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Avifauna associated to a home garden in Valladolid, Yucatan, Mexico

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Biostimulant Effects of Glycine Betaine in Strawberry Exposed to Salinity Stress

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ABSTRACT
Objective: To evaluate the biostimulant effects of glycine betaine (GB) in strawberry plants subjected to salt stress.
Design/methodology/approach: A 2×3 factorial experiment was carried out with a completely randomized experimental design where the effects of foliar application of GB (0, 10, 20 mM) were evaluated in strawberry plants exposed to salt stress induced by NaCl (0 and 50 mM). Plant sampling was done 148 days after the establishment of the experiment. The biochemical and nutritional variables were measured. Analysis of variance was performed on the data obtained and the means were compared using the Tukey test ($p \leq 0.05$).
Results: GB application increased chlorophyll concentration, reduced sugar and proline concentration, and increased P uptake. The 20 mM GB dose maintained the K^+/Na^+ ratio under salt stress.
Study limitations/implications: The reproductive phase was not considered in this study; therefore, the effects of GB on fruit yield and quality are not assessed.
Findings/conclusions: Foliar application of GB in strawberry plants subjected to salinity stress is concluded to improve adaptive responses of a biochemical nature by promoting chlorophyll biosynthesis and nutrient concentration.

Keywords: Biostimulation, osmotic stress, strawberry, quality, resistance to abiotic stress.

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INTRODUCTION

Biostimulation is a technology that improves agronomic attributes related to crop productivity and quality; it also provides plants with better mechanisms to face environmental adversities of an abiotic nature, including drought and salinity stress. Glycine betaine (GB) has potential biostimulant effects on various horticultural crops (Adak, 2019). This non-ionic quaternary amine is synthesized in some plants as an adaptive response to stress situations, producing an osmoprotective effect at the cellular level, which is crucial for the protection of cellular structures and the regulation of vital biological processes (Ali *et al.*,

2020). In particular, GB prevents protein denaturation and changes in lipid fluidity, which allows maintaining the structural integrity of the membrane and ensuring its selective permeability (Adak, 2019; Zulfiqar *et al.*, 2022). GB can also enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as stabilize DNA and histones (Liu *et al.*, 2011). It also allows maintaining the functionality of some components of the photosynthetic apparatus in chloroplasts by protecting protein and lipid complexes, thus allowing greater photosynthetic efficiency even under adverse conditions (Sarikhani *et al.*, 2021; Zulfiqar *et al.*, 2022), and intervenes in cell signaling processes mediated by protein kinases that regulate the expression of abiotic stress response genes (Adak, 2019; Zulfiqar *et al.*, 2022).

The use of biostimulants such as GB contributes to sustainable agricultural production in restrictive environments aggravated by the effects of global climate change. Soil and water salinity is an increasingly worrying problem in agriculture, which can be exacerbates by excessive evaporation caused by extreme heat resulting from global climate change (Atta *et al.*, 2023).

With nearly 600 thousand tons of strawberries produced per year, Mexico is ranked as the country with the fourth highest production of this fruit, only after China, the United States, and Egypt (WPR, 2024). In Mexico, the main strawberry producing states are Michoacán, Guanajuato, Baja California, State of Mexico, and Baja California Sur (SIAP, 2021). Given the economic importance of this crop and the growing salinization problem affecting groundwater and soils in our country, there is a need to generate updated and detailed information on the application of biostimulants and the mechanisms they promote in plants to improve tolerance to osmotic stress and help maintain and even improve crop productivity and quality. The objective of this research was to evaluate the effect of foliar application of GB on strawberry plants subjected to salinity stress, in terms of biochemical and nutritional indicators.

MATERIALS AND METHODS

Location and experimental conditions

The experiment was carried out in a greenhouse in the Plant Nutrition area of the Graduate Program in Soil Science of the Colegio de Postgraduados Campus Montecillo, which is located at 19° 29’ North latitude, 98° 53’ West longitude, at 2250 m.a.s.l. The average temperature during the night was 12 °C, and 27 °C during the day. The relative humidity was 66.95 % and the light intensity during the day was 205 W m^{-2}.

Strawberry plants (*Fragaria*×*ananassa* Duch.) cv. Aromas were used to perform our study. This cultivar is day-neutral and shows better resistance to diseases (Muramoto, 2003; León-López *et al.*, 2014). The plants were established in 30×30 cm black nursery bags with red tezontle as substrate and watered by a stake-drip system. Irrigation was programmed in six 2-min intervals throughout the day, supplying 30 mL per plant.

Treatment design and experimental design

A 2×3 factorial experiment was conducted with a completely randomized experimental design. The study factors and levels were: A) Foliar glycine betaine (GB) at 0, 10, and

20 mM; and B) Salinity, induced by sodium chloride (NaCl) in the nutrient solution, at 0 and 50 mM.

Preparation and application of glycine betaine (GB)

Glycine betaine (Sigma-Aldrich; Toluca, Mexico) was used, with a betaine concentration of 98% and molecular weight=117.15 g mol^{-1}. GB was dissolved in distilled water with 0.1% Tween® 20 as surfactant; the pH was adjusted to 5.5 using 0.1 N NaOH. GB was applied in two foliar sprays, the first 20 d after the NaCl treatments began and the last 20 d before the end of the experiment.

Preparation and application of the nutrient solution plus NaCl

Fifteen days after transplantation, irrigation was started with Steiner nutrient solution at a 50% macronutrient concentration. One month after transplantation, the macronutrient concentration of the Steiner solution was increased to 100% and NaCl application was started to induce osmotic stress due to salinity. Throughout the experiment, the pH of the nutrient solutions was adjusted to 5.5; the electrical conductivity (EC) was 2.0 dS m^{-1} in the nutrient solution, and 5.5 dS m^{-1} in the solution with 50 mM NaCl. The nutrient solutions described were supplemented with the micronutrient concentrations in mg L^{-1}, as follows: 4.99 Fe, 2.33 Mn, 0.47 Zn, 0.43 B, 0.19 Cu, 0.17 Mo.

Variables evaluated

Chlorophyll concentration. Chlorophyll *a*, *b*, and total concentrations were determined using the modified Harborne method (1973). Absorbance was measured at 470, 652, and 665 nm in a spectrophotometer (BioTek®, Gen5™; Vermont, WI, USA), with 100% methanol used as a blank.

Proline quantification. The methodology of Bates *et al.* (1973) was used with modifications. Readings were performed in a spectrophotometer (VELAB VE-5600UV PC; Mexico City, Mexico) at 520 nm absorbance. Toluene was used as a blank and a calibration curve with a proline concentration of 400 nM mL^{-1} as a base.

Total soluble sugars. These were determined by the method described by Southgate (1976). The samples were read at an absorbance of 600 nm, in a spectrophotometer (BioTek®, Gen5™; Vermont, WI, USA). A 1 μg L^{-1} sucrose stock solution was used in the calibration curve.

Concentrations of N, P, K, and Na. They were quantified in leaves by wet digestion of the dry material with a mixture of HNO_3:$HClO_4$ (Alcántar and Sandoval, 1999). The extracts were read in an inductively coupled plasma optical emission spectrometer instrument (Agilent ICP-OES 725-OES; Santa Clara, CA, USA). N was determined using the micro-Kjeldahl methodology (Bremner, 1965).

Statistical analysis

An analysis of variance was performed to assess the main and interaction effects of the study factors, as well as tests for comparing means (Tukey, $p \leq 0.05$). SAS software was used in both analyses.

RESULTS

Chlorophyll concentration

Chlorophyll a concentration was affected by NaCl and GB (Figure 1), but not by their interaction (data not shown).

Salt stress reduced chlorophyll *a* concentration by 9.6% (Figure 1A), while foliar application of 20 mM GB slightly increased this variable, with no differences from the control (Figure 1B). Chlorophyll *a* absorbs most of the light used in the photosystem II (Roca *et al.*, 2016).

The main effects of the study factors and their interaction were not significant for chlorophyll *b* and total. The chlorophyll *b* concentration values ranged between 0.238 and 0.267 mg g^{-1} of fresh matter, while those of total chlorophyll ranged from 0.642 to 0.793 mg g^{-1} of fresh matter. Chlorophyll *b* is an accessory pigment that complements the absorption of light at short wavelengths, that chlorophyll *a* cannot absorb, and transfers the energy produced (Nobel, 2009; Guidi *et al.*, 2017). The maintenance of the concentrations of this pigment in the treatments with and without salinity denotes the protective function of GB in the protein and lipid complexes of the photosystems (Chen and Murata, 2008).

Sugar concentration

The concentration of total soluble sugars was only affected by the main effects of the study factors (Figure 2). Salinity increased the concentration of sugars by 37.4% (Figure 2A). The increase in reducing sugars (sucrose, fructose) represents a strategy for plant tolerance to stress, since they provide protection to cellular, membrane, and protein structures, and also function as an activator of signaling pathways that generate signals that modify gene expression (Singh *et al.*, 2022). On the contrary, the addition of 10 and 20 mM GB reduced this variable by 33.8 and 49.8 %, respectively (Figure 2B). Under salinity conditions, the application of GB caused the highest values of total soluble sugars, compared to the conditions of absence of salinity (data not shown). However, the differences were not significant.

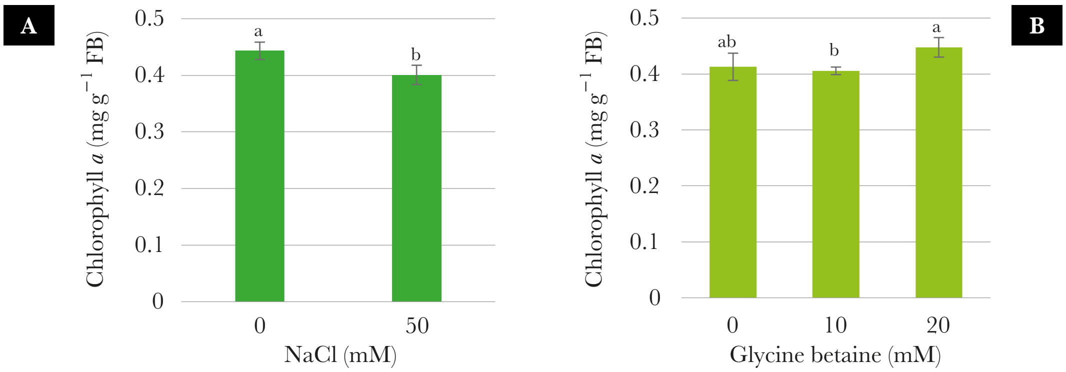

Figure 1. Effect of sodium chloride (A) and glycine betaine (B) on leaf chlorophyll *a* concentration in strawberry plants cv. Aromas. Means ± SD with different letters in each subfigure indicate significant statistical differences (Tukey, $p \leq 0.05$). FB: Fresh biomass.

