions by 5.2% compared to 1990 levels. Subsequently, an Intergovernmental Panel of Experts on Climate Change (IPCC) was established. In 2015, the Paris Agreement was signed, establishing concrete and realistic goals on how to achieve the reduction and mitigation of GHG emissions. In this context, Mexico, with mitigation and compensation goals and actions, committed to: a) achieving zero deforestation by 2030; b) reforesting upper, middle, and lower watersheds with native species; c) increasing ecological connectivity and carbon sequestration through conservation and restoration; d) enhancing carbon capture and coastal protection through the conservation of coastal ecosystems; e) implementing REDD+ (Reducing Emissions from Deforestation and Forest Degradation) synergies; and f) ensuring integrated water management across its different uses (agricultural, ecological, urban, industrial, and domestic).

In this scenario, bamboo forests can provide environmental services as well as tangible and intangible benefits to humanity (Ordóñez-Prado *et al.*, 2024).

Among its ecological functions, bamboo stands out for its rapid growth, soil recovery, maintenance and recharge of water bodies, and carbon sequestration (Cecon and Gómez-Ruiz, 2019; Ordóñez-Prado *et al.*, 2023). Research on bamboo carbon stocks remains relevant both nationally and internationally, as establishing climate change mitigation strategies requires baseline information on fast-growing species with high carbon capture potential.

Studies on this topic report that natural forests and plantations with giant bamboo are capable of producing biomass quantities comparable to those of tropical broadleaf plantations, reaching up to 429 Mg·ha⁻¹ (Ziccardi *et al.*, 2019). Darabant *et al.* (2014) report a total aboveground biomass production of 307 Mg·ha⁻¹ in bamboo plantations established in India. Carbon sequestration is closely linked to biomass production, which can reach up to 392 Mg·ha⁻¹ when considering necromass and roots (Yuen *et al.*, 2017). For bamboo taxa in natural forests, biomass estimates may be lower (Ziccardi *et al.*, 2019).

Many bamboo species are recognized for their potential to capture carbon (Yuen *et al.*, 2017). Their rapid growth and continuous production of shoots foster a high dynamic in various carbon stocks (Liu *et al.*, 2018). The genus *Guadua*, classified as giant bamboo, can reach heights exceeding 20 meters and diameter at breast height greater than 10 centimeters (Clark and Ely, 2011). *Guadua aculeata* Rupr. (carrizo, caña brava) is the most robust native species in Mexico.

Bamboos have the potential to provide economic benefits by being considered in carbon credit markets, which are aimed at offsetting greenhouse gas emissions. In this regard, Pizarro (2021) notes that carbon credits are an alternative for countries implementing them, as they can obtain economic resources by reducing GHG emissions into the atmosphere. For bamboo to be included in carbon markets, research is needed to document its growth, biomass production, carbon sequestration, silviculture, and the development of long-life bamboo products that store carbon over extended periods (Kaam *et al.*, 2023; Ordóñez-Prado *et al.*, 2024).

In Mexico, the state of Puebla is a pioneer in bamboo cultivation, with various plantations established for commercial and restoration purposes (Ordóñez-Prado *et al.*, 2023). In this region, *G. aculeata* is used in local housing construction, the production of artisanal furniture, fences, and supports for agricultural crops (Hernández-Santiago and Torres-Hoyos, 2020). Additionally, its physical-mechanical properties make it ideal for construction (Ordóñez-Candelaria *et al.*, 2014). Despite the importance of bamboo, studies on its carbon sequestration potential are scarce, highlighting the need for information on the capacity of bamboo stands forest to capture and store carbon as a viable alternative for carbon credit markets. Based on the aforementioned context, the objective was to estimate biomass production, quantify carbon sequestration in natural stands forest of the native bamboo *G. aculeata* in the Hueytamalco region, Puebla, Mexico, and assess its economic value in a voluntary carbon credit market.

MATERIALS AND METHODS

Study Area

The study was conducted in natural stands forest of *G. aculeata* bamboo at the “Las Margaritas” Experimental Station (SEMAR, acronym in Spanish) belonging to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located in Hueytamalco, Puebla, Mexico, between the extreme coordinates 20° 02' 35" and 19° 58' 50" N and 95° 21' 27" and 97° 16' 40" W, at an altitude of 450 to 500 meters. SEMAR is one of the best-preserved areas in the region, primarily composed of species representative of the medium-sized evergreen forest. Its orography of small hills and its annual precipitation of 3,000 mm support an abundant water network, providing ideal conditions for the growth of native bamboo *G. aculeata* (Figure 1).

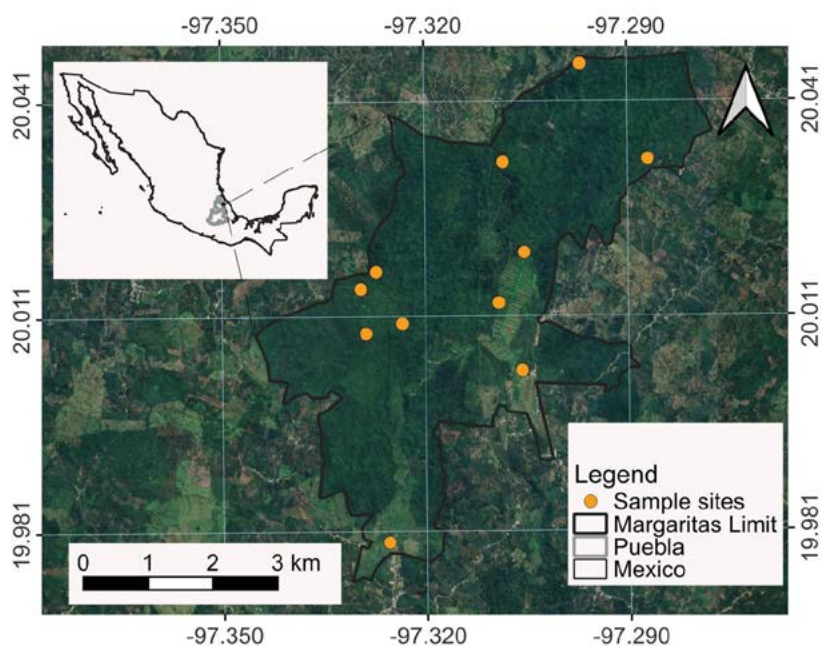


Figure 1. Distribution of bamboo stands at the Experimental Station, Puebla, Mexico.

Field Data

A zoning of the distribution of *G. aculeata* stands forest was carried out using a satellite image (image ©2019 Maxar Technologies) from the online server Google® Earth®, verified through field surveys. A total of 39 natural areas covered by the studied bamboo were identified. The coverage of these stands ranged from 0.5 to 4 hectares (Figure 1). Based on this zoning, a random sampling design was created to establish 12 square plots of 10×10 meters, where the diameter at breast height (Dbh) was measured at 1.3 meters above ground level for each bamboo culm (Figure 2). The Dbh was used to estimate the total aboveground dry biomass per individual, for which the additive equations system proposed by Ordóñez-Prado *et al.* (2024) was applied. The mathematical structure and parameters are shown in equations 1 to 4. Subsequently, the biomass estimated for each structural component of each bamboo was multiplied by the carbon concentration: 46%, 44%, and 42% for culms, branches, and foliage, respectively. This resulted in the stored carbon, scaled to the hectare level.

$$B_c = \exp(-2.44613) Dbh^{2.446172} \quad (R_{adj}^2 = 0.93, RMSE = 4.2849) \quad 1$$

$$B_r = \exp(-5.4065) Dbh^{2.494163} \quad (R_{adj}^2 = 0.63, RMSE = 0.7115) \quad 2$$

$$B_f = \exp(-8.82811) Dbh^{3.7639121} \quad (R_{adj}^2 = 0.42, RMSE = 0.9904) \quad 3$$

$$B_T = Y_c + Y_r + Y_f \quad 4$$

Where: B_c , B_r , B_f y B_T correspond to the estimated dry base biomass weight of the culm, branches, leaves, and total aboveground biomass, respectively.



Figure 2. Natural stands forest of *Guadua aculeata* and fieldwork conducted for biomass and carbon quantification in the study area.

Statistical Analysis

A forest inventory of biomass and carbon in the bamboo stands of the study area was conducted using simple random sampling estimators (Tamarit-Urias *et al.*, 2021). The average biomass per hectare, standard deviation, and 95% confidence intervals were estimated, as well as the sampling error. The biomass inventory was conducted for an area of 64 ha. For the carbon inventory, the biomass of each structural component of the bamboo was multiplied by the respective proportion of fixed carbon expressed in Mg ha^{-1} .

Stored Carbon Value

The annual biomass production of bamboo for mature stands forest with commercial culm production was estimated using seven culms with an average DBH of 10 cm. Based on observations, it was assumed that for this species, on average, seven culms are produced annually in each clump, and the average DBH is 10 cm. A density of 280 plants $\text{clump}^{-1} \text{ha}^{-1}$ was considered. Based on Barnabas *et al.* (2020), to estimate the amount of carbon dioxide equivalent (CO_2e) per hectare fixed by a bamboo species, Equation 5 was applied.

$$\text{CO}_2\text{e} = Bt \cdot CC \cdot FP \quad 5$$

Where: *Bt* is the total aboveground biomass, *CC* is the carbon content proportion in the *Bt*, and *FP* is the proportionality factor, which takes the value of 3.67.

After the annual estimation of the stored CO_2e , the economic value was estimated based on the World Bank report (2024), which indicates that the price range is between US\$0.46 to US\$16 ($\text{Mg} \cdot \text{CO}_2\text{e}$). For Mexico, a value of <US\$20 is estimated (Figure 3). Based on this information, for the purposes of this research, a conservative price of US\$2 (\$36 MXN at the exchange rate) per ton of CO_2e was established.

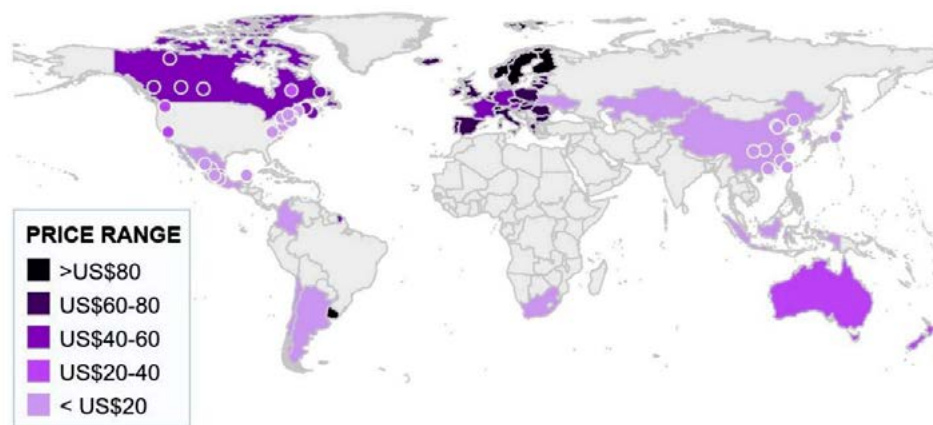


Figure 3. Carbon price around the world for 2024 (The World Bank, 2024).

RESULTS AND DISCUSSION

Carbon stored in bamboo groves

It was determined that the natural stands forest of *Guadua aculeata* store an average of $263.14 \pm 10.13 \text{ Mg ha}^{-1}$ of total aboveground dry biomass; with 91%, the culms are the structural component of bamboo that stores the most biomass, while the branches hold only 5%, and the remaining 4% is found in the foliage. In Table 1, the carbon reservoirs per unit area for the structural components culms, branches, and foliage are shown. The results obtained are higher than those reported for commercial plantations of *B. oldhamii* Munro and *G. angustifolia* Kunth, established in the same study region (Aguirre *et al.*, 2018), which indicates that the *G. aculeata* stands are located in areas with physiographic characteristics ideal for their development. Furthermore, the results are higher than those reported by López-Martell *et al.* (2008), who reported an average of $70.9 \text{ Mg} \cdot \text{ha}^{-1}$ for *B. vulgaris* Schrader ex Wendland in the Granma province, Cuba.

Regarding the stored carbon, an average of $120.34 \pm 6.81 \text{ Mg} \cdot \text{ha}^{-1}$ was estimated in the aboveground biomass, with proportions for each structural component similar to the biomass distribution. Table 1 presents the average values of stored carbon in the structural components and the total. The estimates of carbon content further reinforce and confirm the high potential of bamboo forests to capture and store carbon. Although carbon fixation is slightly lower than in tree species, their rapid growth and constant shoot production rate are strengths that these ecosystems possess for carbon capture. The results obtained are lower than those reported by Fonseca-González and Rojas (2016), who estimated $186.73 \text{ Mg} \cdot \text{ha}^{-1}$ for *D. latiflorus* plantations, while for *G. angustifolia* Kunth and *G. aculeata* Rupr. plantations, they report $117.74 \text{ Mg} \cdot \text{ha}^{-1}$, a value lower than that determined in the present study. These results are also similar to those reported by García-Soria and Del Castillo (2015), who recorded a carbon storage of $121.86 \text{ Mg ha}^{-1}$ for natural bamboo forests of *G. sarcocarpa* Londoño & Peterson in Peru.

Carbon stored value in bamboo forests

It was estimated that the carbon capture in the aboveground biomass of the studied bamboo forests is $23.72 \text{ Mg} \cdot \text{ha}^{-1}$ of carbon per year. This equates to the mitigation of $87.05 \text{ Mg} \cdot \text{ha}^{-1}$ of CO_2e per year. This means that each hectare covered with bamboo is generating 87 carbon credits or carbon offset credits per year. These carbon credits have a value of \$3,133.8 MXN, considering an average cost of US\$ 2.0 per $\text{Mg} \cdot \text{ha}^{-1}$ of CO_2e per year.

Table 1. Biomass and carbon stored in natural *Guadua aculeata* bamboo forests in Huey tamalco, Pue., Mexico.

Component	Dry base biomass (Mg ha^{-1})	Total biomass inventory on the property (Mg)	Carbon content (Mg ha^{-1})	Total carbon inventory on the property (Mg)
Culmo	238.57 ± 8.92	$15,268.41 \pm 570.72$	109.74 ± 6.05	$7,023.47 \pm 387.08$
Ramas	13.91 ± 2.15	890.15 ± 137.80	6.12 ± 1.43	391.66 ± 91.41
Follaje	10.66 ± 1.89	682.38 ± 120.65	4.48 ± 1.22	286.60 ± 78.19
Total	263.14 ± 10.13	$16,840.94 \pm 61.87$	120.34 ± 6.81	$7,701.73 \pm 435.78$

By estimating an average mitigation of $87.05 \text{ Mg}\cdot\text{ha}^{-1}$ of CO_{2e} , this represents an annual value of \$200,448.00 MXN for CO_{2e} capture in *G. aculeata* bamboo forests growing naturally on the “Las Margaritas” Experimental Station and land. The results obtained are conservative compared to those reported by Pathak *et al.* (2015), who estimated that the production of culms is between 11 to 20 per bamboo clump per year. With a density of 124 clumps per hectare, the biomass production calculated by these authors can reach $104.7 \text{ Mg}\cdot\text{ha}^{-1}$ per year (Table 2).

Table 2. Biomass accumulation potential in different bamboo species.

Specie bamboo	Culms produced by clump per year	Clump per hectare	Aboveground biomass tons per hectare
<i>Bamboosa tulda</i>	15	124	70.40
<i>Bamboosa bambos</i>	15	124	75.69
<i>Bamboosa nutan</i>	15	124	41.82
<i>Bamboosa asper</i>	20	124	7.12
<i>Dendrocalamus strictus</i>	11	124	12.94
<i>Bamboosa vulgaris</i>	12	124	53.42
<i>Bamboosa balcoa</i>	15	124	104.70

The annual carbon capture of *G. aculeata* bamboo demonstrates the potential of this highly lignified grass to be considered in voluntary carbon markets. This would allow companies to make their services and products carbon-neutral by acquiring carbon credits from natural forests or bamboo plantations, with 1 carbon credit equivalent to 1 Mg of CO_{2e} . Additionally, it could serve as a source of income for rural community families, contributing to improving their quality of life. In this regard, Pan *et al.* (2023) and Kaam *et al.* (2023) mention that the role of bamboo in climate change mitigation occurs in three dimensions: as a carbon sink in the form of biomass, as carbon storage in bamboo products, and as a contributor to carbon credit projects. However, bamboo species, like many other forest species, face limitations in their incorporation into carbon credit markets due to the absence of internationally accepted methodologies for the precise quantification of carbon reservoirs and the debate on whether bamboo should be considered a tree species or a highly lignified giant grass (as it is currently recognized) (Wu *et al.*, 2015). The estimated potential for annual carbon capture in this study indicates that *Guadua aculeata* is feasible for establishing promising ecological projects as a nature-based strategy and solution (NbS).

CONCLUSIONS

The biomass inventory of *Guadua aculeata* bamboo forests allowed the quantification of the amount of carbon and CO_{2e} stored in this little-studied ecosystem. The study provides basic information about the carbon storage potential of this fast-growing species. A total of 7,701.73 Mg of carbon were quantified as stored in the natural bamboo stands, making them a potential alternative that could help mitigate the effects of climate change. The

estimated annual carbon capture potential and CO_{2e} fixation indicate that the native giant bamboo *Guadua aculeata* is feasible for establishing promising environmental projects as a nature-based solution (NbS), thereby generating additional income for bamboo producers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chitosan beads with neem and encapsulated entomopathogenic fungal spores for pest control

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ABSTRACT

Objective: To synthesize chitosan beads with neem and encapsulated entomopathogenic fungal spores for pest control through their controlled release.

Methodology: Beads were synthesized by means of chitosan suspensions with each of the spores of the entomopathogenic fungi (*B. bassiana*, *M. robertsii*, and *I. javanica*), and the neem extract (Azadirachtin extract in water and in oil), which were crosslinked with sodium tripolyphosphate. The synthesized beads were characterized through the determination of their average diameter, moisture percentage, and morphology, using scanning electron microscopy. Subsequently, the swelling kinetics of each bead were carried out and the first and second order kinetic models were adjusted.

Results: The synthesized beads had stable structures and homogeneous diameters, with a >90% moisture content in all cases. The morphological analysis revealed that the internal structure of the beads has cavernous networks with homogeneously distributed pores. Finally, the swelling kinetics of the beads showed a better adjustment to the second order model.

Study Limitations: The study was limited to three entomopathogenic fungi and two neem extracts.

Conclusions: The neem and the entomopathogenic fungal spores were encapsulated in the chitosan beads, which will allow the controlled release of both the neem and the spores due to their porous structure and swelling capacity.

Keywords: chitosan, neem, *B. bassiana*, *M. robertsii*, *I. javanica*.

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INTRODUCTION

Currently, more than 600 agrochemicals are used to control various organisms that damage crops all over the world. These pesticides benefit agricultural production, but small amounts of these substances can be accumulated in food, water, air, and soil, causing significant exposure in humans and therefore a health risk (Rodríguez Aguilar *et al.*, 2019; Galindo-Reyes & Alegria, 2018; Gyawali, 2018; Ghorab & Khalil, 2016). Health and environmental



risks are not easy to explain, because they not only depend on the type of pesticide, but also involve factors such as exposure period, toxicity, persistence, and the environmental characteristics of affected areas; furthermore, not all the population is fully exposed to the pesticide (Kim *et al.*, 2017; Aktar *et al.*, 2009).