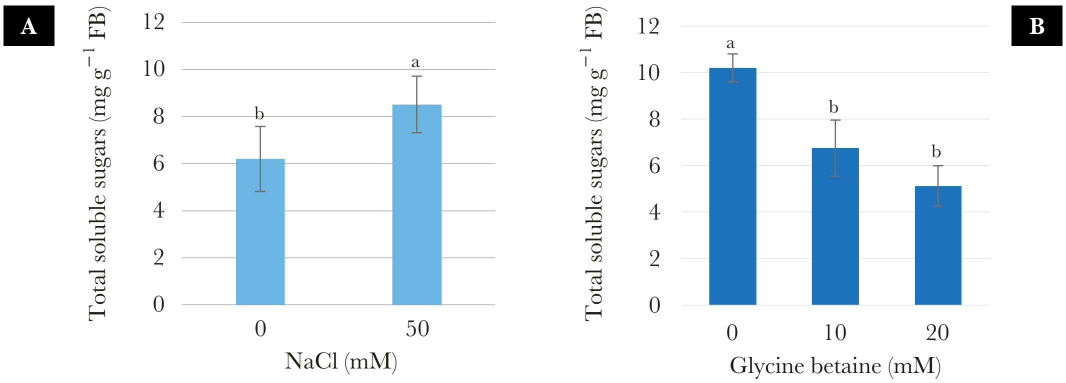

Figure 2. Effect of sodium chloride (A) and glycine betaine (B) on the leaf concentration of total soluble sugars in strawberry plants cv. Aromas. Means ± SD with different letters in each subfigure indicate significant statistical differences (Tukey, $p \leq 0.05$). FB: Fresh biomass.

Proline

The proline concentration was only affected by the NaCl factor, where the addition of a 50 mM dose increased it by 56.5% (Figure 3).

Proline is an osmoprotectant; its synthesis can be stimulated under stress conditions. In strawberry cv. Fern (day neutral) proline concentrations of 16.57 to 33.0 μM g^{-1} are reported and in cv. Camarosa (short day) from 45.0 to 95.5 μM g^{-1} with electrical conductivity levels of 2.0 and 5.0 dS m^{-1}, respectively (Pirlak *et al.*, 2004).

Concentrations of N, P, K, and Na

The leaf N concentrations obtained in this study (15.05-18.90 g kg^{-1} of dry matter), as well as those of P (2.84 to 4.96 g kg^{-1} of dry matter), shown in Table 1, are consistent with those reported by Muramoto (2003). Aguilar-Tlatelpa *et al.* (2019) report leaf K concentrations in the range of 13.3 to 24.3 % for the cultivars Albion, Festival, Jacona, and Zamorana; the values obtained here in the cv. Aromas are much lower (Table 1).

In the absence of salt stress, GB increased leaf N concentration. The addition of 20 mM GB with 50 mM NaCl increased P concentration by 73.8% compared to the treatment without NaCl and without GB (Table 1). N is an important constituent of biomolecules

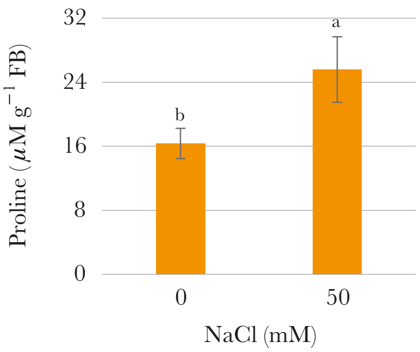

Figure 3. Effect of sodium chloride on leaf proline concentration in strawberry plants cv. Aromas. Means ± SD with different letters in each subfigure indicate significant statistical differences (Tukey, $p \leq 0.05$). FB: Fresh biomass.

Table 1. Effect of glycine betaine (GB) application on the concentration of N, P, K, and Na on leaves of strawberry plants cv. Aromas exposed to salt stress induced by sodium chloride (NaCl).

NaCl (mM)	Glycine betaine (mM)	N	P	K	Na
		g kg^{-1} DB			mg kg^{-1} DB
0	0	15.05±0.67 b	2.84±0.01 d	6.14±0.33 a	597.08±12.33 c
	10	18.90±0.49 a	4.34±0.08 bc	6.13±0.25 a	384.33±9.95 d
	20	17.50±0.48 a	4.43±0.02 b	5.89±0.15 a	316.15±12.23 e
50	0	18.73±0.34 a	4.49±0.02 b	6.04±0.17 a	592.18±8.29 c
	10	18.38±0.21 a	4.18±0.05 c	5.60±0.24 a	1250.78±10.04 a
	20	17.15±0.60 ab	4.96±0.04 a	6.47±0.20 a	664.03±23.96 b

Means ± SD with different letters in each column indicate significant statistical differences (Tukey, $p \leq 0.05$). DB: Dry biomass.

such as chlorophyll, proteins, and nucleic acids, while P is important for energy generation and is involved in the whole cellular metabolism (Alcántar-González *et al.*, 2016).

The leaf concentration of K was not affected by the interaction of the study factors; however, the concentration ratios of this element with respect to Na were not modified in the 50 mM NaCl-20 mM GB treatment with respect to the treatment without NaCl and without GB (Table 1).

Na is not a nutrient in plants, and can be considered a beneficial element. At high concentrations, Na can alter the balance of nutrients in the soil, by affecting the absorption of essential elements such as Ca, K, and Mg. When Na reaches toxic levels for the plant, irreversible damage and death of cells, tissues, and organisms can occur (Lamz *et al.*, 2013; Tarolli *et al.*, 2024).

GB can promote K uptake and reduce Na uptake by stimulating antioxidant activity and the functionality of enzymes such as SOD, CAT, and POD, which are key in ROS detoxification (Ali *et al.*, 2020; Yan *et al.*, 2020). These results were only evident with foliar supply of 20 mM GB, which decreased Na in the presence of salt stress. Although GB did not increase K concentration, it did help maintain stable K levels in plants and thus the K^+/Na^+ ratio.

CONCLUSION

GB increased chlorophyll *a* concentration at 20 mM in plants under salt stress and reduced the concentration of sugars and proline, which appears to be a secondary effect of the decrease in ROS; in addition, it increased N and P uptake, and decreased Na concentration at 20 mM.

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Factors that Impact the Market of Agricultural Tractors in Mexico

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ABSTRACT

Objective: To determine the main factors that impact the market of agricultural tractors in Mexico.

Design/Methodology/Approach: Time series data from 2000 to 2020 were used to estimate two national-scale models for agricultural tractors: one for supply and the other for demand.

Results: The results suggest that, in the long run, the supply and demand for agricultural tractors have an inelastic responsiveness to price changes, with a 0.079 and −0.083 elasticity, respectively.

Study Limitations/Implications: The main limitation is the lack of official data on the historical prices, sales, and production of tractor units in recent years. Consequently, estimates must be employed.

Findings/Conclusions: In addition to the price factor, producer income and interest rate affect demand, with elasticities of 0.178 and −0.002, respectively. Consequently, the government should implement the necessary measures, such as improving income and maintaining low interest rates, to encourage the demand for tractors in Mexico.

Keywords: tractor, supply, demand, elasticity.

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INTRODUCTION

The significance of agriculture as a source of food for the population and a provider of raw materials for the industry, along with the country's rich biodiversity, means that this sector is an indispensable market for the agricultural machinery industry, which encompasses the production and commercialization of agricultural machinery, vehicles, and implements (ICEX, 2019).

The agricultural tractor is the main element of agricultural mechanization and is the main source of power within production units (Ayala-Garay *et al.*, 2013). The 2022 Census of Agriculture recorded 473,000 tractors in operation in the country, serving 1.8 million agricultural production units (INEGI, 2023).

According to the information provided by the Agrifood and Fisheries Information Service (SIAP, 2022), Mexico covers an area of 197 million hectares, out of which 32.4 million are dedicated to agricultural activities. Over 300 products are grown in this surface to nourish a population of 126 million individuals, predominantly through the use of agricultural machinery (INEGI, 2020).

The primary objective of agricultural machinery manufacturers and sellers is to provide equipment for open field and protected agriculture. The responsibility for selecting the necessary machinery lies with the producer. Together, they create a machinery market where supply and demand interact.

The current supply of agricultural tractors in Mexico is comprised of products from multiple manufacturers who also produce and sell related agricultural machinery. In Mexico, the most important manufacturers are John Deere, CNH Industrial, AGCO Corporation, KUHN Group, and Kubota.

According to INEGI (2009), the annual domestic production of agricultural tractors amounted to 12,000 and 11,300 units in 2002 and 2003, respectively. According to John Deere's annual report for 2021, the average annual sales of agricultural tractors in Mexico from 2011 to 2015 amounted to 10,400 units, while from 2016 to 2020 this figure rose to 12,300 units.

The demand for tractors has fluctuated over time. In 2009, 2010, and 2011, this indicator reached 19,000, 14,000, and 10,000 units, respectively (Suárez-López, 2011). Conversely, in 2017 and 2018, it decreased to 14,000 and 12,000 units, respectively (Perea, 2018). Agricultural machinery represents a major input in the context of agricultural production activities. In 2019 alone, the value of primary sector activities reached $589 billion Mexican pesos, accounting for 3.7% of the GDP (CEDRSSA, 2019).

Several studies about agricultural machinery in Mexico share similarities with this research. Aburto-Irigoyen (1984) studied the market and prospects for agricultural tractors. Mora-Flores (1986) conducted a study on the supply and demand of agricultural tractors. Morales-Carrillo and Martínez-Damián (1998) developed an almost ideal demand system for agricultural tractors. Ayala-Garay *et al.* (2012) conducted an analysis of the mechanization situation in the State of Mexico, specifically in the regions of Teotihuacán, Tepotzotlán, and Zumpango. Finally, Terrones-Cordero *et al.* (2020) analyzed the behavior of the primary sector in Mexico from 1980 to 2020.

There is an overall lack of in-depth studies about the situation of agricultural machinery in Mexico. Some studies focus on specific states or regions. Therefore, the aim of this study was to analyze the variables that influence the supply and demand of agricultural tractors in Mexico. Given the crucial role of agricultural machinery (particularly tractors), as a fundamental component of agricultural production, the objective was to identify and analyze the factors affecting this market. The hypothesis was that both supply and demand would have an inelastic response to price changes in the long run.

MATERIALS AND METHODS

In order to achieve the results, an econometric model was developed for the supply and demand for agricultural tractors in Mexico. The model was estimated using the ordinary least squares regression method (Gujarati and Porter, 2010), in the Statistical Analysis Software (SAS, 2013). The geographical scope of the research included the national territory and the 2000-2020 time series were used to estimate the models.

The following econometric models were proposed:

$$QDT_t = \alpha_0 + \alpha_1 PCT_t + \alpha_2 YA_t + \alpha_3 TIR_t + \alpha_4 QDTL_{t-1} + e_t \quad (1)$$

$$QPT_t = \beta_0 + \beta_1 PUE_t + \beta_2 CREA_t + \beta_3 SMIN_t + \beta_4 QPTL_{t-1} + e_t \quad (2)$$

Where for the year t: QDT_t is the quantity of tractors demanded (tractor units); PCT_t is the consumer price of the tractors (pesos per unit); YA_t is the producer income (pesos per hectare); TIR_t is the annual interest rate (%); $QDTL_{t-1}$ is the one-year delay of the QDT_t variable; QPT_t is the tractor quantity offered (tractor units); PUE_t is the unit export price (pesos per unit); $CREA_t$ is the agricultural credit (pesos); $SMIN_t$ is the minimum wage (pesos); and $QPTL_{t-1}$ is the one-year delay of the QPT_t variable.

The long- run econometric models were obtained with the following formulas:

$$QDT_t = \frac{\alpha_0}{\&} + \frac{\alpha_1 PCT_t}{\&} + \frac{\alpha_2 YA_t}{\&} + \frac{\alpha_3 TIR_t}{\&} \quad (3)$$

$$QPT_t = \frac{\beta_0}{\&} + \frac{\beta_1 PUE_t}{\&} + \frac{\beta_2 CREA_t}{\&} + \frac{\beta_3 SMIN_t}{\&} \quad (4)$$

Where α are the coefficients of the demand function; β are the coefficients of the supply function; and & is the autoregressive parameter.

The model formulation was justified by economic theory. According to García-Mata *et al.* (2003), the price of the product and the price of inputs are the factors that determine the quantity supplied. The factors that determine the quantity demanded are the price of the good, the price of substitute or complementary goods, and available income.