Studies on the consumption of pesticides worldwide, carried out from 1990 to 2018, revealed that the amount consumed increased from 2.3 to 4.1 million metric tons during this period (76%). In addition, the demand for pesticides in 2018 was concentrated in 11 countries (78% of total consumption): China is the largest consumer (43%), while Mexico participated with only 1.3% (Centro de Estudios para el Desarrollo Rural Sustentable y la Soberanía Alimentaria [CEDRSSA], 2020). The increase in the use of pesticides in Mexico is linked to the problems that pests cause in crops and their control. The agricultural sector uses 85% of these products, which increases the exposure of farmers and the probability of poisoning (Martínez Valenzuela *et al.*, 2019). The excessive use of highly dangerous pesticides is linked to negative effects on the health of farmers and their families, particularly the children. Pesticides can cause hematological, behavioral, and hormonal changes, as well as genetic, cellular, reproductive, and neuronal damages; additionally, they impact agricultural areas and ecosystems (Quijada & Gómez, 2019; García Hernández *et al.*, 2018).

Given the negative effects of pesticides on the population, it has been currently proposed to replace them with new eco-friendly alternatives—for example, biopesticides derived from materials produced by animals, plants, microorganisms, or minerals (Kumar *et al.*, 2021; Acuña Jiménez *et al.*, 2015; Nava-Pérez *et al.*, 2012). These biopesticides have allowed the rational management and use of natural resources, preserved the biodiversity of ecosystems, lowered costs, and reduced health risk among the population. They can be extracted from plants (azadirachtin extract) or pathogenic fungi (*Beauveria*, *Metarrhizium* and *Isaria* genera) with relevance for the biological pest control (Zelaya-Molina *et al.*, 2022; Figueroa Gualteros *et al.*, 2019).

Azadirachtin extract is obtained from the neem tree (*Azadirachta indica* A. Juss.). This extract is a tetranortriterpenoid used as a natural insecticide, because it impacts more than 400 pest insects (by reducing their feeding, survival, viability of the nymphs, and progeny). In addition, azadirachtin extract is compatible with natural enemies and beneficial microorganisms, without serious impact on the environment (Kilani-Morakchi *et al.*, 2021; Aguilar-Acosta *et al.*, 2020; Esparza-Díaz *et al.*, 2010; Zada *et al.*, 2018; Muñoz-Reyes *et al.*, 2016). Entomopathogenic fungi are also considered major biopesticides. Their spores come into contact and germinate on the cuticle of the insect. Subsequently, they grow inside the host, producing toxins and feeding on the host, which loses mobility and appetite and dies after 7 to 10 days (Litwin *et al.*, 2020; Mantzoukas & Eliopoulos, 2020; Pacheco Hernández *et al.*, 2019; Mora *et al.*, 2017). Entomopathogenic fungi are grouped into 115 genera, among which *Beauveria*, *Metarrhizium*, *Isaria*, *Nomuraea*, and *Hirsutela* are considered parasites capable of infecting pest insects in all the development stages of their biological cycle (Estrada Martínez, 2019; Kovač *et al.*, 2021).

Agricultural producers have sought new alternatives to reduce the excessive spraying of pesticides on crops and, consequently, any type of health or environmental risk.

Therefore, some studies are aimed at the use of natural polymeric matrices (*e.g.*, corn starch, cellulose, alginate, wheat bran, and chitosan), in order to microencapsulate pesticides for their controlled release in agricultural crops (Campos *et al.*, 2015; Korbecka-Glinka *et al.*, 2022; Rosas-García & Luna-Santillana, 2006). Chitin and its by-products are obtained from the exoskeleton of crustaceans and they are used in various areas of the food, medicine, agriculture, cosmetics, and pharmacy industries (Pellis *et al.*, 2022; Triunfo *et al.*, 2021; Triunfo *et al.*, 2022; Hernández Cocoltzi *et al.*, 2009). Chitosan (Ch) is a biodegradable, biocompatible, polycationic, and non-toxic biopolymer derived from chitin, which does not produce a response from the immune system. This molecule has several applications in the food, pharmaceutical, chemical, and agricultural industries. In the last one, chitosan has been used for the dosed release of various agrochemicals or pesticides, through the production of films, beads, microencapsulates, or hydrogels (Rossainz-Castro *et al.*, 2018; Iber *et al.*, 2022; Valderrama Negrón *et al.*, 2017).

This work is focused on the synthesis and characterization of chitosan beads with neem (azadirachtin extract in water and in oil) and encapsulated spores of entomopathogenic fungal strains (*Beauveria*, *Metarrhizium* and *Isaria*) as an alternative pest control. The synthesis of the Ch-neem-spore beads was carried out by adding spores (produced through solid fermentation of the strains) and commercial neem (Azadirachtin extract in water (Aw) and Azadirachtin extract in oil (Ao)) into the chitosan matrix. Once the beads were synthesized, their diameter, moisture percentage, morphology, and swelling kinetics were determined.

MATERIALS AND METHODS

High-density industrial grade, America Alimentos[®] chitosan (degree of acetylation 84.54% and molecular weight of 82.683 g/mol) was used. The reagent grade substances were sodium tripolyphosphate (Sigma), acetic acid (J.T. Baker), Potato Dextrose Agar (PDA) culture medium (MCDLAB), and Tween 80 sterile solution (Sigma). The neem, azadirachtin extract in water (PHC NEEEM), and azadirachtin extract in oil (Azanim, Biokrone) were commercial products. The solutions were prepared with deionized water. The entomopathogenic fungal strains used were *Beauveria bassiana* 885.2, *Metarrhizium robertsii* Xoch 8.1, and *Isaria javanica* CHE-CNRCB303. The glassware was washed with running water and rinsed with deionized water before its use.

Solid fermentation of entomopathogenic fungi

The entomopathogenic fungi strains, *B. bassiana* (Bb), *M. robertsii* (Mr), and *I. javanica* (Ij), were reactivated and propagated in Petri dishes with PDA medium. Then, they were incubated for 16 days in the laboratory at 22 ± 2 °C, $75 \pm 5\%$ relative humidity, and photoperiods of 12 h light/12 h darkness (12:12, L:D). After the propagation of the strains, a suspension was made by cutting 1 cm² of PDA medium with each strain, in order to count the spores. Nine mL of 0.1% (v/v) Tween 80 were added in a test tube, which was stirred for 2 minutes in a Vortex until the elements were homogenized and the spores were detached from the substrate. Afterwards, serial dilutions (1:10, 1:100,

and 1:1000) were made (Rodríguez-Gómez *et al.*, 2017). During the spore counting tests, 0.05 mL aliquots were taken from each sample. The samples were placed in the Neubauer chamber for direct counting, where 13 random squares of the 25 chambers (integrated by the 16 squares) were counted (Marín Cervantes, 2006). The following formula was used to express the number of spores per milliliter of suspension:

$$N = nFD \quad (1)$$

Where: N =number of spores/mL of initial suspension; n =average number of spores counted in the Neubauer chamber; F =factor of the chamber used (25×10^4); D =dilution used for counting.

Rice (*Oriza sativa* L.) was used as a substrate for solid fermentation. First it was washed with running water for two minutes to remove foreign particles; subsequently, it was soaked for 15 minutes and was drained for 10 minutes. The rice was weighed (100 g wet weight) and placed in 500 g high-density polyethylene (HDPE) bags for sterilization in an autoclave at 121 °C (15 psi) for 15 minutes. Finally, the sterile material was cooled for 24 hours for its inoculation.

Prior to inoculation, 1 g of solid fermentation from each entomopathogenic fungal strain was diluted in 5 mL 0.1% (v/v) Tween 80 in a test tube and stirred vigorously. The subsequent inoculation was carried out using 5 mL sterile syringes to inject a spore suspension at a concentration greater than 1×10^8 spores/mL, striving to homogeneously spread the suspension in the substrate bag. Afterwards, the spores were incubated at 22 ± 2 °C, exposing them to photoperiods of 12 h light/12 h darkness (12:12, L:D) during 16 days. The bags were shaken every third day to achieve a homogenous dispersion of the spores in the substrate. At the end of the inoculation, the spores were counted again using the Neubauer chamber (Rodríguez-Gómez *et al.*, 2017).

Inhibition test

The inhibition entomopathogenic fungi by neem extract was tested in a sterile PDA medium, to which different neem (azadirachtin extract in water or in oil) concentrations were added (0, 0.5, 1.0, 1.5, and 2.0 % (v/v)), with a standard volume of 20 mL for the culture medium. Subsequently, the medium was poured into Petri dishes and allowed to solidify. The culture media with different extract concentrations were inoculated in triplicate with the spores of each entomopathogenic fungi, using a platinum loop at three points in the Petri dish. They were incubated at 23 °C, during 20 days, with photoperiods of 12 h light/12 h darkness (12:12, L:D). Finally, the growth of each strain on the dishes was recorded (Castiglioni *et al.*, 2003; Hirose *et al.*, 2001).

Synthesis of Ch-neem-spores beads

The different chitosan-based beads were obtained by dripping a chitosan suspension through a nozzle onto a 2% (w/w) aqueous solution of sodium tripolyphosphate (TTP) under continuous stirring. The control bead (Ch-Control) was prepared with a 4% (w/v) Ch

solution in 1.5% (v/v) acetic acid in water (Sánchez-Duarte *et al.*, 2017). Two different neem extracts were encapsulated in chitosan: azadirachtin extract in water (Aw) and azadirachtin extract in oil (Ao). The Aw or Ao beads were prepared with a 1.5% (v/v) acetic acid in water solution and 1.5% (v/v) neem in water; subsequently, chitosan was added to generate a 4% (w/v) suspension. To obtain chitosan beads with spores of each strain (Bb, Mr, and Ij), spore suspensions with a concentration greater than 1×10^8 spores/mL in 0.1% (v/v) Tween 80 were prepared. Next, a 1.5% (v/v) acetic acid in water solution was prepared, to which the previously prepared spore suspension was added, along with the Ch needed to obtain a 4% (w/v) suspension. To obtain chitosan beads with spores of each strain (Bb, Mr, and Ij) and neem extract (Aw and Ao), a 1.5% (v/v) acetic acid in water solution and a 1.5% (v/v) neem in water solution were prepared. The spore suspension (Bb, Mr, and Ij) was added, followed by the chitosan, to obtain a 4% (w/v) suspension.

Bead characterization

Each of the different characterizations performed is described below.

Viscosity, diameter, and moisture percentage of the beads

Before each suspension was dripped to obtain the beads, the viscosity of 250 mL of each suspension was determined using a calibrated digital rotational viscometer (Cole-Parmer VCPL 150192). The viscosity was determined in triplicate at 22 ± 2 °C (Costa *et al.*, 2015; Singh & Kumari, 2013).

In order to determine the diameter, excess water was removed from the wet beads with absorbent paper; they were then placed in a stereoscopic microscope and the diameter of each bead was measured with a digital vernier. The average diameter was obtained from the measurement of 50 beads (Sánchez-Duarte *et al.*, 2017).

The moisture percentage was determined from 2 g of wet beads, using an oven at 60 °C until constant weight was obtained (Rodríguez Hamamura *et al.*, 2010). The moisture percentage was determined with the following formula:

$$\text{Moisture (\%)} = \left(\frac{\text{Wet bead weight} - \text{Dry bead weight}}{\text{Wet bead weight}} \right) 100 \quad (2)$$

Morphology

The morphology of the different beads was determined in order to verify their structural, superficial, and internal characteristics (texture, roughness, and porosity). First, the beads were lyophilized and some of them were sectioned and covered with a gold bath at 30 mA for 45 s in a Denton Vacuum Desk V system. Finally, each material was observed in a JSM-6390LV scanning electron microscope (JEOL). The operating conditions were: high resolution, accelerating voltage of 20kV, and a high vacuum with bombardment of backscattered electrons on the solid structure of the surface of the beads (Nuñez-Reyes *et al.*, 2019).

Swelling kinetics

In order to determine their swelling kinetics, the lyophilized beads were weighed and placed in double distilled water at 22 °C. Each kinetic was carried out in triplicate. First, the beads were weighed at different times (0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, and 39 min), drying their surface with absorbent paper to remove excess water (Ortega, 2013; Kipcak *et al.*, 2014; Kowalski *et al.*, 2019; Ostrowska-Czubenko *et al.*, 2015). Then, the swelling at each of the different times was quantified using the following equation:

$$W = \left(\frac{\text{Wet weight} - \text{Dry weight}}{\text{Dry weight}} \right) \quad (3)$$

Each kinetic was adjusted to first and second order swelling kinetic models (Katime *et al.*, 2005). These kinetic models had the following variables:

W is the swelling (g of hydrogel/g of xerogel), t is the time (min), W^∞ is the swelling when equilibrium is reached at t equal to ∞ , and k is the maximum swelling rate (min^{-1}). The first order model is based on the swelling kinetics of hydrogels. Assuming a prolonged time or greater shear stress, equilibrium can be reached and consequently a complete swelling can be achieved. The maximum swelling rate k is inverse to shear stress. Likewise, the relationship between the shear stress and the longitudinal osmotic modulus is close to 1. Therefore, the difference equation is integrated as follows:

First order:

$$\ln = \left(\frac{W^\infty}{W^\infty - W} \right) = kt \quad (4)$$

The second order model represents the interval from t equal to zero to an infinite time where the hydrogel reaches equilibrium and is completely swollen. W^∞ is when where a maximum swelling rate (k) is reached.

Second order:

$$W = \left(\frac{ktW^\infty^2}{1 + ktW^\infty} \right) \quad (5)$$

RESULTS AND DISCUSSION

Measurement of production of spores in the substrate

After the solid fermentation of rice, each strain produced spores. The coloration of the rice matches the spores produced by each entomopathogenic fungus. Figure 1 shows that *I. javanica* produced beige spores, *B. bassiana* white spores, and *M. robertsii* green spores.



Figure 1. Production of spores by solid fermentation: a) *I. javanica*, b) *B. bassiana*, and c) *M. robertsii*.

During the spore counting process in the mixture with Tween 80 solution, 1.76×10^8 , 1.96×10^8 , and 8.58×10^8 spores/mL were obtained for *B. bassiana*, *I. javanica*, and *M. robertsii*, respectively. This result shows that the three strains met the desired number of spores ($> 1 \times 10^6$ spores/mL), which matches the number of fungal spores required for germination and infection of pests and the subsequent effect on the insect growth (Acuña Jiménez *et al.*, 2015).

Inhibition test

Regarding the Aw extract, the test showed that the *B. bassiana* and *I. javanica* strains were inhibited by the 1.5 and 2% Aw extracts; meanwhile, the *M. robertsii* strain only showed inhibition with the 2% Aw extract. Regarding the Ao extract, the tests showed that none of the entomopathogenic fungal strains recorded an inhibition effect at the different concentrations (0, 0.5, 1.0, 1.5, and 2.0%).

Viscosity

Table 1 shows the viscosity of each precursor suspension of the different synthesized beads obtained with a 1 rpm constant speed. On the one hand, the viscosities used to

Table 1. Viscosities of the precursor suspensions.

Sample	μ (cP)	Torque (%)
Ch-Control	1780 ± 0.020	29.7
Ch-Aw	1787 ± 0.035	29.8
Ch-Ao	1793 ± 0.027	28.5
Ch-Bb	1494 ± 0.029	24.8
Ch-Mr	1465 ± 0.024	26.3
Ch-Ij	1423 ± 0.019	28.3
Ch-Aw-Bb	1491 ± 0.018	21.4
Ch-Aw-Mr	1405 ± 0.014	26.5
Ch-Aw-Ij	1388 ± 0.017	23.1
Ch-Ao-Bb	1456 ± 0.019	28.2
Ch-Ao-Mr	1416 ± 0.017	24.8
Ch-Ao-Ij	1314 ± 0.020	22.5

obtain the beads with neem (Aw and Ao) increased less than 1% with respect to the Ch-Control. On the other hand, the viscosities used to synthesize the beads with spores recorded an average decrease of 18% with respect to the Ch-Control. This effect is related to the addition of spore suspension in Tween 80, which modifies the pH causing the alteration of the rheology (viscosity) of chitosan (Giraldo, 2015; Labrada-Hechavarría *et al.*, 2018; Prudkin & Perez, 2016). The viscosities of the precursor suspensions used to obtain the beads with neem and spores are lower than those of Ch-Control and beads with neem (Aw and Ao); however, they are similar to those used to obtain the beads that only contain spores.

Diameter and moisture percentage of the beads

According to the data reported in Table 2, the variability of the diameter of the beads is not very dispersed compared to Ch-Control, because these data fall within the range of 2.49 to 2.89 mm (a 0.19 average deviation standard).

The addition of neem (azadirachtin extract) to chitosan decreases the bead size, as can be observed in the diameters of Ch-Aw and Ch-Ao with respect to the Ch-Control. Conversely, the addition of the spore suspension to the chitosan increases its size with respect to the Ch-Control. This phenomenon seems to be related to the decrease in the viscosity of their precursor suspensions, with regard to the viscosity of the suspension that generates the Ch-Control. Although the precursor suspensions have a lower viscosity than the control suspension, the six beads containing neem and some spores are smaller than the Ch-Control.

The moisture percentage in each bead was >90% (Ch-Ao and Ch-Mr recorded >98%). Likewise, in all cases, the data did not express a >0.31 standard deviation: the difference between the Ch-Aw-Bb, Ch-Aw-Mr, and Ch-Aw-Ij beads does not show changes regarding the Ch-Control; meanwhile Ch-Ao-Bb, Ch-Ao-Mr, and Ch-Ao-Ij have lower moisture percentages than the Ch-Control.

Table 2. Diameter and moisture percentage of the beads.

Sample	Diameter (mm)	Moisture (%)
Ch-Control	2.69±0.22	91.91±0.24
Ch-Aw	2.50±0.20	92.81±0.31
Ch-Ao	2.57±0.23	98.10±0.19
Ch-Bb	2.88±0.24	91.30±0.31
Ch-Mr	2.89±0.24	98.76±0.28
Ch-Ij	2.87±0.19	90.93±0.30
Ch-Aw-Bb	2.61±0.18	92.37±0.29
Ch-Aw-Mr	2.58±0.14	92.28±0.17
Ch-Aw-Ij	2.54±0.15	91.94±0.27
Ch-Ao-Bb	2.49±0.18	90.50±0.27
Ch-Ao-Mr	2.61±0.17	90.44±0.25
Ch-Ao-Ij	2.52±0.20	90.24±0.29

Morphological study

Figures 2, 3, and 4 show the micrographs of the external and internal surfaces of each bead. As can be seen in Figure 2, the beads were spherical; however, in some cases, there was minimal deformation (Ch-Ao, Ch-Mr, Ch-Aw-Mr, and Ch-Ao-Ij) or an ovoid appearance (Ch-Aw-Ij, Ch-Ao-Bb, and Ch-Ao-Mr). This phenomenon is not necessarily related to the content of the different beads, perhaps as a result of the lyophilization process. Regarding the morphology of the external surface, the beads showed cracks. In some cases, they were isolated (Ch-Control, Ch-Aw, Ch-Ao, Ch-Bb, Ch-Mr, Ch-Aw-Bb, and Ch-Aw-Ij), and in others they were numerous (Ch-Ij, Ch-Aw-Mr, Ch-Ao-Bb, Ch-Ao-Mr, and Ch-Ao-Ij). This phenomenon is not correlated with the content of the synthesized materials and it could be caused by the sample preparation process.