According to economic theory, the supply of a product (*e.g.*, agricultural tractors) is determined by several factors, including the price of the product itself. Since obtaining the prices of new tractors at the output level from assembly plants proved to be a difficult task, the export unit price was taken as the producer price. Other key factors that influence the domestic supply include the minimum wage for labor and agricultural credit. The latter allows producers to purchase production inputs and encourages the production of tractors.

The quantity consumed of a good is based on its price and the consumer income. Higher income leads to higher demand, and *vice versa*. The producer income variable is the gross income per hectare. Another variable that affects the tractor demand is the interest rate. This variable is important because it drives investment in the agricultural sector.

The data used to develop the model was obtained from several sources. The quantity of tractors produced per year was obtained from INEGI (2009) and John Deere (2021). The export unit price for the 2010-2020 period was sourced from SIAVI (2021), based on the duty rate 87019001, while the price for the 2000-2009 period was obtained from FAOSTAT (2022). Agricultural credit was obtained from FIRA (2021). The minimum wage was obtained from CONASAMI (2021).

To quantify the extent of the demand, the apparent domestic consumption of agricultural tractors was calculated based on the sum of the domestic production and the trade balance. According to the 2007 Agricultural, Livestock and Forestry Census (INEGI, 2007), tractors are classified into four groups: group 1 (up to 60 Hp), group 2 (from 60 to 85 Hp), group 3 (from 85 to 145 Hp), and group 4 (over 145 Hp). The consumer price of tractors was selected according to the power range of group 2 (from 60 to 85 Hp), considering that most of the existing tractors in the country fall into this classification. Once the power range was selected, average prices were taken based on the selected power. Since obtaining nominal prices for all years in the study period proved to be difficult and given the lack of public data regarding the prices of new agricultural tractors sold in Mexico, the prices were estimated in US dollars, based on information from agricultural machinery websites and documents published by marketing companies (COMEXI, 2015; COMEXI, 2020; CNH, 2017; CNH, 2018), as well as information about agricultural tractors provided by government agencies (SEDRAE, 2019). Income per hectare and interest rate were obtained from SIAP (2022) and BANXICO (2022), respectively. Monetary variables were deflated based on 2018 figures from the National Producer Price Index and the National Consumer Price Index (INEGI, 2021a; INEGI, 2021b).

The statistical validity of the models was established using the F-test or global test for a multiple regression, the t-test, and the coefficient of determination (R2) (Gujarati and Porter, 2010).

RESULTS AND DISCUSSION

Table 1 shows the estimated coefficients. The F-test values demonstrate that, at the aggregate level, the explanatory variables have a significant influence in determining the variation in tractor supply ($P \leq 0.0001$) and demand ($P \leq 0.0001$). The coefficient of determination is acceptable for both equations: 0.73 for supply and 0.73 for demand.

The t-asymptotic pointed out that most parameters were individually significant. In other words, the coefficient of the parameters is greater than their respective standard value and the t-statistic is greater than one in absolute terms. In the case of the supply model, the variables have a $t<1$ in absolute terms, except for the lag of the quantity produced. However, in accordance with economic theory, they were allowed in the model as a result of their importance. In the case of the demand model, the variables have a $t<1$ in absolute terms, except for the price and interest rate variable. Nevertheless, according to economic theory, this variable was deemed important enough to be included in the model (Table 1).

The estimated coefficients match the sign outlined in economic theory (García-Mata *et al.*, 2003). On the one hand, the consumer price of tractors is inversely related to the quantity demanded and income has a positive effect. On the other hand, the interest

Table 1. Statistical results of the supply and demand model for tractors in Mexico.

Dependent variable	Intercept	Independent variables				R^2	Prob>F
QPT		*PUE*	*CREA*	*SMIN*	*QPTL*		
Coefficient	4413.05	0.000725	0.000008	−29.341280	0.787830		0.0001
Standard error	2683.83	0.003660	0.000021	30.615180	0.200120	0.73	
t value	1.640000	0.200000	0.400000	−0.960000	3.940000		
Pr>t	0.120900	0.845500	0.694100	0.353100	0.001300		
QDT		*PCT*	*YA*	*TIR*	*QDTL*		
Coefficient	4804.33	−0.001010	0.061410	−1.719000	0.6532		<0.0001
Standard error	2854.31	0.001300	0.047240	144.534730	0.2117	0.73	
t value	1.680000	0.780000	1.300000	−0.010000	3.0860		
Pr>t	0.113000	0.449300	0.213200	0.990800	0.0075		

Source: Developed by the authors with data from the estimated model.

rate is inversely related to the quantity demanded, indicating that a higher interest rate corresponds to a lower demand, because acquiring a new tractor at a higher interest rate is more expensive.

The supply of agricultural tractors is influenced by the producer price, with a positive correlation between the two variables. The agricultural credit variable has a positive impact on the tractor supply, with higher credit leading to a higher supply. Espinoza-Zamorano and Martínez-Damián (2017) have proved that agricultural credit plays a significant role in stimulating the growth of the agricultural sector. Between 1970 and 2013, the ratio of credit to agricultural GDP increased (GDP increase: 5.0% to 28.5%). The minimum wage has an inverse effect on the quantity supplied —*i.e.*, as the wage rises, the supply of the product falls.

The short-run elasticities were calculated using the partial derivatives of each equation, the structural form of the estimated model, and the average values of the variables in question over the 2000-2020 period. Three short- and long-run elasticities were calculated. García-Mata *et al.* (2003) indicate that the long-run model was derived, to ascertain the dynamic trend, using the short-run linear supply and demand models. The speed of adjustment (&) for the long-run model was also calculated with the estimated parameters of the lagged endogenous variables. The estimated parameter was 0.7878 for supply and 0.6532 for demand. The formula (1−&) was used to determine the speed of adjustment, resulting in 0.21217 and 0.3468 for supply and demand, respectively.

Table 2 shows that all the absolute values of the short-run and long-run elasticities are less than one, suggesting that both supply and demand have an inelastic response to changes in determining factors. Additionally, the short-run elasticities are lower than the long-run elasticities in all cases. In conclusion, the reaction of agricultural producers (who act as consumers of agricultural tractors) to price variations is relatively limited.

The long-run price elasticity of the demand coefficient was calculated to be −0.083, indicating that a 10% increase in price would result in a 0.83% decrease in the tractor demand. This value is similar to that reported by Terrones-Cordero and Martínez-

Table 2. Elasticities of supply and demand for agricultural tractors.

Supply	Short run	Long run	Demand	Short run	Long run
E_{PUE}^{QPT}	0.017	0.079	E_{PCT}^{QDT}	−0.029	−0.083
E_{CREA}^{QPT}	0.035	0.164	E_{YA}^{QDT}	0.062	0.178
E_{SMIN}^{QPT}	−0.200	−0.942	E_{Tir}^{QDT}	−0.0006	−0.002

Source: Developed by the authors with data from the estimated model.

Damián (2012) and Terrones-Cordero *et al.* (2020), who report elasticities lower than one, implying that the response of agricultural producers to price changes in the purchase of machinery is low. Morales-Carrillo and Martínez-Damián (1998) calculated the elasticities of tractor demand and reported that 80-100 hp vehicles have an inelastic demand with respect to the price.

The long-run income and interest rate elasticities were 0.178 and −0.002, respectively, indicating that a 10% increase in income will result in a 1.78% increase in demand, if all other factors remain constant. Conversely, a 10% increase in interest rates will lead to a 0.02% reduction in tractor demand (Table 2). Producer income is a key factor that determines their capacity to make investments, including the acquisition of agricultural machinery.

The coefficient of the long-run price elasticity of supply was 0.079, indicating that a 10% increase in price will result in a 0.79% increase in supply, if all other factors affecting the quantity produced remain constant. The long-run elasticities of credit and the minimum wage were 0.164 and −0.942, respectively. These values indicate that a 10% increase in credit will result in a 1.64% increase in output, whereas a 10% increase in the minimum wage will lead to a 9.4% decrease in output. These results assume that all other factors remain constant. The analysis revealed an increase in credit for the agricultural sector (FIRA, 2021). However, this increase has not been substantial enough to drive a significant rise in the tractor supply, resulting in a continued stagnation in supply.

The interest rate is a monetary policy instrument implemented by the Bank of Mexico. It has an inverse relationship with investment —*i.e.*, when this variable is high, producers are unable to mechanize their fields through the acquisition of machinery. A constant interest rate increase in the future would continue to prevent producers from investing in machinery.

The results of the long-run elasticities show that supply and demand have an inelastic response to changes in its determining factors. Therefore, major changes would be necessary to modify the quantity produced and demanded. It is essential to continue supporting agricultural mechanization (*i.e.*, the acquisition of tractors), by means of credits and subsidies. Terrones-Cordero *et al.* (2020) point out that the decision of producers not to acquire more tractors and other agricultural inputs through credit amounts is a consequence of the high interest rates set by the institutions.

To promote demand for agricultural tractors in Mexico, institutions must provide loans at competitive interest rates, consequently boosting tractor sales and expanding the tractor fleet in Mexico.

Given the high price point of current agricultural tractors (in some cases exceeding $400,000 Mexican pesos), producing smaller, less powerful tractors would be advisable. Such tractors are well-suited to the country's plots and affordable for small producers. Institutions, universities, and mechanical design experts should propose equipment capable of performing maneuvers in the varying conditions of each region. Furthermore, the Federal Government must support the establishment of a national technology-based industry that promotes the development of tractors for small producers.

Implementing public policies focused on the agricultural sector is essential. Likewise, the appropriate institutions should ensure the efficient and socially responsible use of allocated resources. These measures will encourage tractor demand through subsidies and low interest rates.

CONCLUSIONS

The estimation of a model of the supply of agricultural tractors in Mexico indicates that this variable has a long-run inelasticity in response to changes in price, minimum wage, and agricultural credit. Tractor supply is expected to continue following a stable trend, with an average of 12,000 units offered and sold each year. Tractor demand is determined by three key factors: consumer price, farm income, and interest rate. The response of the quantity demanded also has an inelastic response to changes in these three factors. Among these factors, income is the variable that most affects the demand for tractors, thereby highlighting the importance of recording the prices of products sold by the producer. One limitation of this research was the lack of public data. Therefore, the competent authorities should collect and provide the necessary data on this market. Further research should be conducted to analyze the adoption of agricultural machinery and additional projects should contemplate the production of smaller tractors that can be easily purchased by producers. The support for agricultural mechanization should continue through the implementation of policies that, on the one hand, maintain low tractor prices and high prices for agricultural products, improving producer income, and, on the other hand, offer low interest rates that encourage greater demand for tractors.

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Colegio de Postgraduados

The Application and advancement of crop simulation models in sugarcane cultivation

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ABSTRACT
Objective: This study aims to describe the approach of agricultural simulation models and their potential application in sugarcane crops in various regions of Mexico.
Methodology: A comprehensive review and classification of scientific articles indexed in major databases, such as SCOPUS or ELSEVIER, was conducted. The focus was on articles related to agricultural and sugarcane simulation models.
Results: A collection of scientific articles concerning agricultural simulation models for sugarcane crops was analyzed. A detailed description of the models' approaches and application for this crop, along with their potential use in certain regions of Mexico was developed.
Study Limitations: The literature of the application of agricultural simulation models for sugarcane in Mexico in recent years is limited.
Conclusions: Crop simulation models are recognized and widely used as indispensable tools in agricultural research. It is crucial to note that many of the models rely on climate information to produce accurate results. Therefore, obtaining reliable and accurate data is essential to achieve trustworthy and useful results applicable to the relevant agricultural areas.

Keywords: agricultural simulation models, sugarcane, yield, APSIM, DSSAT.

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INTRODUCTION

A model is a schematic representation of a concept, an act of mimicry, or a set of equations, exemplify the behavior of a system. This representation of an object, system, or idea is in a form other than that of the entity itself. It is important to consider that it is a simplified version of a part of reality, not an exact copy. Its purpose is generally to help explain, understand, or improve the performance of a system (Murthy, 2007).

Modeling has gained importance in agronomy and other biological areas due to its ability to provide systematic information about both entire biological system and its specific components, such as the agricultural production system (Guevara, 2007). Therefore, the objective of this review is to describe the approach of agricultural simulation models and

Image by Alejandro Sánchez

their potential application in sugarcane crops in various regions of Mexico, through an analysis and classification of scientific articles indexed in major databases such as SCOPUS or ELSEVIER.

Structure and function of simulation models

Simulation models have multiple uses and applications, including crop growth models. These models are based on agricultural systems and scientific principles to generalize and analyze the photosynthetic production process, physiological processes, organ formation, yield, and the relationship between the environment and technology. Following this process, a mathematical model is constructed to perform a quantitative analysis and simulate the crop growth process using a computer. Crop growth modeling is a powerful tool for precise production management, presenting significant theoretical relevance and application value in optimally managed crop (Bo and Jun-Cang, 2010; Ya-Li and Li-Yuan, 2005).

It is important to note that to demonstrate the complex interaction of crop growth with various climatic, hydrological, atmospheric, and agronomic factors, several empirical models have been developed. Initially, these models were based on regression analysis functions, which assume that the variability of crop yields can be explained by a few independent variables (Khan and Walker, 2015).

According to Murthy (2007), models developed in recent years utilize one or more sets of differential equations over time, usually from sowing to final harvest, to estimate agricultural production based on climate and soil conditions, as well as crop management. Authors such as Graves *et al.* (2002) mention that crop growth models prioritize resource management in the agricultural field and have been used to understand, observe, and experiment with cropping systems for the last four decades (Cheeroo-Nayamuth, 2000). They have also been employed as research tools to evaluate the relationships between crop productivity and environmental factors (Adejuwon, 2005).

Approach to crop simulation models

Crop modeling combines the complexities of climate change with the intricacies of physiological functions and other biophysical aspects of crop-soil-atmosphere systems. Historically, it has been reported that the first crop simulation models were developed in the 1980s and were used to simulate the growth of wheat, utilizing conservative physiological functions of the crop. Notably early models included ARCWHEAT1 (Porter, 1984; Weir *et al.*, 1984), the Dutch models SUCROS (Van Laar *et al.*, 1992) and SWHEAT (Van Keulen and Seligman, 1987), and five crop models from the ARS Wheat Yield Project, with CERES-Wheat (Ritchie and Otter, 1985) and WINTER WHEAT (Baker *et al.*, 1985) being the most prominent.

A common feature of these models was that they all operated on a daily time step, either approximating or aggregating processes that occur at a shorter time intervals (Jamieson *et al.*, 2008). The models varied in the details of the physiological processes included and the production constraints addressed. The widely used Australian model, APSIM-Nwheat (Keating *et al.*, 2001), initially relied heavily on CERES-Wheat. Substantial changes were subsequently made to this APSIM-Nwheat to improve its performance and account for a wider range of growth conditions. (Keating *et al.*, 2001; Asseng and Van Herwaarden, 2003; Asseng *et al.*, 2001a, 2001b).

Cheeroo-Nayamuth (2000) reported that the application of crop simulation models began in the 1970s. The most commonly used models were the Environmental Policy Integrated Climate (EPIC) (Williams, 1990), the Decision Support System for Agrotechnology Transfer (DSSAT) (Jones *et al.*, 2003; Jones *et al.*, 1998; Ritchie *et al.*, 1985), and the CROPWAT model (Smith, 1992). However, these models have often been developed for specific localities and are not always applicable to other regions (Adejuwon, 2005). Therefore, when introducing such cropping models in new regions, it is necessary to evaluate their applicability. The use of agricultural models is feasible only if the user has a good understanding of the model's structure, scope, and limitations.

The conventional approach in crop simulation studies has been to run a model for multiple sites and then scale up the results to a regional scope (Iglesias *et al.*, 2000). Additionally, regional yields have been modeled using representative region-specific soil types, crop varieties, and management practices (Moen *et al.*, 1994; Haskett *et al.*, 1995). The fundamental assumption in crop modeling applications is that the model can accurately simulate the processes occurring within the agricultural system (Thorp *et al.*, 2005). It is important to remember that models are only approximate representations of real complex systems (Cheeroo-Nayamuth, 2000).

Application of simulation models in sugarcane crops

In the major sugarcane-producing regions worldwide, systems have been developed to predict sugarcane yield based on agroclimatic information (Scarpari and Beauclair, 2004; Suresh and Krishna-Priya, 2009). These systems use data obtained from remote sensors, and crop simulation models, which frequently integrate climate prediction (Everingham *et al.*, 2015).

A considerable number of models have been developed globally to simulate the yield of sugarcane crops. Among the most important are AUSCANE (Jones *et al.*, 1998), DSSAT/CANEGRO (Jones *et al.*, 2003), QCANE (Liu and Kingston, 1995), APSIM-Sugar (Holzworth *et al.*, 2014; Keating *et al.*, 2001), CASUPRO (Villegas *et al.*, 2005), and the FAO-MZA (Monteiro and Sentelhas, 2014). However, only two of these models, APSIM-Sugar and DSSAT/CANEGRO, are widely accepted.

The number of simulation models applied to sugarcane crops is lower compared to other important crops such as wheat, grass, or soybeans (Marin *et al.*, 2015). For sugarcane, the Decision Support System for Agrotechnology Transfer (DSSAT) is a collection of models that connect the decision support system with crop simulation models. It is used to simulate growth, development, and yield based on soil, plant, and atmosphere dynamics (DSSAT.net, 2019).

The DSSAT comprises several simulation models including CERES, for cereals (barley, corn, sorghum, millet, rice, and wheat), CROPGRO for legumes (dry beans, soybeans, peanuts, and chickpeas), root crops (cassava, potato), and other crops (tomato, sunflower, and grass) (Hoogenboom *et al.*, 2015; Jones *et al.*, 2003; Ines *et al.*, 2001). It also includes the CANEGRO model, which simulates sugarcane growth and development based on agricultural management, daily climate information, crop characteristics, soil properties, radiation efficiency, and proximity to the soil's water deficit (Inman-Bamber *et al.*, 2016).

These models have been widely used by researchers, educators, consultants, extension agents, producers, and policy and decision makers (DSSAT.net, 2019). Furthermore, studies in various parts of the world have employed DSSAT to simulate the effects of climate variability and change on crop production (Basak, 2012; Basak *et al.*, 2009; Karim *et al.*, 1996).

Holzworth *et al.* (2014) mention that the Agricultural Production Systems Simulator (APSIM) has evolved in response to global agriculture demands. It contains interconnected models to simulate crops, soil compression systems and biophysical processes. APSIM has been extensively used by researchers to evaluate agricultural management practices, adaptation strategies to climate change and risk, and competition for agroforestry resources, among other applications.

However, the use of crop simulation models in a specific region may lead to inaccurate crop yield estimates. According to Nain *et al.* (2007), accurate regional crop yield estimation requires precise information on crop types and sowing dates for each field.

The conventional approach in crop simulation studies has been to run a model for several sites and then scale up the results up to a regional scope (Iglesias *et al.*, 2000) or to model regional yields using representative soil types, crop varieties, and management practices (Moen *et al.*, 1994; Haskett *et al.*, 1995). The fundamental assumption in crop modeling applications is that the model can accurately simulate the processes occurring within the agricultural system (Thorp *et al.*, 2005).

Nowadays, crop simulation models are recognized and widely used as an indispensable tool in agricultural research. They enable the development of efficient strategies to improve crop production and adaptation. Moreover, the results obtained assist decision makers in determining optimal crop management measures. It is important to note that many models use climate information to simulate results; therefore, accurate and reliable data are essential to obtain trustworthy and useful outcomes applicable to the relevant agricultural areas.

CONCLUSIONS

With the advancement of technology, it is anticipated that simulation models will integrate the features of various models and combine two or more to optimize their use. Despite its widespread use globally, there is no recent and available information demonstrating the application of these models to sugarcane crops in Mexico.

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Musical education and resilience: A path to economic and emotional empowerment for rural children

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ABSTRACT

Objectives: (1) To identify the various factors that facilitate or hinder the development and consolidation of musical education for rural children; (2) to assess whether child harassment and bullying towards girls exists in a rural community; and (3) to analyze how the practice of music promotes a personal and economic empowerment —factors that facilitate resilience— among boys and girls.

Design/Methodology/Approach: A qualitative research methodology was used. The unit of analysis comprised the child population of the Tepexilotla community, located within the mesophilic cloud forest in the Altas Montañas region of Veracruz. The sample of children was self-selected and comprised the participants in a musical group project that started with flutes. Based on participant and anecdotal observation, four thematic axes were selected: (1) musical education, (2) child harassment and bullying, (3) resilience, and (4) child empowerment.

Results: Initially, 26 children participated —18 boys and eight girls. Boys had a larger learning territory because some girls suffered harassment and/or bullying by their classmates during lessons or on the way from home to school. This conduct led to a gradual drop-out among the girls in the group. After the departure of the music teacher, the presence and institutional accompaniment of the Colegio de Postgraduados strengthened group resilience through music master classes. Currently, most group members have become young independent musicians playing in different musical groups —an activity that enhances their economic, personal, and family empowerment.

Study limitations/implications: The study was conducted only among the child population of a rural micro-region; it is not a quantitative study.

Findings/Conclusions: Gender characteristics as construed under the patriarchy have a detrimental influence on the musical education of girls. On the other hand, they benefit boys by replicating the private space for girls and the public one for boys. Hence, boys have more opportunities for formative education, while girls see their educational development prevented or limited, which harms their future female economic empowerment. However, rural musical training is a splendid educational resource that strengthens economic and cultural empowerment opportunities among rural children and youth.

Keywords: Gender, Feminism, Patriarchy, Inequality, Bullying, Child harassment.

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INTRODUCTION

The right to a formal and informal education has not been exercised under gender equality conditions in rural areas, since education for boys has had priority over school

attendance among girls. In rural communities, the high levels of marginalization, the lack of opportunities, and the lack of middle and high schools push children and adolescents to drop out of school at an early stage. Moreover, women in rural areas tend to marry when they are very young. Patriarchy has a powerful influence on rural areas, limiting access to formal and informal education for girls. Factors such as bullying and harassment also lead to school drop-out. These can cause severe problems: depression, insecurity, sadness, anguish, anxiety, head and stomach aches, as well as self-image and self-esteem issues. The word trauma means "broken skin". Resilience, in turn, is the ability to overcome the effects and traumas (emotional, physical, sexual) that any given problem can produce in a person. Thus, we deem musical practice helpful for children to overcome trauma and advance their independence, self-esteem, and creativity (Alcira, 2019).

Inner strength and control locus (internal control to face difficult situations) are key to resilience (Pimentel-Aguilar, 2008).