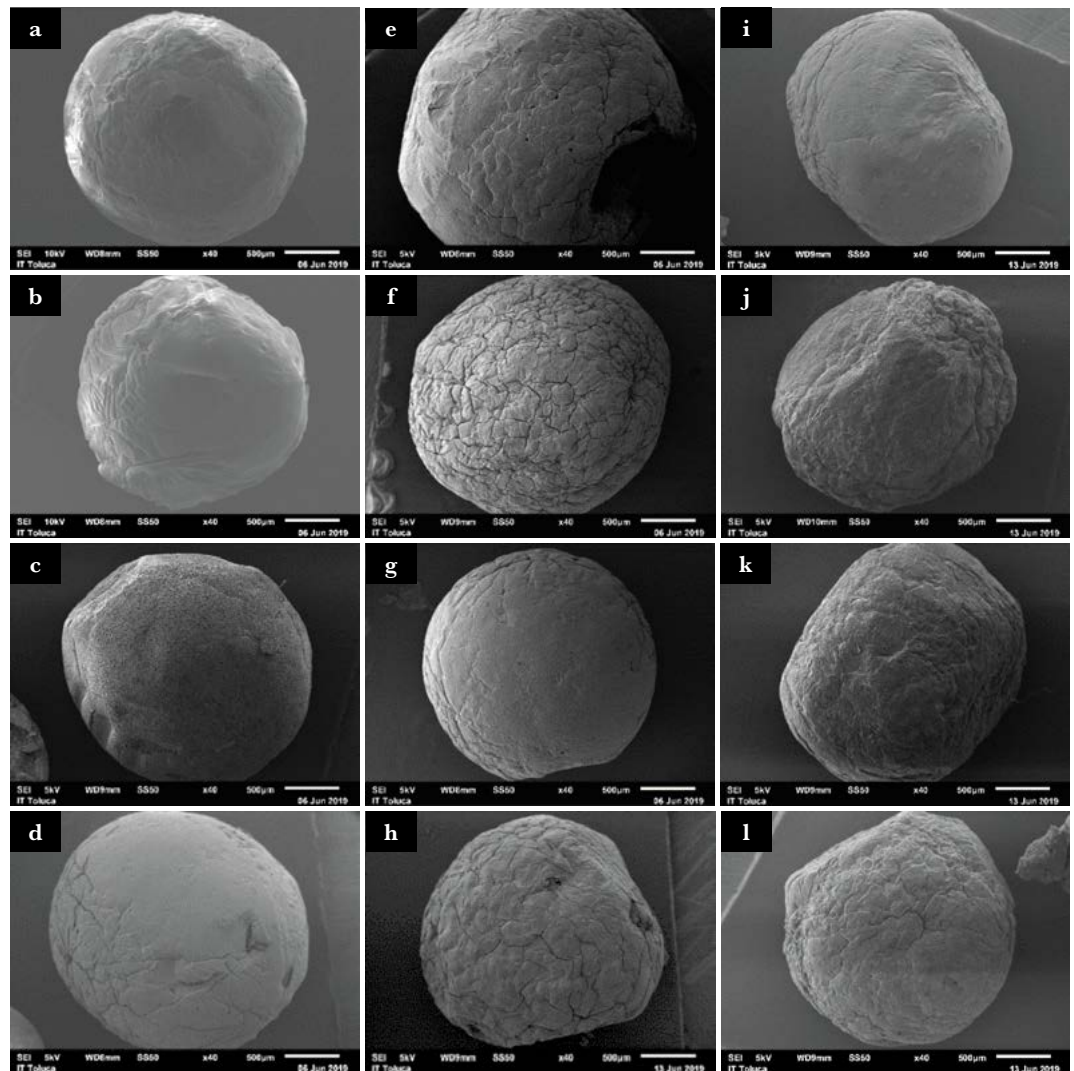


Figure 2. Micrographs at 40x of the external surface of Ch-neem-spore beads: a) Ch-Control; b) Ch-Aw; c) Ch-Ao; d) Ch-Bb; e) Ch-Mr; f) Ch-Ij; g) Ch-Aw-Bb; h) Ch-Aw-Mr; i) Ch-Aw-Ij; j) Ch-Ao-Bb; k) Ch-Ao-Mr; l) Ch-Ao-Ij.

Figure 3 shows the morphology of the external surface of each of the beads. The beads with greater porosity are the Ch-Bb and Ch-Aw-Bb; however, the Ch-Ao-Bb bead does not have a similar surface. The Ch-Aw, Ch-Mr, Ch-Ij, Ch-Aw-Mr, Ch-Aw-Ij, Ch-Ao-Bb, Ch-Ao-Mr, and Ch-Ao-Ij beads have a surface with a fine roughness. When the micrographs are enlarged, fine pores are revealed; they are similar to those observed in Ch-Control. Spherical shapes were adhered to the surface of the Ch-Aw-Bb, Ch-Aw-Mr, and Ch-Ao-Mr beads, which could be indicative of *B. bassiana* (Ch-Aw-Bb bead) and *M. robertsii* (Ch-Aw-Mr and Ch-Ao-Mr beads) spores. Particularly in the Ch-Aw-Mr bead, small spherical protuberances were detected under the surface.

Small cavities can be seen in the images corresponding to the Ch-Mr, Ch-Aw-Ij, and Ch-Ao-Ij beads; they could have been generated by the detachment of the spores that were previously attached to the surface of the beads at the time of the synthesis. A special case

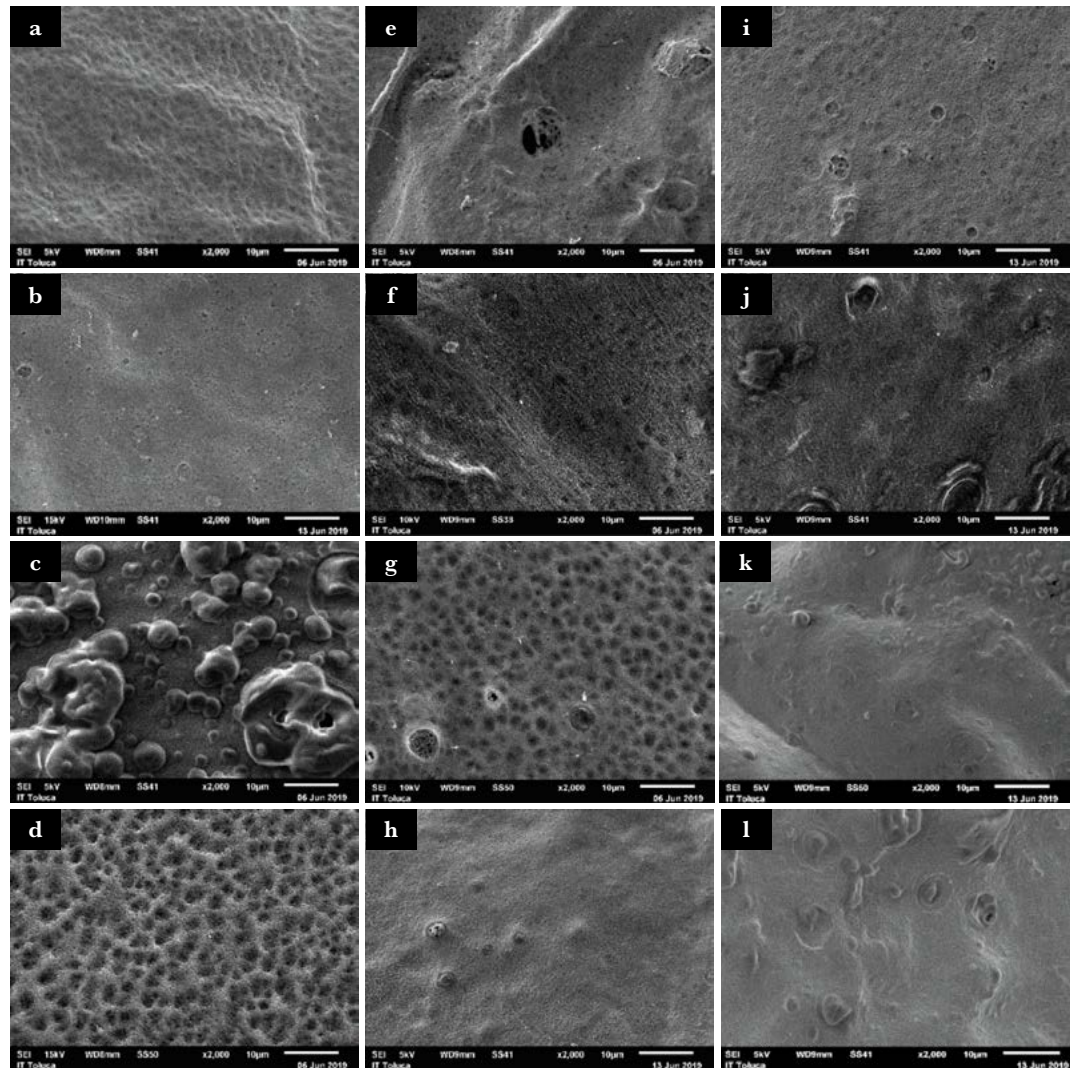


Figure 3. Micrographs at 2000x of the external surface of Ch-neem-spore beads: a) Ch-Control; b) Ch-Aw; c) Ch-Ao; d) Ch-Bb; e) Ch-Mr; f) Ch-Ij; g) Ch-Aw-Bb; h) Ch-Aw-Mr; i) Ch-Aw-Ij; j) Ch-Ao-Bb; k) Ch-Ao-Mr; l) Ch-Ao-Ij.

is the morphology observed on the surface of the Ch-Ao bead: the adhered particles could be part of the oil that was not homogenized with the matrix during the synthesis.

Finally, Figure 4 shows the 3500x magnification of the internal surface of the fractionated beads. The pores of all the beads have different characteristics, such as networks or bone-like meshes with caverns and tubules.

Adjustments to swelling models

Tables 3 and 4 show how each swelling kinetic is adjusted to the first and second order swelling models. According to these tables, the second order model of the swelling kinetics had a slightly higher coefficient of determination (R^2) than the first order model, but there was no significant change in the adjustment of the swelling kinetics. Therefore, the chi-square test was carried out in order to determine if there is a significant difference between

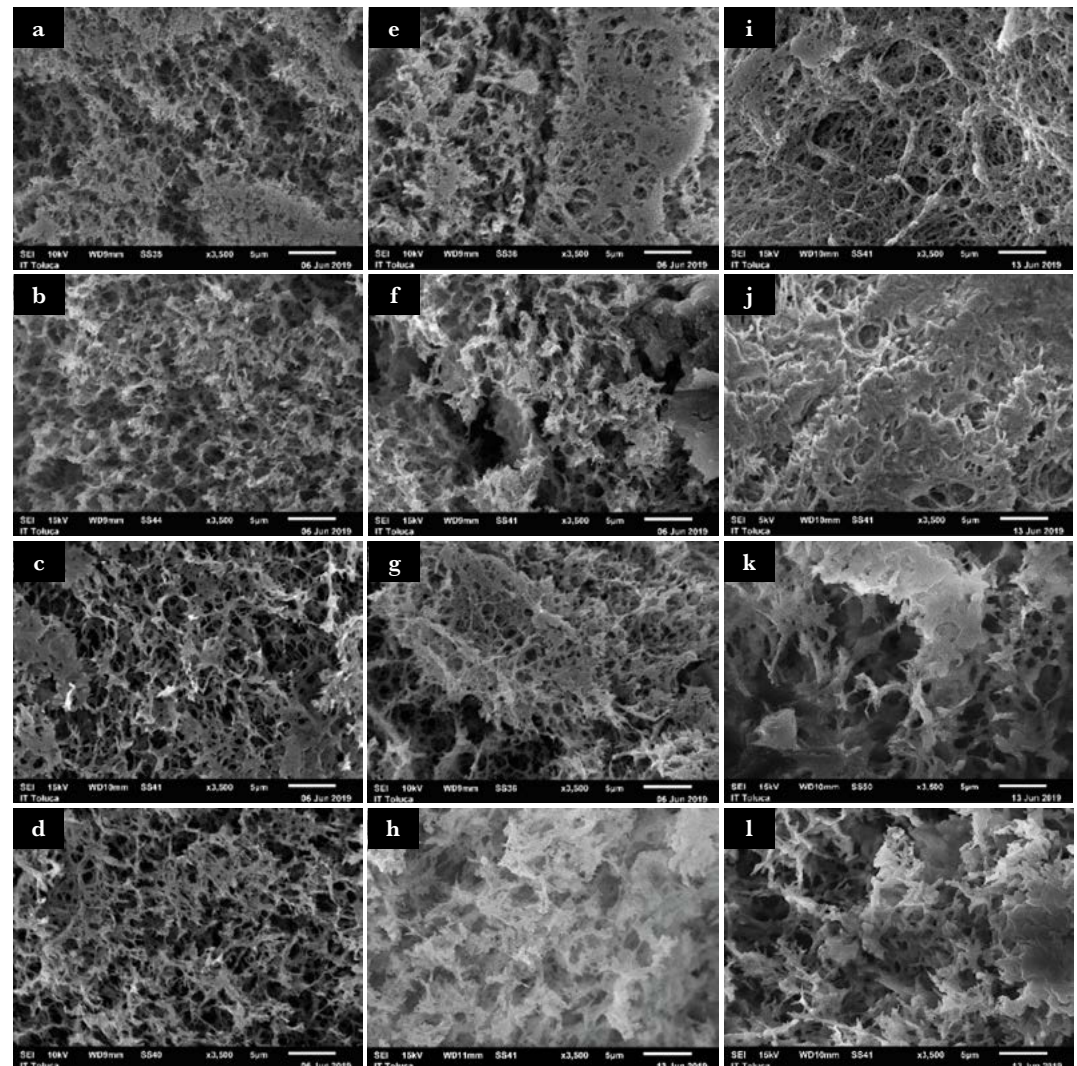


Figure 4. Micrographs at 3500x of the internal surface of Ch-neem-spore beads: a) Ch-Control; b) Ch-Aw; c) Ch-Ao; d) Ch-Bb; e) Ch-Mr; f) Ch-Ij; g) Ch-Aw-Bb; h) Ch-Aw-Mr; i) Ch-Aw-Ij; j) Ch-Ao-Bb; k) Ch-Ao-Mr; l) Ch-Ao-Ij.

Table 3. Adjustments to the first order swelling model.

Sample	W_{∞}	k	R^2	Chi-Sqr
Ch-Control	0.632620	0.73651	0.97683	7.28E-04
Ch-Aw	0.631160	0.89146	0.99419	1.79E-04
Ch-Ao	0.635650	0.93930	0.99698	9.41E-05
Ch-Bb	0.684730	1.03038	0.99875	4.52E-05
Ch-Mr	0.667360	0.90848	0.99804	6.73E-05
Ch-Ij	0.647990	0.92563	0.99311	2.24E-04
Ch-Aw-Bb	0.626830	0.93736	0.99603	1.20E-04
Ch-Aw-Mr	0.623960	1.00813	0.99420	1.75E-04
Ch-Aw-Ij	0.614520	0.92019	0.99462	1.57E-04
Ch-Ao-Bb	0.641050	0.95537	0.99280	2.29E-04
Ch-Ao-Mr	0.649130	0.94973	0.99198	2.62E-04
Ch-Ao-Ij	0.623720	0.80736	0.99134	2.61E-04

Table 4. Adjustments to the second order swelling model.

Sample	W_{∞}	k	R^2	Chi-Sqr
Ch-Control	0.654770	2.65987	0.98913	3.42E-04
Ch-Aw	0.643900	4.74278	0.99835	5.07E-05
Ch-Ao	0.645740	5.93387	0.99925	2.35E-05
Ch-Bb	0.692550	7.65708	0.99985	5.47E-06
Ch-Mr	0.677740	5.60120	0.99975	8.51E-06
Ch-Ij	0.660900	4.78650	0.99750	8.13E-05
Ch-Aw-Bb	0.637390	5.72565	0.99880	3.65E-05
Ch-Aw-Mr	0.634190	6.17635	0.99732	8.07E-05
Ch-Aw-Ij	0.625990	5.30507	0.99821	5.22E-05
Ch-Ao-Bb	0.653170	5.15825	0.99685	1.00E-04
Ch-Ao-Mr	0.662040	4.86027	0.99656	1.12E-04
Ch-Ao-Ij	0.639440	3.75259	0.99739	7.85E-05

the actual values of the swelling kinetics and the adjustment with the theoretical first and second order models. The results showed a better adjustment of the swelling kinetics to the second order model, since there is a greater error (Chi-Sqr) with the first order model. Consequently, there is a better adjustment to the model of second order swelling.

The swelling equilibrium (W_{∞}) in the second order model showed that Ch-Bb recorded the highest value with a lower degree of chitosan crosslinking than the Ch-Control and other beads (Ostrowska-Czubenko *et al.*, 2015). Meanwhile, the maximum swelling rate k in the Ch-Bb bead had a value of 7.65708 min^{-1} ; this result means that it reaches a maximum swelling in a shorter time. This behavior could be caused by a structural change in the chitosan molecule, since some authors have reported that the coupling of some functional groups in the main chain of the biopolymer can improve its physicochemical properties (*e.g.*, solubility) and therefore generate some interesting synergistic characteristics (Carrero Gallardo *et al.*, 2019).

CONCLUSIONS

It is feasible to obtain chitosan beads crosslinked with sodium tripolyphosphate containing in its matrix entomopathogenic fungal spores and neem. Pests in crops could be eliminated by the potential controlled release of neem and fungal spores. This alternative for the biological control of pest insects avoids the use of agrochemicals. The morphological study showed that all the beads have similar porosity characteristics that would allow the neem extract to be released in a controlled manner. Likewise, the swelling kinetics results showed that the maximum swelling of the beads can be achieved in a relatively short time, according to the results obtained in the adjustment of the second order model.

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Training for the production and use of bioinputs in the state of Hidalgo: an analysis of the actors

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ABSTRACT

Objective: The excessive use of synthetic fertilizers and pesticides has had negative effects on agricultural biodiversity. Recently, an alternative that has been suggested is the use of bioinputs, such as biofertilizers, biostimulants and biopesticides, coupled with the need to train agricultural technicians in topics related to the preparation and use of these bioinputs. In response, SADER in collaboration with INIFAP, designed a Training Program of 10 topics related to this subject, aimed at the technical staff of the Agricultural Representations.

Design/methodology/approach: In Hidalgo, approximately 40 participants attended the sessions. Each participant completed both pre- and post-topic evaluations, as well as evaluations of the course and the trainers. This information was compiled into a database and formed the basis of the present document, which aims to describe the program's results with a focus on capacity development (CD) and sustainable agriculture. Capacity development was measured by assessing the change between the initial and final evaluation scores.

Results: The capacity development of the technical staff from the SADER Representation in Hidalgo was low, with an average improvement of only 1.0 point over the 10 courses.

Findings/conclusions: Therefore, it is considered important to reinforce the training by implementing the bioinput production processes presented.

Keywords: Capacity building, sustainable agriculture, soil fertility.

INTRODUCTION

The increase in the human population has been accompanied by a growing demand for food, driving the development of agricultural technologies aimed at increasing productivity. This was the case with the Green Revolution in the mid-20th century, which was based on the use of high-yielding varieties, primarily cereals, as well as the massive application of synthetic fertilizers and pesticides (Mamani & Filippone, 2018). These practices have led to negative effects on agricultural biodiversity, triggering an escalating cycle of ecological and social degradation, resulting in risks and threats (Molina, 2021). In response to these adverse effects, the concept of sustainable agriculture has emerged, promoting agricultural production while conserving natural resources such as soil, water, and biodiversity (Badgley *et al.*, 2007). This shift has been primarily driven by the increasing demand for agricultural and livestock products free of agrochemical residues, necessitating changes in traditional production methods.

An alternative that has gained relevance is the use of bioinputs, such as biofertilizers, biostimulants, and biopesticides, which represent economically attractive and environmentally sustainable options (Mamani & Filippone, 2018). A bioinput is a product based on compounds and/or extracts from microorganisms or plants, or on living microorganisms, capable of improving crop productivity, quality, and/or health without causing negative impacts on the agroecosystem (Gerwick & Sparks, 2014). Throughout

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history, these bioinputs have been used in traditional agriculture based on farmers' empirical knowledge. However, most of them do not have a defined author and have not been incorporated into agronomy curricula. This situation highlights the need to train technicians who advise producers on the production and use of bioinputs, particularly those focused on soil nutrition and fertility.