Stockdale *et al.* (2002) found harmful effects of long-term trauma in their research on bullying among elementary school boys and girls. Rico and Muñoz (2022) examined the musical education programs for early-age children (under six years old) conducted in Colombia. They found that the physical, bodily, and cognitive aspects are essential when developing educational programs. Thus, musical education is presented as an alternative to address the difficulties affecting rural children and youth. Through instrument practice, musical education can enhance children's development and integrate them into a community musical group. In this way, rural children and youth can broaden their horizons regarding their life expectations and increase their tools for personal development, favoring the generation of extra income without leaving school. Due to the social conditions prevailing in the Tepexilotla community, there are several cases of teenage marriage. The high levels of marginalization in the community cause a high level of school drop-outs among boys and girls. The musical project "Sonidos de la Niebla" [Sounds of the Mist] provided an alternative that enriched the education of rural children, particularly girls, in the micro-region. In learning to play an instrument, these girls found an alternative for economic and cultural development. Moreover, musical education encouraged some children to continue playing music in various independent groups. The courses and workshops organized for the "Sonidos de la Niebla" musical group project focused exclusively on rural childhood in the micro-region of Tepexilotla, inviting boys and girls alike.

MATERIALS AND METHODS

The unit of analysis comprised the group of boys and girls who participated intermittently in the children's musical group "Sonidos de la Niebla" between 2015 and 2019. This research was conducted through the music master classes organized by the first author of this article. Each master class session was held over four days and the series of classes

Table 1. Key factors of rural children's resilience and rural musical education.

Interior strength	Locus of control	Internal control

Source: Own elaboration by Silvia Pimentel and Edgar Ramírez.

covered five thematic axes: harmony, choir, rhythmics, ensemble, and instruments. The instrument master classes covered the following subjects: flute, violin, and vocal technique.

The musical group originated in mid-2015, with the teachings of professor and director Apolinar Vázquez de la Cruz, aided by saxophonist Salomé Vázquez as his representative, and musician Miguel Ángel Cruz Alvarado. They taught 32 girls and boys from Tepexilotla and the neighboring communities of Tetla, Carrizal, and other towns. The number of group members varied over time. The children were between seven and 15 years old. A qualitative study was conducted based on the observational recording of photographs, videos, lists, field notes, and historical memories. In 2015, 35 flutes were given to boys and girls, with 28 delivered in groups and the rest extemporaneously and sporadically, answering requests by children who expressed they also wanted instruments.

RESULTS AND DISCUSSION

Method

In 2015, the first author of this article acquired soprano flutes —an act of vital importance to start the project. To be selected by PACMyC [Spanish initials of Support

Figure 1. Flute master class by Jorge N. Loaiza Pimentel in 2015.

Table 2. Stages of the project.

Stage	Objetive
Delivery of flute recorders	To increase the probability of being selected in PACMyC. One of the program evaluators mentioned the need for previous instruments to have the right to participate in the selection.
1st Flute master class	Flute course taught by the teacher Jorge N. Loaiza-Pimentel
Writing of the PACMYC proyect. (by Dr. S. Pimentel)	Be selected and participate to receive financial support and purchase more appropriate instruments for the group.
Initial purchase of 13 flute musical instruments	After being selected in the PACMyC 2016 call, these instruments were acquired with the award subsidy, which were selected by the professor-director of the musical group "Sonidos de la Niebla – Sounds of the Fog, Tepexilotla Veracruz". It continued of teaching face-to-face classes by the teacher-director of the group for 4 months.
Master classes	Harmony, solfeo and rhythmic by: Indra Garduño Reynoso, Edgar Ramírez, Alexis Rivera Palacios y Raquel Fuentes Hernández

Program for Municipal and Community Cultures], the musical groups competing in the call must already have musical instruments. Once support was gained in 2016, various instruments were purchased for the group: 1 drum set, 1 transverse flute, 1 saxophone, 1 guitarrón, 2 vigüelas, 4 violins, and 3 trumpets.

The departure of the teacher and director of the group to Mexico City in 2016 led some boys and girls to drop out. This event occurred approximately four months after the PACMyC project subsidy began.

The master classes were taught over three four-day work sessions. Thus, the musical project continued thanks to the participation of teachers from different musical disciplines, who strengthened the project's musical education in the different aspects referred to in Table 3.

A survey was carried out in 2022 to measure the master classes' impact on those members who had remained in the group the longest. Two girls and five boys between 12 and 22 years old participated. They evaluated various aspects of their experience within the group. The first question asked them to rate their experience in the group,

Table 3. Master classes group work by days.

Master Classes			
Teaching	**Teacher/s**	**Journey**	**Date**
Flute recorder	Jorge N. Loaiza Pimentel	2015	Septembre 6, of 2015
Chorus, ensamble	Alexis Rivera Palacios	working week	November 16, 17, 18, and 19, of 2017
Rythmic, batery, ensamble	Indra Garduño Reynoso	first working week:	16, 17, 18, 19 november of 2017
		2nd working week	15, 16, 17, 18 december 2017
		3rd working week	9, 10, 11 march 2018
Harmony, ensamble	Edgar Ramírez González	1st working week	16, 17, 18, 19 november 2017
		2nd working week	15, 16, 17, 18 december 2017
		3rd working week	9, 10, 11 march 2018
Violin	Raquel Fuentes Hernández	3rd working week	9, 10, 11 march 2018

Figure 2. Handing out flutes to boys and girls from the region in 2015.

Figure 3. Debut of the musical group "Sonidos de la Niebla" at the first Tepexilotla Ecocultural Festival in 2015. From left to right: Silvia Pimentel, Apolinar Vázquez, and Salomé Vázquez.

Figure 4. Third day of master classes in the community kindergarten in March 2018.

Cuadro 4. Members of the group in 2016 during the PACMyC subsidy.

Instrument	Boys	Girls
Violin	3	2
Guitars	2	
Trumpet	2	
Vihüela	3	
Guitarrón	1	
Total niños/niñas	10	2
Representative manager	1	
Rehersals coordinator	1	
Teacher and Group Director	1	

based on four parameters: excellent, good, average, and poor. Five participants rated their experience in the group as good, one rated their participation as average, and one as excellent. Regarding the departure of Professor Apolinar, in a range of happy, sad, and I did not care, five members stated that they felt sad about the teacher's departure, while two said they did not care.

Figure 5. The soprano Alexis Rivera in singing class during the second master class session, 2017.

Table 5. Group development.

Topic	Begining	Development	Last Stage
Musical education	In 2015 the boys and girls initially started with 35 flutes (with the support of Dra. Pimentel), 2 trumpets and a horn (}with the support of Apolinar teacher).	The teacher and director taught classes to boys and girls in person in the community. In 2017 Then,he changed his address to Mexico City, from where he continued teaching classes sporadically via telephone, placing emphasis on trumpets.	Master classes from various teachers. Plus intensive flute course. 3 courses of master classes: harmony, music theory, rhythm, vocal technique, ensemble and violin.
Child harassment y bullying	The study room was in rough construction on the second floor, so an improvised staircase was used for access. This gave rise to the boys peeking out to see the girls' underwear underneath the dress when they went up the stairs.	The girls reported that when they went to the rehersal to practice the instrument, the boys laughed at them and made gestures at them.	A mother and her daughter, who was studying at musical group, reported harassment by the director. This was categorically denied by the director himself.
Resilience and child empowerment	Most of the children (boys and girls) continued to attend music classes with the teacher and group director. La mayor parte de los niños siguieron asistiendo a las clases con el profesor y director del grupo.	Because the teacher changed his home address to Mexico City, some children reported missing him. They later went with a substitute woman teacher who taught a weekly violin class in the community. Low attendance was reported irregularly.	The PACMYC project was fulfilled by bringing in musician teachers who gave master classes in 4-day courses on different weekends. The master classes promoted knowledge on the following topics: Flute, violin, singing, harmony, ensemble and rhythm. Reinforcing the musical ensemble with 2 concerts: one in Córdoba city and a Christmas concert in the community chapel.
Empoderamiento económico	At the beginning, the children's musical group only participated in the Tepexilotla Ecocultural festival. After the first intervention in the Tepexilotla Ecocultural festival (2015), the group's participation was requested in different nearby towns. Events in which they participated with/without financial reward, voluntarily and with great enthusiasm.	Within a few months the group was hired at various events, where the teacher and director shared the income with the group members.	In 2018, the teacher and director of the group decided to convert the group into mariachi. As of 2019, the members play in different musical groups, in other states of Mexico and in Mexico City.

In this survey, six members took one of the master classes offered between 2015 and 2017. All of the participants stated that they would continue playing with Professor Apolinar. On a scale from 1 to 6 designed to measure how useful Professor Apolinar's classes were, five members rated them with a 5, one with a 4, and another one with a 3. Participants were also asked if they found playing a musical instrument of consequence. The ranges were "not at all useful", "moderately useful", and "very useful". Six responded with "very useful" and one with "moderately useful." Moreover, they were asked if playing a musical instrument helped them financially, to which six answers were affirmative and one was negative.

CONCLUSIONS

The benefits provided by musical education in such a remote community are undeniable. These benefits range from cultivating artistic endeavors based on learning to play a musical instrument to developing histrionic skills at an early age. These learnings facilitate interpersonal communication, improve self-esteem and personal self-image, and strengthen cognitive abilities such as attention, concentration, and memory. Rural community musical education prevents violence by putting an instrument in the hands of boys and girls. Moreover, it contributes to family income while providing an informal education alternative that benefits childhood and youth culture. Unfortunately, patriarchal education affects the performance of rural girls due to harassment and bullying.

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Physiological diversity in native Mexican tomatoes (*Solanum lycopersicum* L.)

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ABSTRACT

Objective: This study aimed to evaluate the biodiversity in postharvest fruit quality and photosynthetic attributes of eight native tomato varieties compared to commercial hybrids.

Design/Methodology/Approach: A randomized complete block design with four replicates and four plants per plot was used to allocate treatments. Statistical analysis was conducted with SAS 9.4 using analysis of variance (ANOVA) and mean comparison of by Tukey ($p \leq 0.05$).

Results: Varietal diversity in transpiration rate (E) was detected, but not in net photosynthetic rate (A) nor in water use efficiency (WUE). Variety Oax-131 stood out for its high photosynthetic parameters, such as Amax and its saturation point, as well as by having a carboxylation efficiency similar to the El Cid® hybrid. In terms of fruit postharvest quality, five native varieties had weight losses below the conventional limit of 7%, while the Oax-131 varietey maintained similar fruit firmness to the hybrids during the first 6 days.

Study Limitations/Implications: The study was limited to eight native varieties and the diversity measured in gas exchange rates and photosynthetic parameters may not represent all native varieties.

Findings/Conclusions: The native Oax-131 variety excelled in photosynthetic traits and postharvest quality, demonstrating equal or superior performance compared to commercial hybrids.

Keywords: *Solanum lycopersicum*, native varieties, photosynthetic parameters, postharvest fruit quality.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important crops worldwide, due to its economic significance and nutritional properties, providing essential vitamins, minerals, and antioxidants (SADER, 2022). In Mexico, approximately 48 thousand hectares are cultivated, with an average yield of 69 t ha^{-1}, and a national consumption of 13.4 kg/year per person (SADER, 2020). The predominant tomato types sold in Mexico are saladette (87%) and bola (9%) (SIACON, 2021). However, native tomatoes are cultivated and consumed mainly in local and regional markets, where they are favored for their organoleptic qualities (Parisi *et al.*, 2005) and are integral to many traditional dishes. Native Mexican tomato varieties exhibit a wide range of fruit quality attributes, including flavor,

aroma, color, soluble solids, vitamin C, lycopene and β-carotene content, which contribute to their digestive, antiseptic, diuretic, and anti-inflammatory properties (Juárez-López *et al.*, 2009; Ceballos-Aguirre *et al.*, 2012; SADER, 2022; Vásquez-Ortiz *et al.*, 2010).

Despite these qualities, there is limited information on the physiological performance and resistance of these native tomatoes in adverse conditions (Martínez-Vázquez *et al.*, 2016). Photosynthesis, a critical physiological process, enables plants to capture inorganic carbon (CO_2) and sunlight to produce sugars (Lambers *et al.*, 2008) which feed not only plants but also animals and humans. Photosynthetic and gas exchange studies, including transpiration and water use efficiency (Medrano *et al.*, 2007), may help to identify varieties that are resilient to climate change.

The A/Ci photosynthetic curves relate net photosynthesis rate (A) to increasing concentrations of CO_2 within the leaf (Ci), allowing the estimation of essential photosynthetic parameters (Sharkey *et al.*, 2007; Blanco, 2013), such as compensation point (CP), saturation point (SP), the maximum rate of net CO_2 assimilation (Amax), and Rubisco enzyme carboxylation efficiency (RE). This type of physiological characterization is used to assess varieties by their photosynthetic capacity, which is the main driver of growth and fruit yield. In this case, the photosynthetic parameters are crucial for promoting the preservation of valuable native varieties.

This study's objective was to characterize eight native varieties in terms of their yield potential and postharvest fruit quality, compared with two commercial hybrids, and to quantify essential physiological characteristics in a subset of two native varieties and one hybrid. The hypothesis is that the characterization will define outstanding native tomato varieties that match or surpass commercial hybrids in postarvest quality and photosynthetic capacity.

MATERIALS AND METHODS

The study was carried out during the summer-autumn period of 2022, in a plastic greenhouse located at the Colegio de Postgraduados (19° 28' 05" N, 98° 54' 09" W, at an altitude of 2,243 m). Eight native Mexican tomato varieties from five states were evaluated: two from Puebla state (Pue-105, Pue-55), two from Estado de Mexico (Mex-r92, Mex-12), one from Guerrero state (Gro-78), one from Yucatán state (Yuc-63), and two from Oaxaca state (Oax-131, Oax-130). The hybrids El Cid-F1® and Río Grande® were included as commercial controls.

Experimental management

The seeds from each genotype were germinated in trays and subsequently transplanted into pots, following the protocol outlined by Sandoval (2018). The pots were arranged in double rows, with 25 cm spacing between pots and 50 cm between rows. From this stage onward, irrigation was applied eight times a day with a 100% Steiner nutrient solution at 1 h intervals. The initial daily dosage was 0.2 L per plant, then increasing it every 15 days to a maximum of 1.5 L per plant/day.

The plants were grown with a single stem through periodic prunings of the axillary sprouts and were topped after the seventh cluster. Clusters were thinned to seven or eight fruits. The plants were supported using raffia and hooks to keep them upright. For pest and disease management, the following chemicals were periodically sprayed: Engeo® (1.5 mL L^{-1}) to control whiteflies (*Bremisia tabaci*), Mancozeb® (3 g L^{-1}) to prevent for blight (*Alternaria* sp.), and Kasumin® (2 mL L^{-1}) for preventing bacterial spot.

Experimental design

The 10 genotypes were arranged in randomized complete block designs with four replications and four plants per experimental unit. Harvesting was done as the fruits reached the cutting stage (ripe green stage, physiological maturity). Fruit firmness (F) was measured using a manual texturometer (FORCE FIVE®, Model FDV-30LB × 0.01 LB) in newtons (N), and weight loss (WL) was assessed using a digital scale (Noval, TH- I-EK®, China). The fruits were weighed starting at the ripe green stage and monitored until they reached red ripeness, suitable for consumption.

The three photosynthetic variables —net photosynthesis rate (A), stomatal conductance (gs), and transpiration rate (E)— were measured simultaneously on three plants per variety with a portable photosynthesis device (LI-6400®, LICOR, USA). Measurements were taken on the mature leaf of a raceme with full fruit growth, at 92 days after transplating (dat). All measurements were done between 12:00 and 14:00 hours. Water use efficiency (WUE) was calculated by the A/E ratio.

The curves of the photosynthetic rate (A) performance relative to the intercellular concentration of CO_2 (Ci), known as A/Ci kinetics, were measured one week later (99 dat) using the apparatus LI-6400® (LICOR, USA). It was equipped with a CO_2 dispenser (Model LI-COR P/N 9964-037) and with a mini assimilation chamber designed to measure gas exchange in small leaves —1 cm^2 of leaf area— (Model LI-6400XT QUANTUM), suitable for the small tomato leaflets. The system was programmed to record data when the coefficient of variation remained at or below 2% for a few seconds, as the leaf was exposed to the following external CO_2 concentrations: 400, 200, 100, 0, 200, 400, 800, 1200, and 1600 ppm, in this order. Since the 1 cm^2 mini-chamber lacks the sensors of the standard 6 cm^2 chamber, each external CO_2 value was adjusted under the assumption that Ci=0.5 CO_2. These kinetics were measured on a young mature leaf from each of three plants in two native varieties (Pue-105 and Oax-131) and in the control hybrid (El Cid®). Each curve lasted 40 to 60 min.

Statistical analyses were performed using the GLM procedure of the Statistical Analysis System software (SAS Institute Inc., version 9.4), for the analysis of variance (ANOVA) for each variable, and for the mean comparisons of varieties with the Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The extensive morphological diversity observed in fruit shapes and sizes among the eight native varieties, is shown in Figure 1.

Physiological diversity in gas exchange

A significant diversity ($p \leq 0.05$) was recorded among the studied varieties in transpiration rates (E), with averages ranging from 6.9 to 15.5 mmol H_2O m^{-2} s^{-1}, where

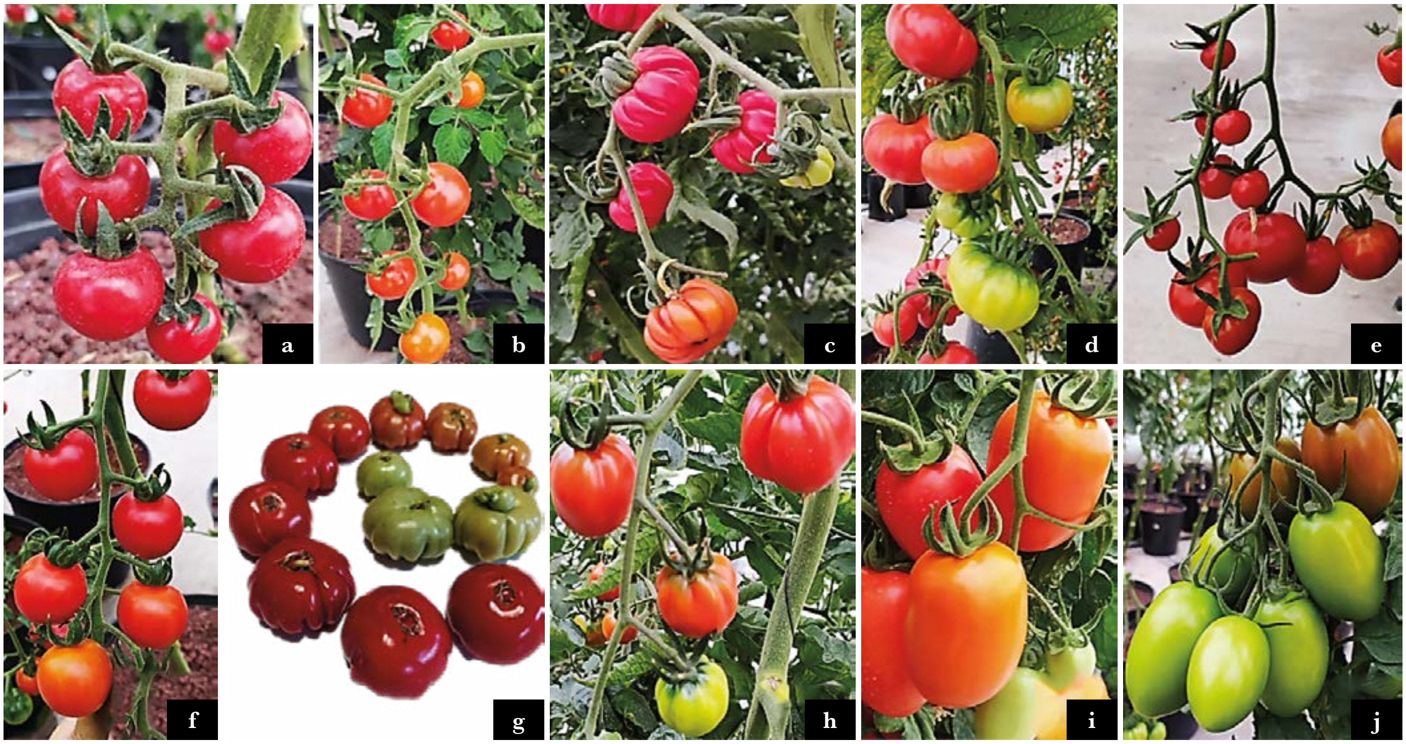

Figure 1. Morphological diversity in eight native varieties of tomato (a-h), and two commercial hybrids (i and j). a) Pue-105 (cherry), b) Pue-55 (cherry), c) Oax-130, d) Oax-131 (pumpkin shape), e) Yuc-63 (cherry), f) Mex-12 (cherry), g) Mex-r92 (pumpkin shape), h) Gro-78 (pepper shape), i) Río Grande® (saladette), j) El Cid® (saladette).

the hybrid El Cid® attained the top E rate, but it was was statistically equaled by two native varieties, Oax-130 and Oax-131 (Table 1). A wide varietal diversity was also recorded in the instantaneous net photosynthesis rates (A), ranging from 23.2 to 41.8 μmol CO_2 m^{-2} s^{-1}. However, no significant differences were found between varieties probably due to the large heterogeneity observed among the individual plants of the same variety (standard deviations ranged from 6.4 to 18.0% relative to their respective means). The overall average for A was 31.7 μm CO_2 m^{-2} s^{-1}, a value comparable to the 34.9 μmol CO_2 m^{-2} s^{-1} reported by Aguiñaga-Bravo *et al.* (2020) in native tomatoes treated with organic fertilizers. Water use efficiency (WUE=A/E) ranged among varieties from 2.8 to 6.0 μmol CO_2 m^{-2} s^{-1}/mmol H_2O mm^{-2} s^{-1}, with no statistical differences among genotypes. Liang *et al.* (2020) reported lower gas exchange rates in a commercial tomato variety in China, with averages of 6.6 for A, 2 for E, and 3.6 for WUE. In Brazil, Gorni *et al.* (2022) also recorded lower values than ours, with 14 and 2.5 units for A and WUE, respectively.

Although transpiration represents the main water use for plants (Taiz *et al.*, 2023), it also plays a crucial role in cooling the leaves and preventing them from overheating, especially during sunny and warm days (Nobel, 1999). Additionally, stomatal transpiration drives the rise of xylem sap from roots to leaves, thus providing water and minerals to the leaves and stem (Taiz *et al.*, 2023). Therefore, stomata exert primary control over water consumption (Medrano *et al.*, 2007), as reflected in the significant correlation (r=0.52***) between E and WUE, which is twice as strong as the correlation between A and WUE (r=0.23*).

Table 1. Mean values of net photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), and water use efficiency (WUE) in a young mature leaf of each tomato variety.