To address this need, the Secretariat of Agriculture and Rural Development (SADER), in collaboration with the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), designed a Training Program covering ten key topics on the production and use of bioinputs and their benefits for soil conservation and fertility (Reyes *et al.*, 2023). This program was aimed at technical staff from the Agricultural Representations in most federal entities of the country, as well as other professionals in the sector.

One of the states that participated in this training was Hidalgo, where INIFAP researchers delivered the ten topics to SADER technical staff during the last quarter of 2022. On average, 40 people participated, each of whom completed an initial and final evaluation for each topic, as well as assessments of the course and trainers. This information was compiled into a database, which served as the basis for this study. The objective of this document is to describe the results of the Training Program for the Production and Use of Bioinputs for Plant Nutrition and Soil Fertility Conservation in the State of Hidalgo, with a focus on capacity development and sustainable agriculture.

MATERIALS AND METHODS

Data compilation

Data from the initial and final evaluations completed by 40 participants from the state of Hidalgo were compiled in an Excel[®] spreadsheet. These evaluations covered the ten topics included in the Training Program: 1. Nutritional needs of plants and soil as a source of nutrients. 2. Biogeochemical processes in soil and their role in nutrient availability. 3. Interactions between microorganisms, plants, soil, and the environment. 4. Tools for determining plant nutritional needs and the use of organic and synthetic fertilizers. 5. Contribution of organic inputs to soil nutrition and quality. 6. Improvement of agricultural crop productivity and health through the use of biological products. 7. Preparation and use of bioles. 8. Production and use of compost. 9. Production and use of vermicompost. 10. Production and use of leachates.

The responses from the satisfaction surveys completed by the 40 participants for each of the 10 topics covered were also integrated, reflecting their opinions regarding the course:

- Were the objectives and content of the training covered?
- How was the relationship between the explanations and the activities performed?
- Do you consider the content of the topics to be appropriate and up-to-date?
- The provided training materials.
- What was learned will be useful in my work activities.
- Are you satisfied with the course?

And their opinion regarding the instructor(s):

- The treatment towards the participants.
- He/she presented the objectives and the work program.
- He/she fulfilled the objectives and the work program.
- Demonstrated mastery and knowledge of the topics.
- Promoted, supervised, and strengthened group integration.
- Conveyed the knowledge clearly.

Analysis of the Initial and Final Evaluation Responses

For each topic, a test with 10 questions was designed and applied at the beginning and end of the topic presentation. The scores from the responses were compiled into an Excel[®] spreadsheet, assigning a value of 1 for correct answers and 0 for incorrect ones. The correct answers were summed, and the total was converted into a grade on a scale from 1.0 to 10.0. The grades for all participants were then averaged to obtain the overall average for each topic.

Analysis of the Course and Instructor Evaluation Responses

To analyze the responses from participants for each topic, a Likert scale (Ospina *et al.*, 2003) was applied. For each question, participants could select from four options: excellent (value of 4), very good (value of 3), good (value of 2), poor (value of 1), and very poor (value of 0). The average value for each participant and for all the questions was calculated to obtain an overall average for each course.

Evaluated Variables

To measure capacity development, the value of the final evaluation was subtracted from the value of the initial evaluation for each course. Additionally, the average, maximum, and minimum percentages of the evaluations for each course and each instructor were calculated.

RESULTS AND DISCUSSION

Based on the topics presented in the 10 courses, the first four address conceptual aspects of the soil-plant-environment relationship (Reyes *et al.*, 2023). Therefore, as shown in Table 1, the initial scores obtained are the lowest, indicating limited mastery of these topics by the participants. The following six topics have a practical orientation, so the participants' prior knowledge was greater, which is reflected in the scores obtained, particularly in the production and use of leachates, where the initial score was 9.0 on a scale of 1 to 10.

In the same Table 1, the values that participants obtained at the end of each course are presented. It can be observed that the courses with greater practical application, such as the preparation and use of bioles, composts, vermicomposts, and leachates, are the ones with the highest scores. This shows the current importance of training and disseminating these topics to transition from conventional agriculture to sustainable agriculture, especially the last three inputs, due to their role as fertilizers and soil enhancers (Aramendis *et al.*, 2023).

Table 1. Average values of the initial and final scores for the 10 courses taught in the state of Hidalgo.

Courses	Initial evaluation			Final evaluation		
	Mean ^a	Max	Min	Mean	Max	Min
1. Nutritional needs of plants and soil as a source of nutrients.	5.0	9.0	1.0	7.0	10.0	2.0
2. Biogeochemical processes in soil and their role in nutrient availability.	5.0	10.0	0.0	7.0	10.0	3.0
3. Interactions between microorganisms, plants, soil, and the environment.	6.0	10.0	3.0	9.0	10.0	5.0
4. Tools for determining plant nutritional needs and the use of organic and synthetic fertilizers.	6.0	10.0	10.0	8.0	10.0	2.0
5. Contribution of organic inputs to soil nutrition and quality.	7.0	10.0	5.0	7.0	10.0	4.0
6. Improvement of agricultural crop productivity and health through the use of biological products.	6.0	10.0	3.0	8.0	10.0	6.0
7. Preparation and use of bioles.	8.0	10.0	4.0	8.0	10.0	4.0
8. Production and use of compost.	7.0	10.0	10.0	8.0	10.0	3.0
9. Production and use of vermicompost.	7.0	10.0	4.0	9.0	10.0	3.0
10. Production and use of leachates.	9.0	10.0	3.0	9.0	10.0	4.0
Promedio general	7.0	10.0	3.0	8.0	10.0	4.0

^aMean=Average. Source: Prepared by the author using data from participant evaluations.

Composts, vermicomposts, and leachates are the most commonly used organic and foliar fertilizers due to their high content of free amino acids or humic and fulvic acids. Some of these inputs are produced by the farmers themselves (Mamani and Filippone, 2018). Therefore, training the technicians who support the farmers has positive effects, and understanding the processes involved in their preparation will improve the efficiency of these inputs, thereby enhancing production.

When analyzing the effect of the 10 courses on the skills acquired by the participants, it is observed in Figure 1 that the topic with the greatest difference between the average initial and final exam scores was related to “nutritional needs of plants and the soil as a source of nutrients.” As mentioned earlier, this is a theoretical topic, and while most participants are agronomists, not all are specialists in Soil. Therefore, the diagnostic evaluation was low, but upon receiving the training, they absorbed the concepts presented, as reflected in the final evaluation. There are other topics that did not show improvements, such as “contribution of organic inputs to soil nutrition and quality,” which had an initial and final score of 7.0, and “production and use of leachates,” which maintained an initial score of 9.0.

When analyzing Figure 1, it is observed that the effect of the training was not significant. The average score across the 10 courses was 1.0, which is a low value. This can be attributed to the fact that capacity development is the result of a combination of resources, practices, knowledge, and relationships (González, 2021). It can be deduced that a short training course is not sufficient to improve the participants’ skills. The topics taught, especially

those with practical application, need to be put into practice. These topics explain how to produce and use inputs and demonstrate the impact on soil improvement or performance. This can only be achieved through direct contact with the producers, which is the main role of most of the course participants. Only then can it be said that the individual has acquired experience, knowledge, and technical competencies (González, 2021).

Another aspect that can be observed in Figure 1 is the training gap. In the case of the production and use of compost, this topic needs to be reinforced, as the average final evaluation score was 7.0. This result represents an area for improvement (González, 2021), because compost is a bio-input that can be produced from manure, a widely available ingredient in the state of Hidalgo, such as in the Tizayuca and Valle de Tulancingo dairy basins (Poméon *et al.*, 2006).

A determining factor for capacity development is the instructor. Participants' opinions regarding these actors were evaluated on a scale from 0 to 4, where zero is very poor and four is excellent. The average of the six questions is shown in Figure 2, which includes the evaluation of: the treatment towards participants, the communication and fulfillment of course objectives, as well as the instructor's command and delivery of the course content. In this figure, it is observed that the instructor with the lowest score was the one who taught the course on the production and use of compost, with participants indicating that they perceived less mastery and knowledge of the topic, as well as difficulty in transmitting it clearly. Although this instructor received the lowest score, they averaged a rating of 3.4, which places them in the "very good" category according to the scale used. On the other

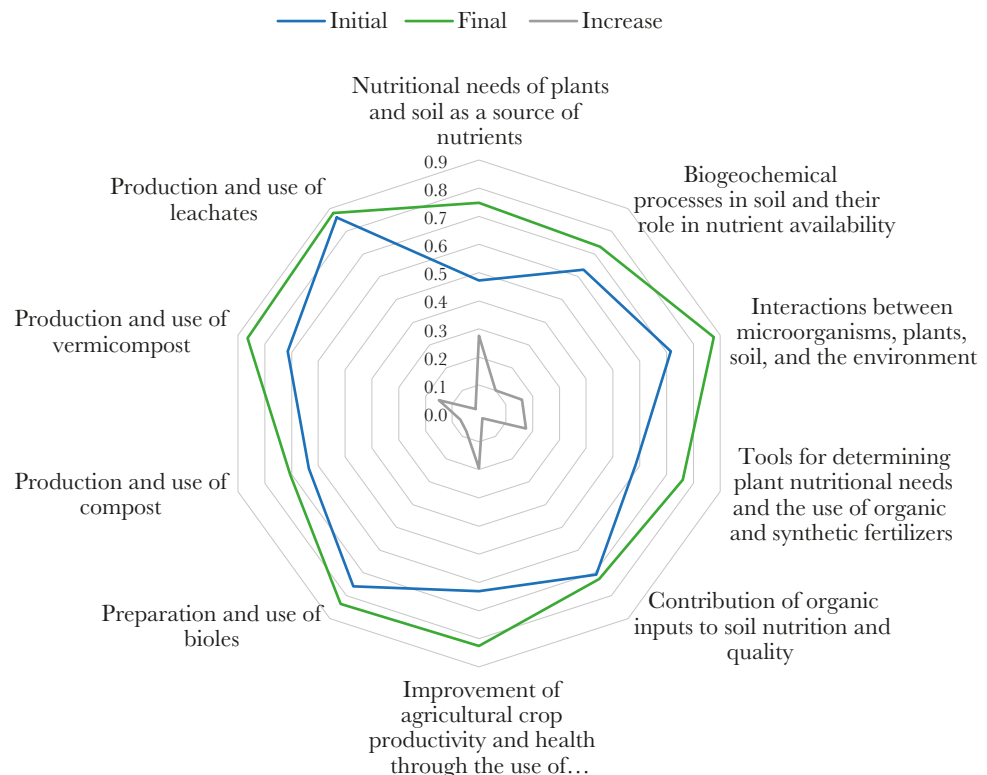


Figure 1. Effect of the training received by participants in 10 courses held in the state of Hidalgo.

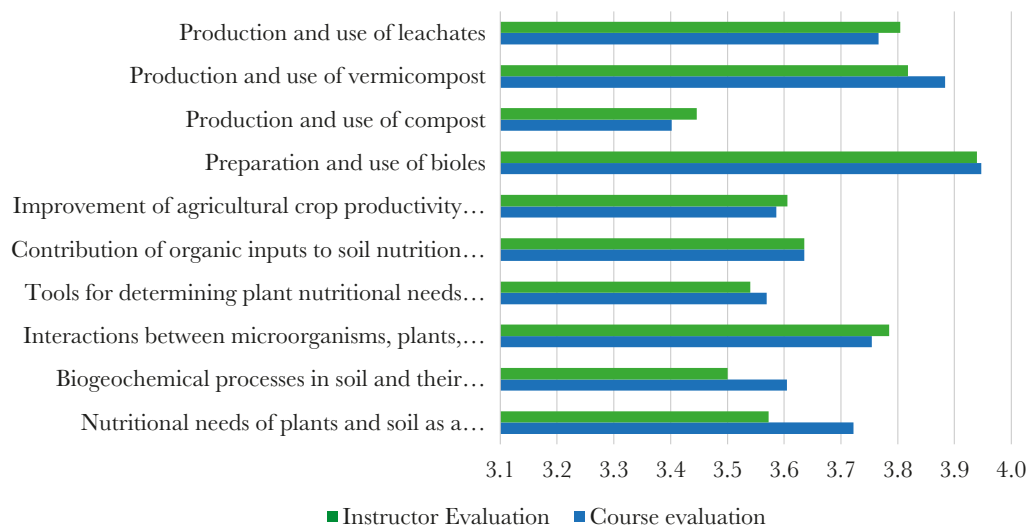


Figure 2. Evaluation of the instructors and the 10 courses held in the state of Hidalgo. Source: Author's elaboration based on the participants' satisfaction survey data.

hand, the instructors who received the highest ratings were those who taught the courses on "preparation and use of bioles" and "production and use of vermicompost." Analyzing the ratings given to all the instructors, it is clear that all instructors received ratings above 3.4, indicating that participants rated them as very good or excellent.

In the same Figure 2, the average rating of the 10 courses provided by the participants is shown, regarding each course. Their opinions were based on whether the stated objectives were met, if the topics were appropriate and up-to-date, if the provided materials were useful and adequate, if what was learned was applicable to the participants' activities, and how satisfied they were with the course. In this figure, it is observed that the course with the lowest rating was "Biogeochemical Processes of the Soil in Nutrient Availability," with the question regarding the provided materials receiving the lowest score. Although this course received the lowest rating, it still averaged a score of 3.5, which places it in the "very good" category.

In contrast, the course with the highest rating was the "Preparation and Use of Bioles." For the six questions that asked for the participants' opinions, it received an excellent rating, with an average of 3.95. Upon analyzing the ratings for all the courses, they all scored higher than 3.5, indicating that the participants rated all 10 courses as very good to excellent.

CONCLUSIONS

The results presented from the participants in the Training for the Production and Use of Bioinputs for Plant Nutrition and Soil Fertility Conservation in the state of Hidalgo show that the capacity development was low, with an average change in the final evaluation score compared to the initial score of only 1.0 on a scale from 1.0 to 10.0 in the knowledge acquired. The initial average score across the 10 courses was 7.0, and at the end, they obtained 8.0. Even in the topics of "Contribution of organic inputs to soil nutrition and

quality” and “Production and Use of Leachates,” the scores obtained in both evaluations were the same.

The participants’ ratings regarding the performance of the instructors, as well as the quality, relevance, and applicability of all the courses, were above 3.4 on a scale of 4, indicating that both the instructors and the courses were rated as very good and excellent by the participants. However, the difference between the initial knowledge of the topics versus the knowledge acquired by the end of the training was low.

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Performance and response to web blight and angular leaf spot of tropical black bean genotypes

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ABSTRACT

Objective: To identify black bean genotypes resistant to web blight and angular leaf spot, with yield significantly superior to that of the Negro Jamapa variety.

Design/methodology/approach: Eleven genotypes were evaluated using a randomized complete block design with three replications. Their reactions to both diseases were rated using the 1 to 9 scale of the International Center for Tropical Agriculture (CIAT). Grain yield was estimated in kilograms per hectare. Analyses of variance were performed on the quantified variables, and a combined analysis was conducted for grain yield. Mean separation was carried out using the Least Significant Difference test at a 0.05 significance level.

Results: With mean disease severity values ranging from 2.0 to 3.33, five lines and the Verdín variety demonstrated resistance to web blight. Nine lines, along with Verdín, also exhibited resistance to angular leaf spot. Among these two groups of outstanding genotypes for disease resistance, Jamapa Plus/XRAV-187-3-4-4 and Jamapa Plus/XRAV-187-3-4-1 were the most productive, with average yields exceeding 2100 kg ha⁻¹, statistically similar to those of Verdín and Jamapa Plus/XRAV-187-3-1-2.

Limitations on study/implications: Although angular leaf spot did not significantly affect bean yield, the disease incidence and the severity in the regional control were sufficient to evaluate and classify the genotypes' reaction to this disease under natural infection conditions.

Findings/conclusions: The three lines derived from the Jamapa Plus/XRAV-187-3 cross and the Verdín variety exhibited resistance to both diseases and achieved an average yield significantly higher than that of the Negro Jamapa variety.

Keywords: *Phaseolus vulgaris* L., diseases, genetic resistance, productivity.

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INTRODUCTION

Fungal diseases are one of the main biotic factors that commonly affect bean cultivation in the tropical and subtropical regions of the state of Veracruz, Mexico (López-Salinas *et al.*, 2006). Among these, angular leaf spot, caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, stands out due to its prevalence in both residual moisture plantings during the autumn-winter cycle and rainfed plantings in the summer cycle. The latter are primarily



carried out in the Las Altas Montañas region in the central part of the state, at altitudes above 800 m (Tosquy-Valle *et al.*, 2012; Becerra-Leor *et al.*, 2021).

Angular leaf spot is characterized by causing angular lesions on the lower leaves, bordered by reddish-brown veins, where spores form and serve as the inoculum for secondary leaves. The lesions can spread across the entire leaf blade, causing defoliation. On the pods, the lesions are oval and circular, dark brown or reddish in color. Environmental conditions with high relative humidity (>70%), alternating with dry or low-humidity days and temperatures between 18 and 25 °C, promote infection and the development of angular leaf spot (Garza-García *et al.*, 2009), which can cause yield losses exceeding 70% in beans (Tosquy-Valle *et al.*, 2013).

Web blight, although less frequent in the state, also causes significant damage (Tosquy-Valle *et al.*, 2012). This disease is caused by the fungus *Thanatephorus cucumeris* (Frank) Donk in its perfect (sexual) stage, which infects the plant through basidiospores, while its imperfect (asexual) stage is known as *Rhizoctonia solani* Kühn (Becerra-Leor *et al.*, 2021). Symptoms include grayish-green to dark brown spots on the leaves, resembling hot water burns. The fungal mycelium spreads to other plant organs, growing in a web-like manner until it completely covers the plant. The leaves adhere to each other, leading to rapid and severe defoliation, while young pods may be entirely destroyed; in mature pods, lesions merge, causing severe damage (Araya-Fernández & Hernández-Fonseca, 2006; Atilio & Reyes, 2008).

The development of web blight is favored by periods of high precipitation, relative humidity above 80%, and temperatures between 20 and 30 °C (Gálvez *et al.*, 1989). This fungus can affect multiple bean plant organs at any phenological stage and, in severe cases, can cause total crop loss within one to two weeks, especially in lowland tropical areas (Rodríguez *et al.*, 1999; Pedroza-Sandoval *et al.*, 2010).

In Veracruz, web blight occasionally occurs in bean plantings in the central zone and the Las Altas Montañas region (Tosquy-Valle *et al.*, 2024), and more frequently during the autumn-winter cycle in the municipalities of Martínez de la Torre and Tlapacoyan, located in the northern zone, where it has caused significant reductions in grain yield (Rodríguez-Rodríguez *et al.*, 2016).

Most farmers in Veracruz use low-yielding landrace varieties, others of unknown origin, and some improved varieties such as Negro Jamapa, which was developed over six decades ago by the former INIA (Rosales-Serna *et al.*, 2004), all of which are susceptible to these diseases (Ugalde-Acosta *et al.*, 2014; Rodríguez-Rodríguez *et al.*, 2016; Tosquy-Valle *et al.*, 2024). Moreover, they generally do not apply fungicides due to the increased production costs (Tosquy-Valle *et al.*, 2013). The use of fungicides, the implementation of agronomic practices such as zero tillage, and the cultivation of improved varieties with high-yield potential and resistant or tolerant to web blight and angular leaf spot can significantly reduce yield losses and increase crop productivity (Rodríguez *et al.*, 1999; Tosquy-Valle *et al.*, 2013).

The objective of this study was to identify black bean genotypes resistant to web blight and angular leaf spot under field conditions, with higher grain yield than the Negro Jamapa cultivar, which is commonly used by farmers in Veracruz, Mexico.

MATERIALS AND METHODS

During the 2019-2020 autumn-winter agricultural cycle (September-February), a bean trial was conducted under residual moisture conditions in two locations within the municipality of Orizaba, in the Las Altas Montañas region of central Veracruz, Mexico: Rincón Grande (18° 51' N, 97° 06' W, altitude 1,248 m) and Rincón Chico (18° 50' N, 97° 05' W, altitude 1,191 m). The soils in both locations have a sandy loam texture, a high organic matter content (5.96% and 5.90%, respectively), and a moderately acidic pH (6.51 and 5.85, respectively).

Eleven advanced black bean lines were evaluated: five derived from the Negro Papaloapan/SEN 46 cross, three from the Negro Citlali/XRAV-187-3 cross, and three from the Jamapa Plus/XRAV-187-3 cross, along with the varieties Negro Medellín (López-Salinas & Acosta-Gallegos, 2002), Verdín (Tosquy-Valle *et al.*, 2016) and Negro Jamapa, as the regional control. All these materials were developed for the tropical areas of southeastern Mexico by the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP).

The genotypes were planted at a density of 250,000 plants ha⁻¹, in a randomized complete block design with three replications and experimental plots consisting of three 5-meter-long rows, with the useful plot corresponding to the entire central row. Agronomic management was carried out according to the recommendations of INIFAP for bean cultivation in Veracruz (López-Salinas *et al.*, 2017).

During the trial, rainfall was recorded, and at the R8 stage (pod filling) of the genotypes, the severity of web blight was visually assessed in Rincón Grande and angular leaf spot in Rincón Chico. The general 1 to 9 scale from CIAT was used to evaluate the reaction of the black bean germplasm to fungal pathogens (van Schoonhoven & Pastor-Corrales, 1987), where a reaction score of 1 to 3 is considered resistant (no or very mild symptoms), 4 to 6 is intermediate (visible symptoms causing limited economic damage), and 7 to 9 is susceptible (severe to very severe symptoms, causing significant yield loss or plant death). After harvest, the grain yield was determined in each useful plot, adjusted to 14% moisture content, and expressed in kilograms per hectare.

Individual analyses of variance were performed for the quantified variables and a combined analysis for grain yield. The Least Significant Difference test at a 5% probability level (LSD, $\alpha=0.05$) was applied for mean separation (SAS Institute, 1999). Correlation analyses were also performed between the average severity values of each disease and the grain yield of the genotypes at each trial site to determine if these diseases caused significant damage to bean production (Olivares, 1994).

RESULTS AND DISCUSSION

Significant differences ($p\leq 0.01$) between treatments were detected in the individual analyses of variance for all quantified variables (Table 1), indicating that the genotypes showed differences in their reaction to web blight and angular leaf spot, as well as in their productive capacity.

In Rincón Grande, the high relative humidity (>85%) due to constant rainfall during the reproductive phase of the crop, and moderate to high temperatures (between 19 and

Table 1. Mean squares and statistical significance of quantified variables in the black bean genotypes trial in two environments of the Las Altas Montañas region, central Veracruz state. Fall-winter cycle of 2019-2020.

SV	DF	Web blight ¹	Grain yield ¹	Angular leaf spot ²	Grain yield ²
Treatments	13	4.690**	171881.53**	2.703**	420000.00**
Blocks	2	2.952	32834.00	2.167	3056.00
Error	26	0.773	24293.85	0.731	47894.15
Total	41				
CV (%)		22.11	13.46	26.79	7.93

SV: source of variation. DF: degrees of freedom. 1: Rincón Grande. 2: Rincón Chico. **: Significant at 0.01 level. CV: coefficient of variation

30 °C), favored the incidence of web blight starting from the beginning of the pod-filling stage, which significantly reduced grain yield ($r = -0.810^{**}$). These results confirm what was reported by Takegami *et al.* (2004) and Costa-Coelho *et al.* (2014), in the sense that greater damage caused by web blight results in lower bean grain yield. The three lines derived from the cross Jamapa Plus/XRAV-187-3, along with the lines Negro Papaloapan/SEN 46-3-2 and Negro Papaloapan/SEN 46-2-6 and the variety Verdín, showed resistance to this disease, with average severity ratings between 2.0 and 3.33 (Table 2).

Table 2. Resistance reaction of black bean genotypes to web blight and angular leaf spot in two environments of the Las Altas Montañas region of Veracruz, Mexico. Fall-winter cycle of 2019-2020.

Genotype	Scale of 1 to 9 [†]	
	Web blight	Angular leaf spot
Negro Papaloapan/SEN 46-2-6	3.33 cde	2.67 cd
Negro Papaloapan/SEN 46-3-2	2.00 e	4.00 abc
Negro Papaloapan/SEN 46-7-7	4.33 bcd	4.00 abc
Negro Papaloapan/SEN 46-7-10	4.33 bcd	3.00 bcd
Negro Papaloapan/SEN 46-7-12	4.33 bcd	2.00 d
Negro Citlali/XRAV-187-3-1-5	5.67 ab	2.67 cd
Negro Citlali/XRAV-187-3-1-6	5.33 ab	3.00 bcd
Negro Citlali/XRAV-187-3-1-8	4.67 abc	2.67 cd
Jamapa Plus/XRAV-187-3-1-2	3.33 cde	3.33 bcd
Jamapa Plus/XRAV-187-3-4-1	2.00 e	2.00 d
Jamapa Plus/XRAV-187-3-4-4	3.00 de	3.33 bcd
Negro Medellín	4.33 bcd	5.33 a
Negro Jamapa (Regional control)	6.00 a	4.33 ab
Verdín	3.00 de	2.33 d
Environment average	3.97	3.19
LSD (0.05)	1.476	1.435

[†] Severity values using the general scale of 1 to 9 (van Schoonhoven & Pastor-Corrales, 1987). Means with the same letters in each column of averages are statistically similar according to the Least Significant Difference (LSD, 0.05).

In contrast, the variety Negro Jamapa was the most affected by web blight, with damage statistically similar to that shown by the three lines derived from the cross Negro Citlali/XRAV-187-3 and significantly higher than the rest of the genotypes (Table 2). The resistance of Verdín and the susceptibility of Negro Jamapa to web blight have already been documented (Rodríguez-Rodríguez *et al.*, 2016; Tosquy-Valle *et al.*, 2023).

In Rincón Chico, angular leaf spot appeared towards the end of the pod filling stage, so it did not significantly affect yield ($r = -0.454$ ns). These results are consistent with those reported by López-Salinas *et al.* (2006) in Medellín, Veracruz, and Tosquy-Valle *et al.* (2012) in Tecamachalco, Puebla, who also found a negative and significant relationship with grain yield in the presence of this disease from pod formation to filling.

Most of the genotypes had average severity ratings between 2.0 and 3.3, indicating that they only showed mild symptoms of angular leaf spot (van Schoonhoven & Pastor-Corrales, 1987), while the variety Negro Medellín was the most affected with an average severity value of 5.33, statistically similar to that of the variety Negro Jamapa and the lines Negro Papaloapan/SEN 46-3-2 and Negro Papaloapan/SEN 46-7-7, and significantly higher than the rest of the genotypes (Table 2).

Among the genotypes resistant to web blight in Rincón Grande, the three lines derived from the cross Jamapa Plus/XRAV-187-3 and the variety Verdín also stood out for their grain yield, which was statistically similar to that of the line Papaloapan/SEN 46-7-12. In contrast, the two lines derived from the cross Negro Citlali/XRAV-187-3 and the variety Negro Jamapa (which showed the greatest damage caused by this disease) were the least productive (Table 3).

In turn, in Rincón Chico, the lines Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1, and Negro Citlali/XRAV-187-3-1-6, as well as the variety Verdín, were the most productive with a grain yield greater than 3000 kg ha^{-1} , statistically similar to each other, while Negro Jamapa and Negro Medellín, which showed higher severity values for angular leaf spot, were the least productive (Table 3).

According to the combined analysis, yield also varied significantly between environments, genotypes, and their interaction ($P \leq 0.01$). The highest average yield was obtained in Rincón Chico (Table 3), largely due to the favorable humidity (total rainfall of 374.1 mm) and temperature (average of $17.8 \text{ }^\circ\text{C}$) conditions during the crop cycle, even though angular leaf spot was present, it did not significantly affect bean yield. In contrast, in Rincón Grande, despite beans were sown in soil rich in organic matter with a pH close to neutrality, optimal for crop development (Edmeades *et al.*, 2012; Kyomuhendo *et al.*, 2020), the presence of web blight significantly reduced bean yield.

In the genotype factor, eight lines and the Verdín variety were placed in the outstanding group for average grain yield, with Jamapa Plus/XRAV-187-3-4-4 and Jamapa Plus/XRAV-187-3-4-1 being the most productive. The first line has shown significantly outstanding average grain yields in previous evaluations conducted in different environments with fungal and viral disease incidence in the states of Veracruz and Chiapas (Tosquy-Valle *et al.*, 2019). On the other hand, the Negro Jamapa variety was the least productive, mainly due to significant damage from web blight and angular leaf spot, with its average

Table 3. Grain yield of black bean genotypes evaluated in two environments in the Las Altas Montañas region of Veracruz, Mexico. Autumn-winter cycle of 2019-2020.

Genotype	Rincón Grande [†]	Rincón Chico [†]	Average
Negro Papaloapan/SEN 46-2-6	1166.67 cd	2718.67 def	1942.67 abc
Negro Papaloapan/SEN 46-3-2	1233.33 bcd	2933.33 bcd	2083.33 ab
Negro Papaloapan/SEN 46-7-7	1222.00 bcd	2654.67 def	1938.33 abc
Negro Papaloapan/SEN 46-7-10	1117.67 d	2503.33 ef	1810.50 bc
Negro Papaloapan/SEN 46-7-12	1326.67 abcd	2662.00 def	1994.33 ab
Negro Citlali/XRAV-187-3-1-5	789.00 f	2531.00 ef	1660.00 bc
Negro Citlali/XRAV-187-3-1-6	715.33 f	3227.67 ab	1971.50 ab
Negro Citlali/XRAV-187-3-1-8	1131.00 cd	2528.33 ef	1829.67 bc
Jamapa Plus/XRAV-187-3-1-2	1388.67 abc	2827.00 cde	2107.83 ab
Jamapa Plus/XRAV-187-3-4-1	1462.33 ab	3260.33 ab	2361.33 a
Jamapa Plus/XRAV-187-3-4-4	1497.67 a	3315.00 a	2406.33 a
Negro Medellín	1069.00 de	2364.33 fg	1716.67 bc
Negro Jamapa (Regional control)	849.00 ef	2021.00 g	1435.00 c
Verdín	1244.33 abcd	3093.67 abc	2169.00 ab
Environment average	1158.05 b	2760.02 a	1959.03
LSD (0.05)	353.66	367.38	518.91

[†]: Yield in kg ha⁻¹. Means with the same letters in each column are statistically similar according to the Least Significant Difference (LSD, 0.05).

yield statistically similar to that of the Negro Medellín variety and the lines Negro Citlali/XRAV-187-3-1-5, Negro Papaloapan/SEN 46-7-10, and Negro Citlali/XRAV-187-3-1-8 (Table 3).

The highly significant effect of the interaction between both factors indicated that the productive response of some genotypes varied with the evaluation environment; this is the case of the line Negro Citlali/XRAV-187-3-1-6, which in Rincón Grande showed a lower grain yield, primarily due to damage caused by web blight, while in Rincón Chico, under adequate humidity and temperature conditions during the crop cycle, it was among the most productive. Similarly, the line Negro Papaloapan/SEN 46-7-12, which in Rincón Grande, in the presence of web blight, obtained a significantly outstanding grain yield, statistically similar to the most productive line (Jamapa Plus/XRAV-187-3-4-4), whereas in Rincón Chico, despite only showing mild symptoms of angular leaf spot, it was one of the least productive (Table 3).

CONCLUSIONS

Under the environmental conditions of the Las Altas Montañas region of Veracruz, Mexico, the lines Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1, and Jamapa Plus/XRAV-187-3-1-2, as well as the variety Verdín, showed resistance to web blight and angular leaf spot, and were the ones with the highest average yield, significantly superior to that of Negro Jamapa, the regional control.

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Analysis of production costs and net income of wheat (*Triticum aestivum* L.x.) in the state of Guanajuato

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ABSTRACT

To analyze and compare the structure of production costs (PC) and net income (NI) obtained from the production of wheat cultivars (*Triticum aestivum* L.x.): Cortázar S94 (WCC) and Faisán S2016 (WCF) in Guanajuato, a questionnaire was designed to obtain PC information. This questionnaire was applied to a sample of 85 producers, calculated using maximum variance sampling proportions with a reliability of 94% and a precision level of 0.1. The total production cost (TPC) was obtained by adding the variable costs and fixed costs; the average costs and unit production cost (UPC) were calculated. The NI was estimated by the difference between gross income and TPC. The information was analyzed using basic statistics and to compare the cultivars, an analysis of variance was performed under a completely randomized model, along with a comparison of means using the Student's t-test. The costs of land preparation, sowing, weed control, pest control and harvesting of the WCF were higher ($p < .0001$) than those of the WCC; however, fertilization costs were higher ($p < .0001$) for WCC. The TPC were higher ($p < .0001$) for WCF with \$33,202 vs. \$27,242 for the WCC. However, there were no significant differences in the UPC or the NI. It is concluded that, although WCF presented a higher yield, better price and higher PC than WCC, the NI obtained for the production of both cultivars were statistically equal, which may imply that the producer decides to produce either cultivar indistinctly.

Keywords: Cultivars, production costs, profit.

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INTRODUCTION

Wheat cultivation ranks eighth in terms of the sown area in the country. In 2022, a total of 598,539.2 hectares were planted (SIAP, 2023). It is the second most important cereal in the Mexican diet, with an average *per capita* consumption of 57.4 kg (SAGARPA, 2017). Wheat grain production is divided into durum wheat, primarily used for pasta production, and bread wheat, used for making bread, pastry products, and flour. Durum wheat accounts for the largest share of the cultivated area, with 62%, while soft wheat occupies the remaining 38% (SIAP, 2023). In 2022, Mexico produced 3.6 million tons

of wheat, of which only 30% was bread wheat, an amount insufficient to meet national demand, requiring the importation of 75% of the country's consumption (SAGARPA, 2017). The main wheat-producing states, in order of importance, are Sonora, Guanajuato, Baja California, Michoacán, and Sinaloa, with Guanajuato being the leading producer of bread wheat (SIAP, 2023).

In the Bajío region, the main problems affecting wheat production are water scarcity, yellow rust, and dry rot caused by *Fusarium* spp. (Solís *et al.*, 2024). In addition to these issues, there is an increasing demand from the flour industry for wheat varieties that better adapt to their industrial processes, helping to reduce costs and offering greater resistance to diseases such as those mentioned above. An example of this type of seed includes the Cortázar S94 and Faisán S2016 cultivars, both of which have the largest cultivated area in the state, despite a 20-year gap between their release. This raises the question of why they have remained on the market. Therefore, this document aims to analyze and compare the structure of production costs and the net income obtained from the production of the Cortázar S94 and Faisán S2016 wheat cultivars in Guanajuato State.

MATERIALS AND METHODS

Data collection

Secondary information on the Cortázar S94 (C S94) and Faisán S2016 (F S2016) cultivars was gathered and reviewed, which facilitated the design of a questionnaire applied to a sample of wheat producers in Guanajuato. The questionnaire consisted of the following sections: (i) general information about the producer; (ii) production costs per hectare (Ha^{-1}); and (iii) yields obtained. The sample size was determined using the Maximum Variance Proportion Sampling design (Infante y Zarate, 1990), applying the following formula:

$$n = Np(1 - q) / (n - 1)(\beta / z1 - \alpha)^2 + p(1 - q)$$

Where n is the simple size, N is the population, Z is the confidence level, β is the precision level, p is the probability that the sample is representative, and q is the probability that the sample is not representative. Considering a population (N) of 6,667 wheat producers, a confidence level of 94% ($Z^2 = 1.88$), and a precision level of 0.1, the sample size resulted in $n = 85$ surveys applied from June to August 2022.

To obtain the total production cost (TPC), the following expression was used:

$$TPC = TVC (\text{Total Variable Cost}) + TFC (\text{Total Fixed Cost})$$

The TVC was calculated by summing the monetary value of the following components: (i) land preparation (PrepL), which includes subsoiling, plowing, harrowing, cross-harrowing, border formation, and leveling; (ii) sowing (Sow), which includes mechanized sowing, labor, and seed; (iii) fertilization (Fert); (iv) weed control (WeedCon); (v) pest control (PestCon); and (vi) disease control (DisCon). For all components, both the cost

of inputs and the labor required for their application were considered; (vii) irrigation, which includes water fees and labor; and (viii) harvesting (Harv), which includes the cost of mechanized harvesting, labor, and transportation. For the TFC, an administrative cost was considered, representing a self-assigned salary for the producer for this activity, which on average accounts for 3% of the total gross income (TGI), in addition to other fixed costs (OfC).

The following expression was applied to each component of both variable and fixed costs:

$$\sum_{ji}^{i=1} Q_{ij} * Pr_{ij}$$

For the total cost:

$$TPC = (TCPrepL + TCSow + TCFert + TCWeedCon + TCPestCon + TCDisCon + TCirrigation + TCHarv) + (TFC)$$

The average variable and fixed costs were also calculated, which correspond to the quotient of each component divided by the yield. The unit production cost (UPC) was obtained by dividing the total production cost (TPC) by the yield.

The net income (NI), also known as profit, was calculated as the difference between the gross income (GI) and the total production cost (TPC) ($IB_{cultivar} - CTP_{cultivar}$). The GI with $GI = PreSale_{cultivar} * Yield_{cultivar}$.

Statistical Analysis

The survey data were compiled in an Excel[®] spreadsheet, and basic statistics (mean, relative frequencies, and standard deviation) were obtained. To compare the cultivars, an analysis of variance (ANOVA) was performed under a completely randomized model, and a means comparison was conducted using the Student's t-test to identify differences in costs, profits, and cultivars, using JMP[®] 17.0 (SAS Institute, 2024).

RESULTS AND DISCUSSION

Cultivar Description

The Cortázar S94 cultivar (C S94) has a spring growth habit, is semi-dwarf, and reaches a height of 90 cm. It has an intermediate vegetative cycle, with 78 days to flowering and 133 days to physiological maturity. Its leaves are dark green, and its stem is hollow, cream-colored, and strong, making it moderately resistant to lodging. In the Bajío region, it is resistant to stem rust, moderately susceptible to yellow stripe rust, and leaf rust. Its yield ranges from 6.8 to 8.7 tons/ha under optimal climatic conditions and agronomic management, with a potential yield exceeding 9 tons/ha (Solís *et al.*, 1996).

In terms of quality, this cultivar has a soft endosperm, with hardness values ranging from 62 to 65%, making its flour suitable for cookie production. It has a test weight that varies from 73 to 79 kg/hl and a flour yield of 64.6 to 72.8%, indicating excellent milling

performance. Its W value is 127, classifying it as a weak gluten cultivar, and the protein content in the flour ranges from 9.2 to 10.6% (Solís *et al.*, 1996).

The first records indicating the development of the wheat hybrid Faisán S2016 (F S2016) date back to the 2007-2008 period in Celaya, Guanajuato, although some genetic improvement activities also took place in Texcoco, State of Mexico. The validation and technology transfer stages occurred between 2013 and 2017. The technology was transferred and adopted between 2018 and 2019 through various technology transfer mechanisms (Ledezma *et al.*, 2024).

The F S2016 is a soft wheat cultivar whose flour is suitable for the cookie industry or for blending to improve strong gluten wheats. It is a spring growth habit cultivar, semi-dwarf, with a height of 92 cm; it has an intermediate vegetative cycle, ranging from 78 days to flowering and 132 days to physiological maturity (Solís *et al.*, 2020).