Tomato variety	Net photosynthesis rate, A (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance, gs (mol H_2O m^{-2} s^{-1})	Transpiration rate, E (mmol H_2O m^{-2} s^{-1})	Water use efficiency, WUE (μmol CO_2 m^{-2} s^{-1}/ mmol H_2O m^{-2} s^{-1})
Pue-105	25.2±2.1 a	0.6±0.3 a	6.9±5.0 c	6.0±3.0 a
Pue-55	23.2±2.8 a	0.7±0.2 a	9.2±5.0 bc	2.8±1.5 a
Oax-130	33.6±3.1 a	1.0±0.2 a	14.0±4.9 ab	2.9±1.2 a
Oax-131	33.4±3.1 a	0.8±0.3 a	12.5±4.7 ab	3.1±1.5 a
Yuc-63	28.6±4.8 a	0.5±0.1 a	9.4±4.5 bc	3.4±1.3 a
Mex-12	34.9±3.9 a	0.6±0.1 a	10.5±6.0 abc	5.6±3.9 a
Mex-r92	34.4±2.2 a	0.8±0.2 a	12.6±5.4 ab	3.8±1.9 a
Gro-78	26.6±4.8 a	0.5±0.2 a	9.2±4.8 bc	3.3±1.2 a
El Cid®	41.8±3.2 a	1.0±0.1 a	15.5±4.5 a	2.9±2. a
Río Grande®	35.6±4.9 a	0.6±0.2 a	11.5±3.6 abc	3.4±3.0 a
Mean	31.77	0.73	11.17	3.76

Note: The literals in each column represent groupings based in mean separation using the Tukey test ($p \leq 0.05$) conducted for each variable.

Diversity in CO_2 assimilation parameters

The curves of A in response to the intercellular concentration of CO_2 (Ci), as illustrated in Figure 2, allow for the estimation of essential parameters of the photosynthetic process, such as the maximum potential rate of CO_2 assimilation (Amax); the efficiency of the Rubisco enzyme (RE) in fixing CO_2; the compensation point (CP), which represents the minimum concentration of this gaseous input required to get a net photosynthesis rate above zero; and the saturation point (SP) which is the Ci at which Amax is reached.

The photosynthetic kinetics (curves) obtained in three tomato varieties (Figure 2) indicate that these tomatoes reached their maximum A rates (Amax, SP) when the intercellular carbon concentration (Ci) was 800 μmol/mol (Figure 2 A, C, and D, at a saturation point (SP) of 800, equivalent to 1600 μmol/mol of CO_2 in the outer air, Co). The results suggest that the two native tomato varieties and the control hybrid have the capacity to assimilate the excess of CO_2 present in the air. Similarly, Kozai (2016) found that SP fluctuated between 1000 and 1500 ppm in mature tomato leaves. Therefore, it would be possible that other native Mexican tomatoes could achieve even higher Amax and SP values.

The native variety Oax-131 is particularly noteworthy, with an Amax of 70 μmol m^{-2} s^{-1} (Figure 2 C) which surpasses the commercial hybrid El Cid® by 21% in CO_2 assimilation rate under high concentrations of this gas. Variety Oax-131 also exhibits a compensation point (CP) of 128 μmol/mol (ppm) (Figure 2 D), nearly as low as the control hybrid, whose CP is 118 ppm (Figure 2 F). The CP represents the minimum concentration of CO_2 required for tomato leaves to achieve a net carbon gain.

These finding also show that the CP of tomato is higher than in other C3 plants, in which the reported CP values range from 50 to 100 ppm (Taiz *et al.*, 2023). Regarding the efficiency of the Rubisco enzyme (RE in Figure 2) that catalyzes CO_2 assimilation, the Oax-131 variety (Figure 2D) nearly matched the efficiency of the El Cid® hybrid (Figure 2

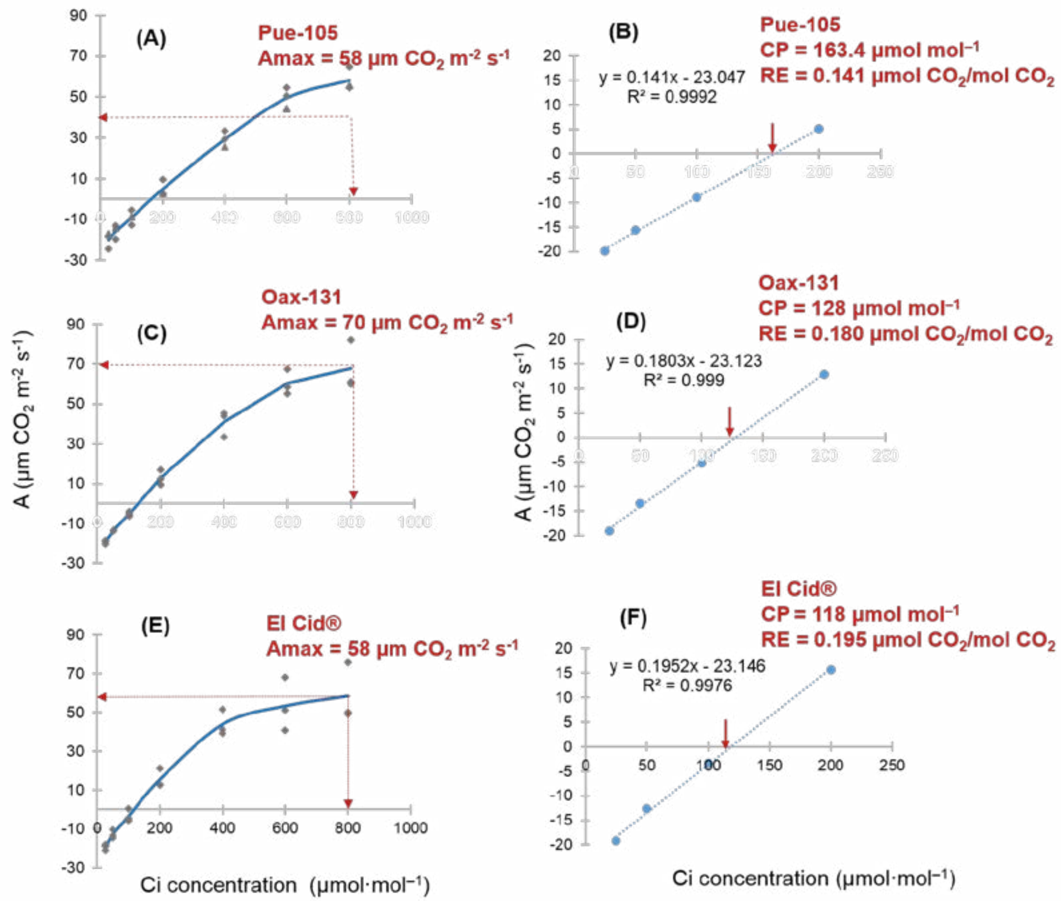

Figure 2. Curves of net photosynthetic rates (A) of tomato leaves in response to intercellular CO_2 concentration (Ci, in μmol mol^{-1}) of two native varieties (Pue-105, A and B; Oax-131, C and D), compared to the commercial hybrid El Cid® (E and F). Measurements were done at 99 days after transplant, during fruit growth. The arrows in (A), (C), and (E) indicate the values of Amax (maximum A, in μmol CO_2 m^{-2} s^{-1}), and their corresponding saturation points for CO_2 concentration (Ci, in μmol mol^{-1}). Arrows in (B), (D), and (F) mark the compensation points (CP, in μmol mol^{-1} of Ci), and their Rubisco efficiencies (RE, in mmol mol^{-1} of Ci).

F), with values of 180 *vs.* 195 μmol CO2 m^{-2} s^{-1} respectively. In the current atmospheric air (420 ppm of CO_2, Co, equivalent to about 210 ppm Ci), both this hybrid and Oax-131 achieved an instantaneous photosynthesis rate (A) of 20 μmol CO_2 m^{-2} s^{-1}, which is similar to the averaged rate for C3 plants.

Diversity in postharvest quality of the fruit

Among the most important quality traits of harvested fruits are shelf-life length, fruit firmness (FF) maintenance, and minimal weight losses (WL). In all the varieties studied here, the firmness (F) monitored from physiological maturity (harvest) to consumption maturity (12 days later), decreased faster during the first 6 to 8 days of ripening (Figure 3). However, there were notorious differences among varieties on initial firmness and on the rate of firmness loss. The two hybrids evaluated here outperformed all the native varieties in F. But among the native varieties, Oax-130 and Oax-131, with kidney-shaped and pumpkin-type fruits, stood out for having the highest initial firmness (7.95 and 9.03 N

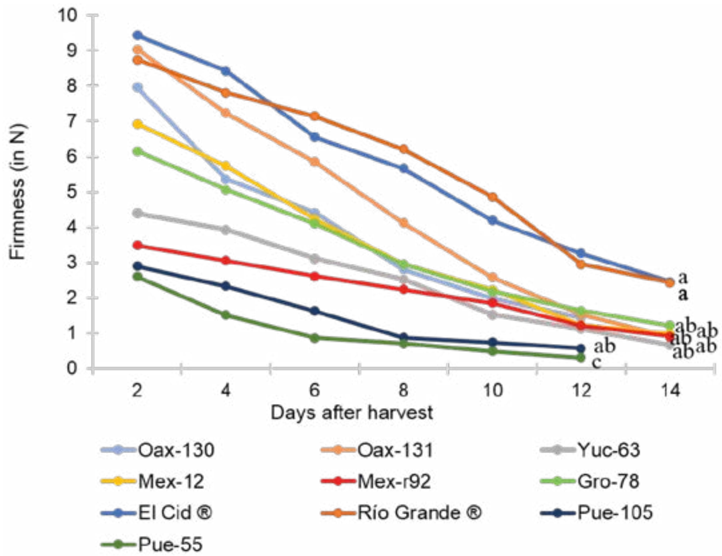

Figure 3. Loss of fruit firmness (in N) monitored after harvest in eight native tomatoes varieties and two commercial hybrids, from two days after the harvest day (at physiological maturity, green fruits) to the day when fruits reached consumption maturity (full red color). Varieties marked with the same letter are not statistically different (Tukey, 0.05; LSD=0.9).

respectively), which was close to the control hybrids (8.74 and 9.43 N). For native tomatoes from Spain, Pérez-Díaz *et al.* (2020) reported lower F values, ranging from 0.58 to 3.77 N on day 0, which further decreased to 0.34 and 0.93 N by day 14.

Fruit weight losses (WL) are attributed to water losses through the peduncle (Bouzo and Gariglio, 2016). On this regard, the WL of native varieties for 14 days, ranged from 5.2 to 10.4% of the initial weight (Figure 4), except for variety Gro-78 whose WL (3.5%) was statistically similar ($p \leq 0.05$) to that of the two commercial hybrids (3.3 and 3.9%). In contrast, other native varieties with cherry-type fruits showed higher WL (Yuc-63, Pue-

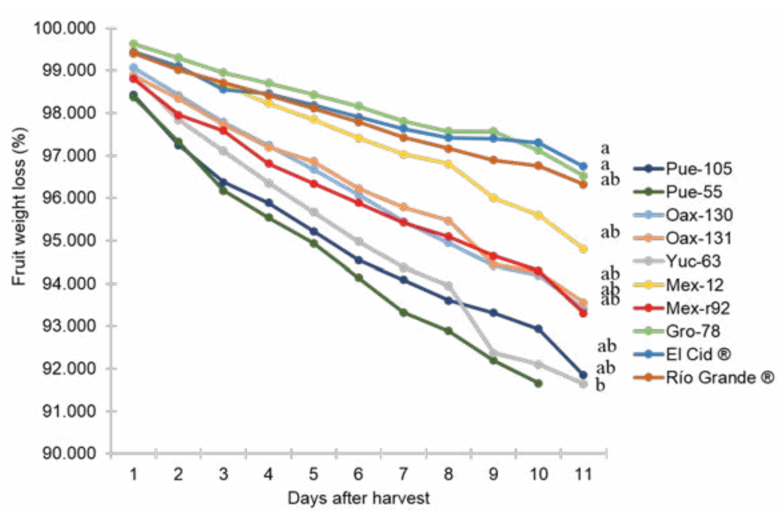

Figure 4. Weight loss (%) of fruit in eight native varieties and two commercial hybrids, measured from the harvest day (physiological maturity, green fruits) until reaching consumer maturity (full red color), in days after harvest. Varieties marked with the same letter are not statistically different (Tukey, 0.05; LSD = 0.9).