It is moderately resistant to yellow rust and shows great stability across a wide range of sowing dates and irrigation schedules. Its yield has exceeded that of C S94—the most widely cultivated cultivar in El Bajío—by up to 21%. F S2016 has excellent cookie-making quality and exhibits commercial quality parameters that ensure its market value as a raw material for products requiring weak gluten, with strength properties, and as an improver for strong, tenacious gluten flours (Solís *et al.*, 2020).

Producer Characteristics

Surveys were conducted in the municipalities of Cortázar (2%), Cuernavaca (1%), Irapuato (41%), Pénjamo (1%), Salamanca (36%), and Valle de Santiago (18%), with 53 surveys corresponding to C S94 and 31 to F S2016. The average age of producers cultivating C S94 was 67 years, while for F S2016 it was 57 years. The educational level of producers was primarily elementary education, with 52% for C S94 and 32% for F S2016; secondary education accounted for 15% and 42%, respectively. Additionally, 10% of F S2016 producers had completed high school, and 13% held a university degree.

The average area dedicated to wheat production was 5.80 ha for C S94 and 12.5 ha for F S2016, with a minimum of 0.7 ha and a maximum of 45 ha for C S94, and a minimum of 1.3 ha and up to 104 ha for F S2016. Both cultivars are grown under irrigated conditions. C S94 is cultivated with 56% gravity irrigation and 44% pumping irrigation, whereas 97% of F S2016 is grown using pumping irrigation.

Cost Structure of Cortázar S94 and Faisán S2016 cultivars

The production costs of C S94 and F S2016 in Guanajuato were different and statistically significant at $p < .0001$ for land preparation, sowing, and irrigation, with higher costs associated with F S2016 production. Fertilization was the only cost category significantly higher for C S94 compared to F S2016, with a value of \$9,369, which is \$149 more than the amount reported for wheat production in Sonora (Retes *et al.*, 2022), which was \$9,220 (Table 1).

The concepts of disease control and harvesting showed no significant differences. The variable Total Cost (TC) of F S2016 was significantly higher ($p < .0001$) than that of C S94 and represents 95% of the total production costs for both cultivars. This total cost was

significantly higher for F S2016 than for C S94 by more than \$5,000 (Table 1) and lower than the amount reported which was \$29,903.

The percentage structure of production costs for C S94 and F S2016 shows that the highest percentage was allocated to fertilization for both cultivars, although higher in C S94. In the case of F S2016, irrigation stands out as the second most significant cost, followed by land preparation. Regarding the control of weeds, pests, and diseases, the percentages do not represent more than 6% of the total production cost (Figure 1).

In terms of variable and fixed costs, the highest percentage corresponds to variable costs, which are directly related to the production volume, such as the amount of labor inputs and mechanized operations. Fixed costs represent a smaller percentage and only

Table 1. Production Costs, Cortázar S94 and Faisán S2016 in Guanajuato.

Concept/Statistical	Average		SD		Prob>F
	VTC	VTF	VTC	VTF	
Land preparation, \$/He	4,156 ^b	6,119 ^a	226	298	<.0001*
Planting, \$/He	3,758 ^b	4,477 ^a	94	124	<.0001*
Fertilization, \$/He	10,741 ^a	9,370 ^b	300	396	0.0070*
Weed control \$/He	1,260 ^b	1,604 ^a	65	84	0.0018*
Pest control, \$/He	692 ^b	816 ^a	35	47	0.0153*
Disease control, \$/He	1,075	1,062	53	78	0.8891
Irrigation, \$/He	4,445 ^b	6,932 ^a	226	298	<.0001*
Harvest, \$/He	2,157	2,337	105	138	0.3024
Total variable cost, \$/He	2,5806 ^b	3,1470 ^a	661	872	<.0001*
Fixed costs, \$/He	1,436 ^b	1,732 ^a	45	60	0.0002*
Total costs, \$/He	2,7242 ^b	3,3202 ^a	684	902	<.0001*
Average variable cost, \$/He	4,097	4,585	102	134	0.0049*
Average fixed cost, \$/He	225	249	5	7	0.0066*
Unit production cost, \$/Ton	4,322	4,834	103	136	0.0035*

Levels not connected with the same letter are significantly different from $p \leq 0.01$. Source: Own elaboration with information from a sample of 85 surveys applied in 2022.

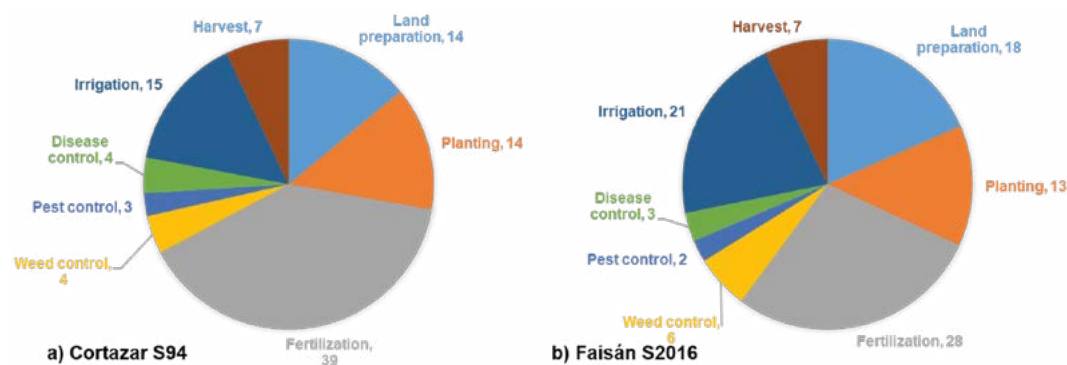


Figure 1. Cost structure as a percentage for Cortázar S94 and Faisán S2016 in Guanajuato, Mexico. Source: Elaborated by the author with field data, 2022.

include the cost associated with the payment that should be allocated to the administrator, an activity performed by the producer.

The average variable and fixed costs allow for visualization of how much of these costs were allocated to wheat production. The sum of both results in the average total cost per ton per hectare. The UPC allows for direct comparison with the selling price and quick estimation of the profit per ton sold at that price for each producer. In this comparison, it was significantly different, but only by \$512.00 (Table 1). In aggregated production costs, it is not possible to observe the components that integrate them, such as the quantities of activities, inputs, and labor that are part of production. Therefore, the main cost components carried out in their production units by wheat producers in the state of Guanajuato are presented separately.

In land preparation, mechanized activities are carried out, such as subsoiling, fallowing, tracking, cross-tracking, making embankments, and leveling; of these, only the embankments are carried out by more than 50 producers of WCC S94, followed by tracking, and only one producer reported performing other mechanized activities (Table 2). In contrast to C S94, in F S2016, tracking is done by all 31 producers in the sample, and only 10 do cross-tracking (Table 2). Regarding the average unit cost for both cultivars, fallowing is the most expensive activity for both C S94 and F S2016, but it is only carried out by 23 producers.

In land preparation, mechanized activities are carried out, such as subsoiling, fallowing, tracking, cross-tracking, making embankments, and leveling; of these, only the embankments are carried out by more than 50 producers of C S94, followed by tracking, and only one producer reported performing other mechanized activities (Table 2). In contrast to C S94, in F S2016, tracking is done by all 31 producers in the sample, and only 10 do cross-tracking (Table 2). Regarding the average unit cost for both cultivars, fallowing is the most expensive activity for both C S94 and F S2016, but it is only carried out by 23 producers.

The amount of labor used in production is similar for both cultivars, as is its unit cost. On average, one day of labor is used for planting, pest, disease, and weed control in C S94, while it differs by an additional day of labor for fertilization in F S2016. Three of

Table 2. Mechanized activities of Cortázar S94 and Faisán S2016 in Guanajuato.

Concept	Cortazar S94				Faisán S2016			
	n	Act. Average	Costo/Act.		n	Act. Average	Costo/Act.	
			Average	SD			Average	SD
Subsoil	14	1	1,162	465	17	1	1,221	412
Fallow	35	1	1,843	347	23	1	1,961	422
Tracking	49	2	921	236	31	2	996	232
Cross tracking	15	1	943	140	10	1	1,020	193
Boards	52	1	710	200	28	1	896	274
Pair	12	1	767	267	23	1	752	217
Other labors	1	1	600		18	1	750	226

Source: Elaborated by the author with field data, 2022.

the 54 interviewed producers used labor for harvesting, and none did so for the harvest of F S2016 (Table 3). The highest unit labor costs were reported by producers of F S2016, reaching up to 800 pesos for pest and disease control, which were lower for C S94.

The inputs used were seeds, fertilizers, pesticides, and herbicides, of which the amounts of fertilizers for both cultivars, both in the first and second fertilization, were more than 300 kg, with maximum amounts of up to 800 kg, applied by more than 90% of the producers of both cultivars. The unit cost of the seed was higher for F S2016, and the minimum and maximum prices for the disease control products were the same for both cultivars (Table 4).

The yield obtained was significantly higher for F S2016, as was the selling price. However, the net income or profit earned by the producers was 447 pesos less than that obtained from the sale of C S94, and no statistically significant differences were found (Table 5). Gross income was significantly different, a situation that changes when calculating the profit or net income, which already deducts the TPC.

Table 3. Labor employed in the production of Cortázar S94 and Faisán S2016 in Guanajuato.

Concept (Wages)	Cortazar S94				Faisán S2016			
	n	Wages Average	Cost/Wag.		n	Wages Average	Cost/Wag.	
			Average	SD			Average	SD
Planting	52	1	276	55	31	1	274	43
Fertilization	54	1	279	55	31	2	263	46
Irrigation	54	4	410	91	31	4	460	92
Weed control	52	1	295	72	31	1	358	158
Pest control	50	1	279	81	31	1	300	127
Disease control	24	1	295	53	11	1	277	82
Harvest	3	7	343	12	-	-	-	-
Other	14	1	289	49	16	1	284	96
Administration	54	1	1,313	213	31	1	1,478	284

Source: Elaborated by the author with field data, 2022.

Table 4. Inputs used in the production of Cortázar S94 and Faisán S2016 in Guanajuato, Mexico.

Concept Kg o L ha ⁻¹	Cortazar S94				Faisán S2016			
	n	Units Average	Cost/Uni		n	Units Average	Cost/Uni	
			Average	SD			Average	SD
Seed	54	39	11	2	31	36	16	3
1 st fertilization	54	357	15	3	31	317	12	5
2 nd fertilization	54	385	13	3	31	403	13	4
Pest and weed control	49	1	334	54	30	1	409	234
Disease control	23	1	793	143	11	1	760	276
Irrigation (amount)	54	4	747	195	31	4	1,318	583

Source: Elaborated by the author with field data, 2022.

Table 5. Yield and profit of Cortázar S94 and Faisán S2016 in Guanajuato, Mexico.

Concept/Statistical	Unit	Average		SD		Prob>F
		C S94	F S2016	C S94	F S2016	
Planting area	He	5.8 ^b	13 ^a	1.8	2.4	0.026*
Yield	Ton	6.36 ^b	7.022 ^a	0.2	0.2	0.0097*
* Price	\$/Ton	6,868 ^b	7,018 ^a	31.3	41	0.0050*
Gross Income	\$/Ton	43,767 ^b	49,280 ^a	1,094	1,443	0.0031*
Net income	\$/Ton	16,525	16,078	894	1,179	0.7634

The levels not connected by the same letter are significantly different at $p \leq 0.01$. *Current prices. Source: Prepared by the author with data from a sample of 85 surveys conducted in 2022.

CONCLUSIONS

The cultivar Faisán S2016 showed higher yield, better price, and higher production costs than Cortázar S94. However, the net income obtained from the production of both cultivars was statistically the same, which may imply that the producer may choose to produce both cultivars interchangeably. The production cost structures as percentages were similar for both cultivars, as well as the percentages of variable and fixed costs, and the quantity and unit costs of mechanized activities, inputs, and labor.

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Milk Production in Dual-Purpose Cattle Systems under a Humid Subtropical Climate Af(c)

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ABSTRACT

Objective: To evaluate the effects of environmental factors year, season, and parity on milk production and lactation duration in Holstein×Zebu and Brown Swiss×Zebu cows in a humid subtropical region.

Design/methodology/approach: Data from 2010 to 2021 were analyzed from the dual-purpose production unit El Paraíso de Ayototxco, located at 240 meters above sea level in the municipality of Ayototxco de Guerrero, Puebla, under a humid subtropical climate Af(c). The studied traits included total milk yield (TMY), daily milk yield (DMY), milk yield per day of calving interval (MYDCI), and lactation duration (LD). Data were analyzed using the GLM procedure in SAS. The final model included year, season, and parity as fixed effects.

Results: A total of 934 lactations were evaluated, with an average LD of 339.65±94.75 days and a TMY of 3,392.6±1,185.9 kg. The average DMY and MYDCI were 9.94±1.99 kg and 7.43±2.36 kg, respectively. Year and parity significantly affected all traits (P<0.05), whereas the calving season was significant only for MYDCI. The parity effect showed that first-lactation cows had lower TMY, DMY, and MYDCI, while cows with more than three calvings exhibited the highest performance in these traits. The average values for TMY, DMY, MYDCI, and LD in the validation module were 3,392.6 kg, 9.94 kg, 7.43 kg, and 339.65 days, respectively. A productive improvement was observed over the years, attributed to the adoption of new technologies.

Findings/conclusions: Year and parity significantly influenced all analyzed variables, while calving season affected only MYDCI.

Keywords: Validation, adoption, lactation, Holstein×Zebu.

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INTRODUCTION

In Mexico, the bovine population is approximately 35,998,885 head, of which, according to data from the past decade, dual-purpose cattle aged two years or older account for 28.4% of this category, equivalent to 2,466,477 head. Additionally, Mexican



livestock farming utilizes approximately 110 million hectares, 28.3% of which corresponds to tropical areas, which are predominantly covered with forage used for meat and milk production in dual-purpose systems. These systems are primarily developed in tropical regions and have historically been characterized by low milk production (Calderón *et al.*, 2016).

Cattle farming in Mexico faces an increasingly critical situation. There has been a milk production deficit since the 1970s, resulting in the importation of approximately 20% of the annual national consumption. This crisis is further exacerbated by the rising costs of inputs compared to the low prices of livestock products (meat and milk), a trend particularly evident in the tropical and subtropical regions of the country. These regions contribute approximately 20% of the national milk production (González, 1993; SADER, 2023), mainly through traditional systems with low technological adoption. These systems often involve indefinite crossbreeding between *Bos taurus* and *Bos indicus* cattle (Koppel *et al.*, 2002).

In tropical regions, milk and meat production depend on the season of the year, with the highest production occurring during the rainy season. Although productivity indicators remain low, studies on dual-purpose livestock in the tropics have shown the feasibility of improving prospects for increasing milk and meat production in Mexico (CIPEP-INIFAP-SARH, 1989a, 1989b).

In response to these challenges, the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) has conducted applied research by establishing projects in the tropics to develop and evaluate technologies. This has led to the creation of technological packages to enhance the efficiency and competitiveness of dual-purpose production systems (CIPEP-INIFAP-SARH, 1989a).

The dual-purpose production unit 'La Doña' operates with Holstein×Zebu, American Brown Swiss×Zebu, and Simmental×Zebu cattle under grazing conditions at the Las Margaritas experimental site. This unit has achieved an average milk production per lactation of 3,190 kg over 322 days, a calving interval of 446 days, an average calf weight of 293 kg at one year of age, and heifers reached first calving at an average age of 29.7 months and a weight of 443 kg (SAGARPA-INIFAP-CIPEP A.C., 2004).

In summary, the adoption of various technologies has contributed to increased milk production. The Las Margaritas experimental site in Puebla, established in 1981, has been dedicated to generating and validating technologies to improve dual-purpose production systems. These efforts aim to address the technological demands of producers and enhance their productivity (SAGARPA-INIFAP-CIPEP A.C., 2004). Validated technologies have been transferred to producers, and a demonstration module was established with a collaborating farmer in 2008. This initiative sought to implement a dual-purpose production unit as a validation and demonstration model based on technologies developed at La Doña. Initially, over 50% of the recommended technologies were adopted, with gradual increases over time. By 2017, a newly implemented technology, fertilizing pastures with a recommended formula (138:69:00), led to increased milk production and an increase of 2.4 times in yield per unit of land. Another significant technological advancement has been achieving the recommended proportion of European genetic influence in the herd, ranging

between 3/4 and 5/8, as suggested in the technology package. Currently, it is estimated that over 80% of the recommended technologies have been adopted in this production unit.

Therefore, the objective of this study was to evaluate the effects of selected environmental factors such as year, season, and parity on milk production traits (total milk yield per lactation (TMY), daily milk yield (DMY), milk yield per day of calving interval (MYDCI), and lactation duration (LD)) in Holstein×Zebu and Brown Swiss×Zebu cows at the El Paraíso de Ayotoxco production unit in Puebla's humid subtropical region.

MATERIALS AND METHODS

Study Area

Data from 2010 to 2021 were collected from the dual-purpose technology validation module El Paraíso de Ayotoxco, located at coordinates 19° 59' 54" to 20° 08' 48" N and 97° 21' 18" to 97° 27' 42" W, at an altitude of 240 meters above sea level in the municipality of Ayotoxco de Guerrero, within the Sierra Oriente region of Puebla, Mexico. The climate of the study area is classified as humid subtropical Af(c), with an average annual temperature of 23 °C and mean annual rainfall ranging between 2,200 and 2,500 mm. Three distinct seasons can be identified: 1) Rainy season, which occurs from June to October and is characterized by heavy rainfall; 2) Windy season, which runs from November to March and is characterized by drizzle and a drop in temperature; and 3) Dry season, in April and May, which experiences isolated showers and an increase in temperature.

Animals and Management

The females included in this study are *Bos taurus taurus* crossed with *Bos taurus indicus* (ranging from 3/4 to 5/8 European) and belong to a validation module covering a total area of 158 hectares (ha). Of this area, 100.5 ha are planted with grasses of the *Brachiaria* genus, predominantly *Brachiaria brizantha* (Insurgente). Within this pasture, 49 ha are allocated to milking cows, 23 ha to dry cows, 16 ha to heifers under reproductive management, 8.5 ha to developing heifers, and 4 ha to nursing calves. Additionally, 54.5 ha are covered with native grasses (*Axonopus* and *Paspalum* spp.), where 50 ha are used for heifers under reproductive management, 3 ha for developing heifers, and 1.5 ha for weaned calves. Lastly, 3 ha are cultivated with Japanese sugarcane (*Saccharum sinense*), which is used as supplemental forage. During the critical season, each female received a daily ration of 15 to 20 kg of freshly chopped Japanese sugarcane (*Saccharum sinense*). Furthermore, milking cows were provided with 2 kg of concentrate (16% CP and 70% TDN) during each milking session. All females had *ad libitum* access to a mineral supplement and water.

Milking cows, dry cows, and developing heifers undergo rotational grazing, with rest periods of 30 and 45 days during spring-summer and autumn-winter, respectively. Since October 2017, pasture fertilization has been carried out using the formula 138-69-00 kg/ha of N and P in the paddocks designated for lactating cows and nursing calves. The diet of dry cows consists of pasture consumed directly from the field, along with *ad libitum* access to mineral salts and water. Additionally, from November to May, they receive 15 to 20 kg/animal/day of freshly chopped Japanese sugarcane. Calf nutrition includes pasture grazing, supplemented with milk obtained from one-quarter of the udder plus residual milk, until

they reach a body weight of 100 kg. After this point, they consume only supplemental and residual milk, along with 850 g/day of concentrate (18% CP) throughout the rearing period. Weaned heifers are managed under a rotational grazing system and receive 1.5 kg/animal/day of concentrate (18% CP), along with *ad libitum* mineral salts and water. Developing heifers, until they reach the reproductive management stage (RMS), are also managed under rotational grazing, with their diet consisting of pasture, mineral salts, and water available *ad libitum*. Reproductive management in cows follows a continuous mating system with natural mating. Estrus detection is conducted in the morning and afternoon during milking, and rectal palpation is performed every three months.

Heifers begin reproductive management at approximately 330 kg and remain in continuous mating system with natural mating. Similarly, rectal palpation is performed every three months.

Cows began milking four days after calving, once it was confirmed that they no longer produced colostrum. Milking was performed mechanically with the “assistance” of the calf. The calf was used to stimulate milk letdown until it reached approximately 11.5 months of age, at which point it was weaned. Milking was conducted twice daily using a mechanical system equipped with a two-stall setup powered by an electric and/or gasoline engine. The reproductive management of cows followed a continuous mating system with natural mating.

Design and Experimental Unit

The studied traits were: total milk yield per lactation (TMY), daily milk yield (DMY), milk yield per day of calving interval (MYDCI), and lactation duration (LD).

Statistical Analysis

The traits were analyzed using the GLM procedure in the SAS software. The fixed effects included in the final model were year, season, and parity number. Sequential analyses were performed for each trait to determine the final models, removing non-significant interactions from the preliminary model at $P < 0.05$.

Evaluated Variables

Lactation milk yield was measured using daily production records for each cow, obtained with Waikato-type commercial milk meters installed in each stall of the milking parlor. These records were collected from the beginning to the end of lactation. The start and end dates of lactation were recorded to determine lactation duration in days. Daily milk yield (milk yield per day in lactation) was calculated by dividing the total milk production per lactation by the lactation duration. Finally, milk yield per day of calving interval was determined using calving date records for each cow. The total milk production per lactation was divided by the number of days in the inter-calving period to obtain this value.

RESULTS AND DISCUSSION

The descriptive statistics for the different traits are presented in Table 1. A total of 934 lactations were evaluated, with an average LD of 339.65 ± 94.75 days and TMY of

Table 1. Descriptive statistics for milk production traits.

Variable	N	Mean	SD	Min	Max	CV
LD ^A	934	339.65	94.75	120.00	727.00	27.90
TMY ^B	933	3392.56	1185.94	787.50	7926.00	34.96
DMY ^C	933	9.94	1.99	4.37	17.24	19.98
MYDCI ^D	814	7.43	2.36	1.26	14.92	31.73

^ALD=Lactation duration, ^BTMY=Total milk yield per lactation, ^CDMY=Daily milk yield, ^DMYDCI=Milk yield per day of calving interval.

3,392.56±1,185.94 kg. DMY and MYDCI averaged 9.94±1.99 kg and 7.43±2.36 kg, respectively. According to a previous study (Calderón *et al.*, 2016), LD was the same; however, the other traits showed lower values (TMY=2,905 kg, DMY=8.5 kg, and MYDCI=6.6 kg). This improvement can be attributed to the increasing adoption of technology developed by INIFAP within the demonstration module, which has consequently enhanced productive indicators.

Considering the overall means from the present study and the previous one (Calderón *et al.*, 2016), LD was higher (340 days) than the values reported in other studies conducted in southeastern Mexico, which range from 214 to 255 days (Hernández *et al.*, 2001; Parra *et al.*, 2005; Magaña *et al.*, 2006; Magaña *et al.*, 2016). Similarly, TMY reported by these authors ranged from 1,322 to 1,862 kg, which is lower than the values obtained in both the present study and the previous one (Calderón *et al.*, 2016). Consequently, DMY in this study (9.94 kg), despite the longer lactation period (340 days), also exceeded the 5.81 kg reported by Parra *et al.* (2005) and the 6.71 kg documented by Magaña *et al.* (2016). Notably, MYDCI also surpassed the values reported by these authors.

The effects of year and parity number were significant in all models, whereas calving season was significant only for MYDCI. This finding aligns with previous reports (Vite *et al.*, 2007; Simón *et al.*, 2010), which found significant differences ($P<0.05$) between lactations. Except for MYDCI, no other significant associations were found between calving season and the evaluated production traits. Least squares means and their standard errors for milk production traits are presented in Table 2, Figure 1, and Table 3.

Table 2. Least squares means and standard errors for productive traits by parity number.

Parity Number	Trait			
	LD ^A	TMY ^B	DMY ^C	MYDCI ^D
1	342.14±6.43 ^{ab}	2908.04±76.54 ^b	8.40±0.11 ^c	5.91±0.14 ^c
2	347.59±6.56 ^{ab}	3415.24±78.09 ^a	9.75±0.11 ^b	7.15±0.15 ^b
3	355.40±7.37 ^a	3673.37±87.74 ^a	10.31±0.12 ^a	7.79±0.17 ^a
4	341.39±8.77 ^{ab}	3454.35±104.44 ^a	10.05±0.15 ^{ab}	7.68±0.20 ^{ab}
>5	326.76±7.20 ^b	3382.21±85.83 ^a	10.31±0.12 ^a	7.95±0.17 ^a

^{a,b,c} Means within the same column with different superscripts differ significantly ($P<0.05$). ^ALD=Lactation duration, ^BTMY=Total milk yield per lactation, ^CDMY=Daily milk yield, ^DMYDCI=Milk yield per day of calving interval.

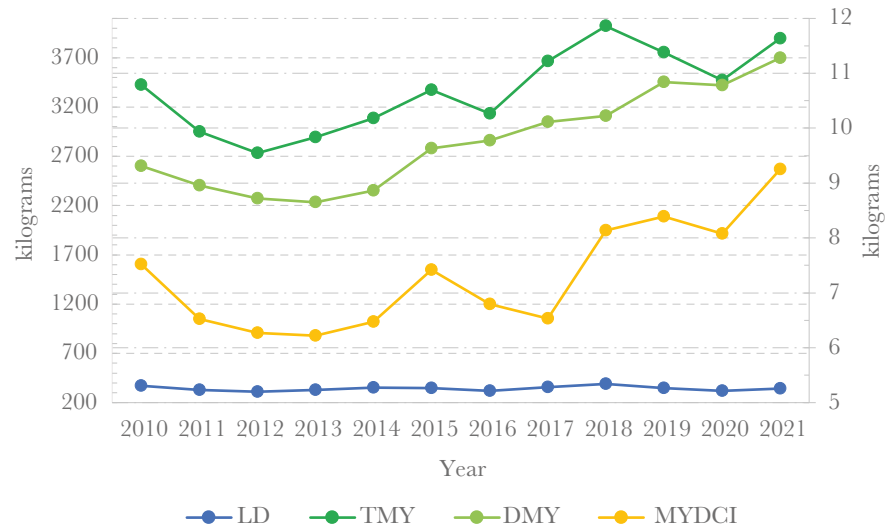


Figure 1. Productive traits (LD=Lactation duration, TMY=Total milk yield per lactation, DMY=Daily milk yield, MYDCI=Milk yield per day of calving interval) by calving year.

As shown in Table 2, total milk yield (TMY), daily milk yield (DMY), and milk yield per day of calving interval (MYDCI) were lowest during the first lactation and increased progressively with each subsequent lactation. Cows with more than three lactations showed the highest milk yield. In contrast, lactation duration (LD) decreased as the number of lactations increased, with the shortest duration observed in cows with more than five lactations. Calderón *et al.* (2016) reported no significant differences in LD and TMY but found results consistent with the present study for DMY and MYDCI. Except for LD, all other traits reported lower values compared to the present study, with peak production observed during the third lactation (TMY=3,006 kg, DMY=9.13 kg, and MYDCI=6.8 kg). Similar trends have been observed in previous studies, such as López *et al.* (2009), who also reported lower milk yield in the first lactation compared to subsequent ones. Furthermore, milk production reported in both South American studies (Santana *et al.*, 2022) and research from southeastern Mexico (Magaña *et al.*, 2016) was lower than in the present study.

The effect of calving year (Figure 1) was significant ($P<0.05$) for all evaluated traits. A steady increase in total milk yield (TMY), daily milk yield (DMY), and milk yield per day of calving interval (MYDCI) was observed over the years. This trend became more evident after 2017, coinciding with the implementation of fertilization technology. In contrast, lactation duration (LD) fluctuated from year to year (ranging from 310 to 390 days), however, these variations did not negatively affect DMY or MYDCI.

Table 3 shows that cows calving during the rainy season had a significantly higher ($P<0.05$) MYDCI (7.64 kg) compared to those calving during the windy season (7.34 kg) and the dry season (6.92 kg). Similar to this study, lactation duration (LD) was not significant in a previous study (Calderón *et al.*, 2016). However, that study did report differences in total milk yield (TMY), with lower values during the rainy season, a pattern not observed in the present study, although the numerical trend remained similar.

Table 3. Least squares means and standard errors for productive traits by season of measurement.

Season	Trait			
	LD ^A	TMY ^B	DMY ^C	MYDCI ^D
Rainy	337.17±4.75	3355.13±56.59	9.87±0.08	7.64±0.11 ^a
Windy	344.03±4.99	3365.10±59.46	9.78±0.08	7.34±0.12 ^{ab}
Dry	346.77±7.95	3379.69±94.70	9.63±0.13	6.92±0.18 ^b

^{a,b} Means within the same column with different superscripts differ significantly ($P < 0.05$). ^ALD=Lactation duration, ^BTMY=Total milk yield per lactation, ^CDMY=Daily milk yield, ^DMYDCI=Milk yield per day of calving interval.

CONCLUSIONS

Calving year and parity significantly affected all evaluated variables, while calving season influenced only MYDCI. The effect of lactation number indicated that first-lactation cows had lower TMY, DMY, and MYDCI, whereas cows with more than three calvings exhibited the highest performance in these traits. The mean values for TMY, DMY, MYDCI, and LD in the evaluated validation module were 3,392.56 kg, 9.94 kg, 7.43 kg, and 339.65 days, respectively. A productive improvement was observed compared to earlier years, primarily attributed to the adoption of new technologies, particularly pasture fertilization, and an increased proportion of European genetics, ranging between 3/4 and 5/8 European.

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Production and quality of cassava (*Manihot esculenta* Crantz) organically and chemically fertilized and intercropped with sapodilla

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ABSTRACT

Objective: To evaluate the yield and quality of organically nourished cassava (*Manihot esculenta* Crantz) produced in an intercropping system with sapodilla.

Design/methodology/approach: A cassava crop was established in Veracruz, Mexico, under a completely randomized block design in split-plot arrangements. The large plot corresponded to sapodilla densities (156 and 312 trees/ha), and the small plot to mineral and organic fertilization and a control. The variables measured were: total plant height, aerial and root biomass, number of roots per plant, external and internal root color, average root diameter and length. Additionally, starch content was estimated by the specific gravity method.

Results: The results show that at low density, better-quality cassavas are obtained, and average yields of 13 roots/plant with an average total weight of 2.914 kg and a starch content of 29.58%. On the other hand, organic and mineral fertilization showed similar behaviors with the exception of root diameter, luminosity, internal hue angle and starch content, where organic fertilization showed values of 34.49 mm, 85.67, 87.36° and 29.71%, respectively.

Limitations on study/implications: The results obtained can be applied in tropical regions with vertisol soils with a sandy crumb texture with dark color, pH of 6.5 and good surface drainage; they also serve as a basis for further exploring these production systems.

Findings/conclusions: It is concluded that planting density and nutrition influence the quality and yield characteristics of cassava.

Keywords: Mandioca, Bokashi, Production systems.

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INTRODUCTION

Cassava is a food of great nutritional importance. Around 330 million tons are harvested annually worldwide (Food and Agriculture Organization of the United Nations [FAO], 2024), making it the fourth staple food in the diet of 1 billion people, primarily those with limited resources (Díaz & López, 2021). The crop has grown since 2000, with a faster rate in Africa and Asia than in the Americas, where it originated, resulting in an annual per



capita consumption of 60 kg by 2013 (Díaz & López, 2021; Howeler *et al.*, 2013; Suárez & Mederos, 2011). In Mexico, 24,000 tons are produced across seven states (Agricultural and Fisheries Information System [SIAP], 2024), with the roots primarily intended for food purposes.

Cassava is characterized by having a root capable of storing large amounts of starch, which can be harvested from eight months to three years after planting without losing its nutritional qualities (Díaz & López, 2021; Salvador *et al.*, 2014). This crop is consumed fresh, roasted, boiled, or fried, but it is primarily consumed as flour (Salvador *et al.*, 2014). This makes starch content an important parameter when defining the quality of this root.

Cassava cultivation is easy to manage, as it shows good tolerance to drought and low-fertility soils, making it accessible to subsistence farmers (Laranjeira *et al.*, 2023). However, it should not be overlooked that proper management can impact the quality and yield of cassava, such as genotype selection, fertilizer use, and the design of the production system to be employed (Laranjeira *et al.*, 2023; Souza *et al.*, 2024). Additionally, it should be considered that there is a continuous search at the international level for sustainable management practices that minimize environmental impact while supporting food security for families (Howeler *et al.*, 2013).

In Mexico, cassava is primarily cultivated in marginal areas and small plots, particularly in the state of Veracruz, where the average regional chemical nutrition is 146-46-60 kg of NPK/ha (Durán *et al.*, 2023). However, it is unknown how the use of alternative nutrient sources and intercropping with fruit trees may influence its yield and quality. Therefore, the objective of this study was to evaluate the yield and quality of cassava (*Manihot esculenta* Crantz) organically nourished and produced in an intercropping system with sapodilla.

MATERIALS AND METHODS

The experiment was conducted in the intercropping system orchard with sapodilla at the Cotaxtla Experimental Field of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) in Medellín, Veracruz, Mexico. The orchard is located at the geographic coordinates 18° 56' 12" north latitude and 96° 11' 32" west longitude, at an altitude of 16 meters (Google Earth, 2024). The region is characterized by an average annual precipitation of 1350 mm and an average annual maximum and minimum temperature of 31.8 °C and 21.8 °C, respectively (Martínez-Ruiz *et al.*, 2017). The soil of the orchard is vertisol with a sandy crumb texture and dark color, a pH of 6.5, and good surface drainage (Alemán, 2018).

The cassava crop (*Manihot esculenta* Crantz) of the San Andrés native variety was evaluated in an intercropping system with sapodilla (*Manilkara zapota* (L.) P. Royen) of the Betawi variety. The sapodilla was established in 2005 at two planting densities of 156 and 312 trees/ha, with spatial arrangements in real frame at distances of 8×8 m and 8×4 m, respectively. The sapodilla trees are maintained with crown pruning at a 2 m radius and 3 m height, leaving a 4 m space for intercropping.

In June 2023, a harrow was used in the available 4 m for intercropping, and three beds of 0.5 m in height were formed, with a center-to-center distance of 2 m. Cassava was

planted in the beds using cuttings of 30 cm in length, which were collected from another orchard within the same Experimental Field. The cuttings were planted at a 45° angle, in a staggered pattern, and at a density of 6600 plants/ha. The cassava was managed as a rainfed crop; however, due to the uneven precipitation throughout the cycle, supplementary irrigation was applied every third day from June 13 to July 19.

Nutritionally, the NPK dose of 146-46-60 was applied using the mineral fertilizers DAP, urea, and potassium chloride at planting, or with bokashi at planting and five applications of efficient microorganisms (EM) at a dose of 50 mL per plant every 15 days starting on October 11.

The cassava was harvested in January 2024, seven months after planting. For the development of this study, the organic fertilizers “bokashi” and “Efficient Microorganisms” (EM) were prepared, both of which are obtained through fermentation processes. The former is used as a solid fertilizer, and the latter as a liquid fertilizer.

To prepare the bokashi, chicken and cow manure, pangola grass, soil, and sand were used in volumetric proportions of 2:2:2:1:1. The materials were mixed with a 1.5% molasses solution until a moisture content of 75 to 80% was reached.

The moisture content was verified using the fist test, as described by Méndez and Viteri (2007). The process continued as outlined by Rebolledo *et al.* (2012). After the fermentation process, an analysis was conducted, which showed that the bokashi was characterized by a pH of 6.5, a C/N ratio of 8.18, and a composition of: 20.04% organic matter, 11.62% C, 1.42% N_{total}, 0.23% N_{nitrate}, 1.47% P₂O₅, 1.42% K₂O, 3.64% CaCO₃, 0.8% MgO, 1.02% Fe²⁺, 0.04% Zn²⁺, 0.00% Cu²⁺, 0.06% Mn²⁺, and 0.03% B²⁺.

The bio-input EM is a solution containing several beneficial microorganisms, so plant material with fungal strains must be collected from undisturbed areas. The preparation of 200 L of EM was carried out following the process of Rebolledo *et al.* (2012); the materials used were 2 kg of corn flour, 2 kg of bran, 0.5 kg of charcoal, and 1 kg of plant material with strains; the fermentation medium was a 10% molasses solution.

A completely randomized block design was used in a split-plot arrangement, where the main plot corresponds to the sapodilla planting densities with two levels, and the subplot corresponds to the nutritional management of cassava with three levels, with four replications. The experimental unit consisted of plots measuring 20 m². The resulting treatments can be seen in Table 1.

Table 1. Nutritional treatments evaluated in an intercropped cassava study with sapodilla.

Treatment	Sapodilla density plantation	cassava nourish
1	156 trees ha ⁻¹	0
2	156 trees ha ⁻¹	2 kg bokashi plant ⁻¹
3	156 trees ha ⁻¹	146-46-60 kg ha ⁻¹ of N P K
4	312 trees ha ⁻¹	0
5	312 trees ha ⁻¹	2 kg bokashi plant ⁻¹
6	312 trees ha ⁻¹	146-46-60 kg ha ⁻¹ of N P K

Regarding the evaluated variables, total plant height (m) was measured five months after establishment using a stadia rod from the base to the apex. The aboveground biomass (kg), consisting of stems, branches, and leaves, was weighed fresh with a hand scale accurate to the gram. The total root biomass (kg) of the sampled plants was also weighed in the field using a scale with gram precision, and the number of marketable roots per plant was recorded. These roots were then transported to the tropical fruit sample preparation laboratory at the Cotaxtla Experimental Field of INIFAP.

The internal and external color of the roots was measured using a WR10QC colorimeter. The L a b parameters were converted into hue and chroma values according to McGuire (1992). From the total roots per plant, a subsample of three representative roots was selected. The length (cm) and average diameter (mm) of these roots were determined, with the diameter measured at three points per root—upper, middle, and lower sections—using a vernier caliper.

The starch content was determined using specific gravity, a method employed to obtain the dry matter and starch content in roots through a standardized correlation (Aristizábal & Sánchez, 2007). This variable was measured according to the procedure described by Aristizábal and Sánchez (2007), where the selected roots were individually weighed both in air and submerged in water. The obtained values were applied in Equation 1.

$$SC = \frac{Pfr_{ai}}{Pfr_{ai} - Pfr_{ag}}$$

where SC is the specific gravity; Pfr_{ai} corresponds to the weight of the roots in air and Pfr_{ag} is the weight of the roots in water.

The percentage of dry matter was determined according to Equation 2.

$$\%MS = (SC \times 158.26) - 142.05$$

Finally, this value was used to calculate the percentage of starch with Equation 3:

$$\%AL = \%MS \times 0.875$$

where $\%AL$ is the starch percentage and $\%MS$ is the dry matter percentage (Aristizábal and Sánchez, 2007).

The statistical analysis was performed using Minitab 19 software. The assumptions of normality were verified with the Anderson-Darling statistic, autocorrelation was checked using the Durbin-Watson test, and homogeneity of variances was assessed with Bartlett's test for the studied variables. Subsequently, analysis of variance ($\alpha=0.05$) was performed, and mean comparisons were made using Tukey's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The studied variables met the statistical assumptions of normality, independence, and homogeneity of variance ($p > 0.05$). The descriptive statistics are shown in Table 2.

The results by factor of the studied variables, considering the planting density of sapodilla and the nutrition, are shown in Tables 3 and 4, respectively.

The aerial biomass is important in the livestock sector, where it has been considered a viable alternative to meet the forage demand that has been affected by climate variability (Gómez *et al.*, 2016). In the present study, the aerial biomass variable did not show

Table 2. Descriptive statistics of parameters evaluated in cassava nourished with different sources and intercropped with sapodilla established at two planting densities.

Parameter	Mean	SD	Mdn	Min.	Max.	Range	Kurtosis
Aerial biomass (kg/plant)	2.84	1.16	2.71	0.64	5.31	4.67	-0.56
Root quantity (number/plant)	10.75	4.36	10.00	4.00	23.00	19.00	-0.01
Root biomass (kg/plant)	2.43	1.16	2.35	0.56	5.50	4.94	0.35
Root length (cm)	30.85	10.87	30.10	9.50	73.00	63.5	0.94
Root diameter (mm)	32.91	5.15	33.05	16.37	43.97	27.6	0.29
External luminosity	41.02	3.09	41.38	32.85	48.51	15.66	0.16
External color saturation	21.91	1.89	21.45	18.10	27.24	9.14	0.16
External hue angle (°)	56.61	1.91	56.33	53.62	64.84	11.22	7.11
Internal luminosity	84.28	2.32	84.64	77.39	88.11	10.72	0.77
Internal color saturation	11.72	1.32	11.97	9.05	14.23	5.17	-0.73
Internal hue angle (°)	86.72	1.69	86.53	81.86	89.29	7.43	-0.01
Starch content (%)	26.85	8.09	29.57	14.18	44.58	30.4	-0.86

SD: standard deviation; Mdn.: median; Min: minimum; Max: maximum.

Table 3. Evaluated parameters in cassava grown as intercropped with sapodilla established at two planting densities.

Parameters	Low density ^a	High density
Aerial biomass (kg/plant)	3.31 a	2.36 b
Root quantity (number/plant)	13.11 a	8.38 b
Root biomass (kg/plant)	2.914 a	1.93 b
Root length (cm)	32.54 a	29.16 a
Root diameter (mm)	34.62 a	31.20 b
External luminosity	41.60 a	40.43 a
External saturation	22.66 a	21.15 b
External hue angle (°)	56.85 a	56.36 a
Internal luminosity	83.91 a	84.64 a
Internal saturation	11.51 a	11.93 a
Internal hue angle (°)	85.99 b	87.44 a
Starch content (%)	29.58 a	24.11 b

^a Different letters between columns indicate statistically significant differences between groups. Low density: 156 trees per hectare, High density: 312 trees per hectare.

Table 4. Parameters evaluated in cassava cultivated with different nutritional sources.

Parameter	Inorganic ^a	Organic	Control
Aerial biomass (kg/plant)	3.55 a	2.93 a	2.02 b
Root quantity (number/plant)	11.66 a	10.75 a	9.83 a
Root biomass (kg/plant)	2.63 a	2.37 a	2.26 a
Root length (cm)	32.83 a	32.84 a	26.87 b
Root diameter (mm)	31.52 b	34.49 a	32.72 ab
External luminosity	40.82 a	41.61 a	40.62 a
External saturation	22.41 a	21.82 a	21.50 a
External hue angle (°)	56.95 a	56.32 a	56.54 a
Internal luminosity	83.05 b	85.67 a	84.11 ab
Internal saturation	12.33 a	11.44 a	11.39 a
Internal hue angle (°)	85.83 b	87.36 a	86.96 ab
Starch content (%)	24.90 b	29.71 a	25.94 ab

^a Different letters between columns indicate statistically significant differences between groups. The nutrition treatment involved the application of 146-46-60 with Urea, DAP, and K₂Cl for the inorganic treatment, and bokashi with EM for the organic treatment. The absolute control had no additional nutritional sources other than those present in the soil.

interactions between plots ($p=0.9453$); however, it is observed that the sapodilla planting density ($p=0.0041$) and fertilization ($p=0.0027$) significantly affect this variable, producing a higher biomass when cassava is intercropped at a lower density and when a fertilizer is applied. The cassava crop intercropped with sapodilla at a density of 156 trees/ha shows an aerial biomass production of 21.8 t/ha. On the other hand, in the nutrition factor, the inorganic and organic treatments were statistically equal to each other and higher than the control, with an average production of 21.38 t/ha. The results obtained are higher than those reported by Gómez *et al.* (2016), who evaluated cassava at different densities, finding a production of 11.1 t/ha at a density of 70 thousand fresh aerial biomass of cassava, which was established in the Colombian Caribbean and harvested in the first months of the year. This difference is likely due to the fact that the cassava was harvested between 75 and 105 days.

The number of commercial roots did not show interactions ($p=0.1127$) or significant differences based on the nutrition factor ($p=0.4654$), but it did show significance regarding the sapodilla planting density ($p=0.0004$). The 152 trees/ha density presented a higher number of roots than the 312 trees/ha density (Table 3). The number of commercial roots found in this study (Table 2) is similar to those reported by Amara *et al.* (2024), who characterized a yam germplasm bank in Sierra Leona. The root biomass refers to the total yield of commercial roots. This variable did not show interaction ($p=0.4642$) nor was it significant regarding nutrition ($p=0.7018$), but it was significant in relation to its cultivation in an intercropping system at different densities ($p=0.0116$). The intercropped cassava cultivation in sapodilla at a density of 156 trees/ha yields 19.23 t/ha of commercial roots, while increasing the planting density of sapodilla results in significantly lower yields of 12.73 t/ha (Table 3). The plant weight obtained in the present study is lower than that

reported by Amara *et al.* (2024), which could be attributed to multiple factors such as genotype, planting density, planting-to-harvest time, and harvest season. However, it is similar to the findings by Cohelo *et al.* (2017), who, when studying the spatial distribution of cassava, found that simple row plantations intercropped with beans produced 2.29 kg/plant.

The root length was not affected by the interaction of the studied factors ($p=0.7275$) or by planting density ($p=0.0991$), but it was affected by the applied nutrition ($p=0.0252$). The roots of cassava plants fertilized with both organic and inorganic sources were similar to each other and superior to the control, which showed values 18% lower (Table 4).

Root diameter shows an interaction between factors ($p=0.0441$), where lower density and organic nutrition result in thicker roots. The factors planting density of sapodilla ($p=0.0002$) and nutrition ($p=0.0291$) also showed significance in the model. The mean comparisons indicate that when the density is higher, roots are 10% thinner than those at the lower density (Table 3); moreover, organic nutrition (Table 4) results in thicker roots compared to those fertilized with inorganic sources. This is likely because the organic fertilizer acts as a soil conditioner, providing better conditions for the roots to thicken more easily.

Regarding the external color, the lightness and hue angle were not affected by the factors studied ($p>0.05$). However, the color saturation of the cassava peel was significantly affected by the sapodilla planting density ($p=0.0178$). At the lowest density, there is higher saturation, meaning the color appears with greater purity.

In the internal part of the cassava root, the luminosity parameter is affected by the nutrient source ($p=0.0199$), and the hue angle is affected by both density ($p=0.0052$) and nutrition ($p=0.0397$), while other factors did not significantly affect these variables ($p>0.05$). The color saturation was statistically similar across all studied conditions ($p>0.05$). The results indicate that the shades of white in the pulp vary, with a higher value at the lowest density, while these values of 87° can be reached with organic nutrition. It is also evident that organic nutrition provides a brighter pulp compared to inorganic sources, which result in more opaque tones.

Starch content is the quality variable linked to the use of cassava for flour production, as it is a carbohydrate that primarily provides its chemical and mechanical qualities. The factors studied showed that their interaction significantly affects the starch ($p=0.0003$) present in the cassava, as well as density ($p>0.0000$) and nutrient source ($p=0.0104$). A lower density allows for cassava to be obtained with a higher starch percentage (Table 3). On the other hand, organic nutrition was similar to the control and was statistically superior to inorganic nutrition. The results obtained are consistent with the values reported by Aristizábal and Sánchez (2007), who provide reference values for starch content ranging from 17.5% to 39.4%.

CONCLUSIONS

It is concluded that planting density and nutrition influence the quality characteristics and yield of cassava. A lower planting density in an intercropping system with cassava allows for greater production of aerial biomass, root biomass, and starch. Organic nutrition is a

good option for cassava cultivation, as it provides the same benefits as inorganic nutrition in the morphological characteristics of cassava, but offers better qualities in starch content and internal coloration.

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Identification of Native Maize (*Zea mays* L.) within the Area of Influence of Pico de Orizaba National Park

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ABSTRACT

Objective: This study aimed to investigate the morphological diversity of native maize varieties from the area of influence of Pico de Orizaba National Park.

Design/Methodology/Approach: An exploration was conducted from December 2023 to May 2024 in the municipalities of Zongolica, Los Reyes, Astacinga, Atlahuilco, Tlaquilpa, Ixtaczoquitlán, Acultzingo, Amatlán de los Reyes, Chocamán, Huatusco, Calcahualco, Alpatlahuac, Mariano Escobedo, Rafael Delgado, Veracruz, Mexico. Quantitative characteristics of the ear were recorded, including ear length (EL), ear diameter (ED), number of rows (NR), number of grains per row (GPR), ear weight (EW), and cob diameter (CD).

Results: Ten original racial types were identified: Arrocillo, Cacahuacintle, Celaya, Cónico, Coscomatepec, Elotes Cónicos, Olotillo, Ratón, Tuxpeño, and Pepitilla. Among the interracial mixtures found: arrocillo-Cónico-Pepitilla-Tuxpeño, Celaya-Coscomatepec, Celaya-Coscomatepec-Ratón, Celaya-Elotes Cónicos, Celaya-Ratón, Cónico-Arrocillo, Cónico-Celaya, Cónico-Elotes Cónicos, Cónico-Pepitilla, Coscomatepec-Celaya-Pepitilla, Coscomatepec-Celaya, Olotillo-Tepecintle, Pepitilla-Cónico-Arrocillo, Ratón-Celaya, and Ratón-Tepecintle. The most frequent races were Cónico and Ratón. The most frequent native maize colors were white, cream, and yellow.

Limitations of the study/Implications: It is recommended to work with producers using participatory breeding tools for specific ecological niches.

Findings/Conclusions: The observed morphological diversity indicates a constant dynamism of seed exchange among maize producers who conserve this valuable resource.

Keywords: *Zea mays* L., biological diversity, conservation, traditional system, original races.



INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals worldwide. The primary destination of the grain is for direct human consumption, in addition to many derivatives used in industrial products (Najar *et al.*, 2018). Globally, the annual production volume of 1.163 billion tons produced on approximately 206 million hectares, with an average yield of 5,878.6 kg ha⁻¹ (FAOSTAT, 2023). Of the total grain produced, Mexico contributes about 2.3% (26.6 million tons), ranking it among the top ten countries with the highest production volume (SIAP, 2023).

On the other hand, there are more than 300 maize races in the world, most of which are cultivated on the American continent (Wellhausen *et al.*, 1951). Consequently, Mexico is considered a center of origin, diversification, and domestication of maize, resulting in approximately 59 native races (Ortega *et al.*, 2013). Specifically for the state of Veracruz, Sierra *et al.* (2014b) reported around 17 original races and 640 interracial mixtures, making it one of the states with the greatest biodiversity (CONABIO, 2011). The regions with the greatest presence of native maize include Los Tuxtlas, the high and low Sierra of Zongolica, Huatusco, Ixtaczoquitlán, Coscomatepec, Mariano Escobedo, Acultzingo, Tuxpan, Papantla, among other municipalities (Sierra *et al.*, 2014b).

The majority of studies focused on the diversity of native maize in Mexico have concentrated primarily in the central-southern region of the country (Aragón-Cuevas *et al.*, 2005; Herrera-Cabrera *et al.*, 2004; Hortelano *et al.*, 2011; Sierra-Macías *et al.*, 2014a; Flores-Pérez *et al.*, 2015; Guillén-de la Cruz *et al.*, 2014). There is still a need for more detailed exploration of the genetic-morphological variation within maize races, particularly in regions near Pico de Orizaba, highlighting the necessity for updated information on the diversity of these native maize varieties. Therefore, the objective was to study the morphological diversity and racial identification of native maize populations from the area surrounding Pico de Orizaba National Park.

MATERIALS AND METHODS

Location of the study area and germplasm used

Fifty native maize populations were used, preliminarily classified visually by researcher Mauro Sierra Macías. These were collected from December 2023 to May 2024 in the municipalities of Zongolica, Los Reyes, Astacinga, Atlahuilco, Tlaquilpa, Ixtaczoquitlán, Acultzingo, Amatlán de los Reyes, Chocamán, Huatusco, Calcahualco, Alpatlahuac, Mariano Escobedo, and Rafael Delgado. These municipalities are located within two important polygons: Pico de Orizaba National Park and the Metlác-Río Blanco Protected Natural Area (Figure 1), covering an area of 6,350.85 km² in the central-southern part of the State (19° 22' 41.29" N, 97° 19' 59.42" W and 18° 21' 53.57" N, 96° 28' 47.10" W; and an altitude ranging from 70 to 5,560 meters above sea level).

Evaluated traits

The sample consisted of 20 to 30 ears of maize, ensuring that no duplicates were obtained. A passport sheet was used as a basis to collect all the information of the cultivar, including the producer's name and the collection site. At each location, geographic

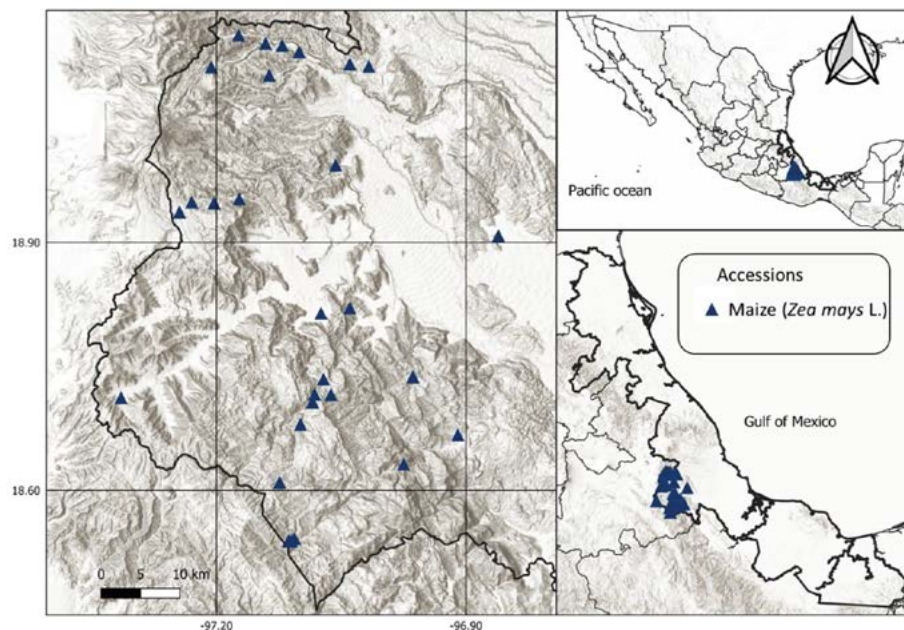


Figure 1. Origin of the 53 maize collections gathered in the area of influence of Pico de Orizaba National Park.

coordinates (latitude, longitude, and altitude), the common or local name of the cultivar, and the farmer's name were recorded. Information was collected on the quantitative and qualitative traits of the ear and grain. The following were quantified: ear length (EL), ear diameter (ED), number of rows (NR), number of grains per row (GPR), ear weight (EW), and cob diameter (CD).

Statistical Analysis

The generated data were integrated and systematized in a Microsoft Excel[®] spreadsheet. A principal component analysis (PCA) was performed using the evaluated variables. A graphical representation of the collections with the principal component one (PC1) and two (PC2) was created to visualize the differences and similarities among the 53 collections. All analyses were performed using the INFOSTAT statistical software, version 2008 (Di Rienzo *et al.*, 2008).

RESULTS AND DISCUSSION

Racial identification

The classification of the maize populations at the race level identified the following racial groups: Cónico Group (Figure 2), Early Maturing Tropical Maize Races (Figure 3), Tropical Dent Maize Group (Figure 4), and Late-maturing Maize Group (Figure 5). According to Sánchez *et al.* (2000), races are grouped into racial complexes, which in turn are associated with specific geographic and climatic distribution.

Within the racial groups, 10 original race types were identified: Arrocillo (Figure 2a), Cacahuacintle (Figure 2b), Cónico (Figure 2c), Elotes Cónicos (Figure 2d), Ratón (Figure 3), Celaya (Figure 4a), Tuxpeño (Figure 4b), Pepitilla (Figure 4c), Olotillo (Figure 5a),



Figure 2. Cónico Group. a) Arrocillo, b) Cacahuacintle, c) Cónico, d) Elotes Cónicos.



Figure 3. Early Maturing Tropical Group. Ratón Race.



Figure 4. Tropical dented group. a) Celaya, b) Tuxpeño, c) Pepitilla.



Figure 5. Late maturing maize group. a) Olotillo, b) Coscomatepec.

and Coscomatepec (Figure 5b). These races are consistent with those reported by Sierra-Macías *et al.* (2014) for the state of Veracruz. Similar studies by Temoxtle-Mellado *et al.* (2023) identified 10 native maize races for the Altas Montañas region of Veracruz.

Among the interracial mixtures found were: Arrocillo-Cónico-Pepitilla-Tuxpeño, Celaya-Coscomatepec, Celaya-Coscomatepec-Ratón, Celaya-Elotes Cónicos, Celaya-Ratón, Cónico-Arrocillo, Cónico-Celaya, Cónico-Elotes Cónicos, Cónico-Pepitilla, Coscomatepec-Celaya-Pepitilla, Coscomatepec-Celaya, Olotillo-Tepecintle, Pepitilla-Cónico-Arrocillo, Ratón-Celaya, and Ratón-Tepecintle. Some crosses involve more than three races; however, the Celaya and Cónico races are involved in most of them. Valadez *et al.* (2008) identified eight interracial crosses for the state of Tamaulipas, indicating that the distribution of the crosses follows a pattern similar to that of the races involved in them.

The most frequent races were Cónico (Figure 2c) and Ratón (Figure 3). The races with the least distribution were Arrocillo, Olotillo, and Tuxpeño. The predominant native maize colors were white, cream, and yellow. Homogeneous collections of blue, purple, black, and red grains were also observed (Figures 2a, 2d, 4a, and 5b).

In most cases, the collected races and their distribution correspond to those reported by Sierra *et al.* (2014b); however, it is important to note the presence of the Cacahuacintle race in the border region of Veracruz and Puebla (Figure 2b), given that this race is distributed in specific ecological niches in areas of the central Mexican highlands, Puebla, Tlaxcala, and Hidalgo, at altitudes higher than 2,000 meters above sea level (Flores-Hernández *et al.*, 2022). This suggests a strong exchange of seeds between producers from Veracruz and Puebla, as well as an interest in increasing their income through the revaluation of different forms of consumption such as pozole, cookies, pinole, atole, among others (Fernández-Aulis *et al.*, 2019).

Principal Component Analysis

The eigenvalues and eigenvectors of the principal component analysis showed that the first three principal components (PCs) explained 90% of the total variation, 42% for PC1, 29% for PC2, and 18% for PC3, respectively. The characters that most defined PC1 were LMZ (0.82), NGRAN (0.66), and PMZ (0.95). Previous studies report similar

CONCLUSIONS

There is broad morphological diversity, expressed in the different races reported here, which are conserved by producers in various traditional systems near Pico de Orizaba. Races with specific characteristics were found, such as Tuxpeño and Coscomatepec, which express a large ear size and are an important part of maize breeding programs at the national and global levels. Likewise, Cacahuacintle is adapted to more humid ecological niches than its place of origin. Finally, this diversity of races is attributed to different geographic, environmental, social, economic, and cultural factors in the agriculture practiced in the Las Montañas region of the state of Veracruz.

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