105, and Pue-55, with WL of 10.4, 8.2, and 7.9 %, respectively). According to Bouzo and Gariglio (2016), small fruits tend to lose more weight because they have a larger ratio of fruit area/volume, possibly due to a thinner and more permeable epidermis. Ballesteros (1995) noted that a WL of 7% significantly lowers the quality and nutritional value of the tomato fruits, giving them a withered appearance.

CONCLUSIONS

Among the eight native Mexican tomatoes varieties evaluated here, Oax-131 stood out for its exceptional physiological traits, including its photosynthetic metabolism with enhanced CO_2 capture capacity and high carboxylation efficiency (*i.e.*, Rubisco efficiency), compared to the best hybrid control. Additionally, this native variety has a rather low fruit weight loss during postharvest as well as a high water use efficiency, achieving levels comparable to those of the top-performing control.

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Colegio de Postgraduados

Physicochemical and biological properties of honey samples from *Melipona beecheii* Bennett collected in Hopelchén, Campeche

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ABSTRACT

Objective: To determine the physicochemical and biological parameters of *Melipona beecheii* honey produced in the state of Campeche, as the basis for a future proposal for the establishment of an official Mexican standard for Melipona honey.

Design/Methodology/Approach: Samples of *Melipona beecheii* honey from the community of Hopelchén, Campeche, were analyzed. The results showed an acid honey with 3.89±0.13 pH, 49.85±1.74 free acidity, 68.09±2.12 total acidity, 76.95% °Brix, 21.38% humidity, 6.43±0.84 HMF, 8.58±0.25 diastase index, and 187.33±7.9 color intensity.

Results: The honey had a low phenolic (28.45±1.14 mgEAG/kg) and flavonoid (0.072±0.01 mg/kg) content. The low phenolic content interfered with the antioxidant activity, revealing an EC50 of 0.76. The honey showed antibacterial activity against *Staphylococcus aureus*, *Pseudomona aeruginosa*, and *Pseudomonas syringae*. A high-performance liquid chromatography (HPLC) was used to determine the chromatographic profiles of the honey; the said profiles were evaluated by chemometrics to classify the honey, according to the similarity of the chromatographic profiles.

Study Limitations/Implications: Despite being produced at a close distance from each other, the great variability among the small number of samples from the state of Campeche requires the characterization of the honeys by geographic zones.

Findings/Conclusions: The parameters determined in this study help to understand the variability in values, which is crucial for establishing quality and authenticity standards for honey. These standards will not only be applied to honey produced by the *M. beecheii* species, which is widely used in southeastern Mexico, but also to other types of honey.

Keywords: Honey, *Melipona beecheii*, physicochemical parameters, chemometrics.

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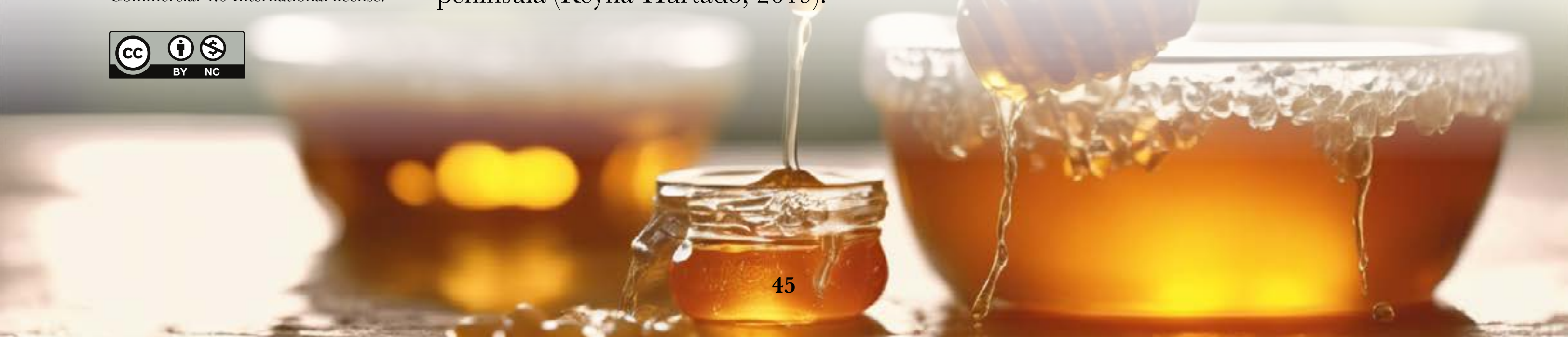
INTRODUCTION

The distinct geology of the state of Campeche, Mexico, located in the southwestern part of the Yucatan Peninsula sets it apart from the rest of the country. It is home to two important natural reserves (the Petenes Biosphere Reserve and the Calakmul Reserve) that host a vast floral diversity, including more than 80% of the plant species of the entire peninsula (Reyna-Hurtado, 2019).



This floral diversity provides the region with different goods and services, among which the role of the melliferous and polliniferous flora in the beekeeping sector stands out (Villalobos-Zapata and Mendoza-Vega, 2016). Campeche is currently ranked as the second producer of honey nationwide (SIAP, 2021) and its honey is appreciated in the international market due to its organoleptic properties. Its composition and sensory attributes vary depending on the geographic region, climatic condition, floral origin, and type of bee (Hadju *et al.*, 2021; Santos *et al.*, 2021).

Honey production in Campeche and the Yucatan Peninsula has its origins in meliponiculture, a technique practiced by the ancient Mayans through the breeding of stingless bees, also known as meliponines (Quezada-Euán, 2018). Stingless bees have ecological, cultural, and economic importance as pollinators and as a food and medicine source (Yurrita *et al.*, 2017). There are 17 species of stingless bees in the Yucatan Peninsula (Ayala *et al.*, 2012). Eight species have been recorded in the Petenes Reserve of Campeche alone (Fernández *et al.*, 2018). However, the beekeeping practiced in the state (mainly in Calkiní, Hecelchakán, and Hopelchén) is based on the *Melipona beecheii* species, known in the Mayan language as "Xunan-Kab" or "Koolel-Kab" (Reyes-González *et al.*, 2014).

Although far less Melipona honey is produced than *Apis mellifera* honey, the demand and cost ($4,000.00-$8,000.00/liter*) for *M. beecheii* honey has increased significantly; this phenomenon has caused a recent resurgence of interest in preserving meliponiculture in countries such as Argentina, Brazil, Mexico, India, Indonesia, Thailand, Malaysia, and Australia (Zulhendri *et al.*, 2022).

A special characteristic of Melipona honey is its fermented flavor resulting from its high humidity percentage (Fernández *et al.*, 2018; Anguebes *et al.*, 2016), higher acidity and ash content (Sharin *et al.*, 2021), lower glucose and fructose composition (Pucciarelli *et al.*, 2014), and lower enzymatic activity (Nordin *et al.*, 2018). Additionally, its mineral composition is mostly made of potassium, followed by calcium, sodium, magnesium, and manganese (Biluca *et al.*, 2016).

No quality regulation exists for the commercialization of *A. mellifera* honeys, because they are not included in the Official Mexican Standards (NOM).

The FAO Codex Standard for Honey (Codex Alimentarius Commission, 2001), which establishes the definition, composition, and labelling of honey, does not cover honey produced by meliponines. This honey tends to be more acidic and have a higher water content (Vit *et al.*, 2004) and, consequently, does not comply with the parameters established in the said standard, emphasizing the importance of developing regulations for the honey produced by stingless bees.

To tackle this lack of regulation, the Kelulut (Stingless Bee) Honey - Specification (Malaysian Standard MS2683:2017) was published as the first national standard for meliponines in Malaysia. In 2019, the Secretariat of Regulation and Health Management and the Secretariat of Food and Bioeconomy of Argentina published the 17/2019 joint resolution in its Official Bulletin regarding the honey of Yateí bees (*Tetragonisca fiebrigi* Schwarz). This was the second standard proposed to regulate honey produced by meliponines in Argentina (Vit *et al.*, 2023). Consequently, the objective of this work, rather than to carry out a regional study of a particular honey, is to determine the physicochemical and biological parameters of *Melipona beecheii* honey produced in Campeche. This research would be the basis for a future proposal to establish a NOM, similar to the Malaysian and Argentinian standards.

MATERIALS AND METHODS

Honey samples

The honey samples of *M. beecheii* were collected in May and July in the town of Ich ek, municipality of Hopelchén, Campeche. Six samples were obtained: five came from bee colonies located less than 500 m apart from each other and one was a commercial mixture of the honey from the five bee colonies. In the first case, the objective was to achieve homogeneity in the samples, while in the second case the honey mixture was prepared by producer. The samples were stored in plastic jars at 4 °C awaiting their analysis. After analyzing the pollen under a phase contrast microscope, the floral origin of the samples was classified as multifloral.

Physicochemical analysis

Degrees Brix, moisture, pH, acidity, and hydroxymethylfurfural (HMF) content were determined following the NMX-F-036-NORMEX Mexican standard (2006). The Bianchi method was used to determine diastase activity, while color intensity was evaluated using the method described by Kek *et al.* (2014). All analyses were performed in triplicate. Analytical grade reagents and solvents for high-performance liquid chromatography were used. The °Brix and moisture of honey were determined by refractometry, using an RHB-92t portable refractometer. The results were set forth in °Brix and moisture percentages. The pH of the honey was determined with a Corning Pinnacle® 540 precision pH meter. The procedure established on section 8.3 of the NMX-F-036-NORMEX-2006 official standard was followed for the measurement, modifying the weight of the sample mentioned in the methodology, given the reduced quantity of honey. Free and lactonic acidity were added to calculate total acidity. The results were expressed in milliequivalents of acid per kilogram of honey (meq kg^{-1}).

Hydroxymethylfurfural

The methodology established by White (1979) and Kek *et al.* (2014) was followed, processing 5.0 g of honey in 25 mL per sample. The readings were performed on a Beckman Du® 650 spectrophotometer and the results were expressed in mg kg^{-1}.

Diastase activity

The Bianchi method (1990) was used to determine this variable, putting 2.0 g of honey in 2 mL of a buffer solution (17.4 g CH_3COONa+2.1 mL CH_3COOH/100 mL H_2O, pH 5.3). Nine serial dilutions were prepared with a 50% dilution factor, using a 1% NaCl solution as the blank. The results were reported in Gothe units.

Color intensity

A 50% (w/v) honey solution with warm water (45 °C) was prepared to determine this variable. The solution was filtered through a 0.45 μm MF-Millipore™ membrane, and the filtrate was analyzed using a Beckman DU® 650 UV-Vis spectrophotometer at 450 nm and 720 nm. The results were expressed in milliabsorbance units (mAU).

Total phenols

Total phenolic content was determined using the Folin-Ciocalteu method as modified by Kek *et al.* (2014). Measurements were taken at 765 nm. A calibration curve for gallic acid (20, 40, 60, 80, and 100 μg/mL) was developed. The results were expressed as milligrams of gallic acid equivalents (GAE) per kilogram of honey.

Flavonoids

Four milliliters of a 25% honey solution were mixed with 300 μL of 5% $NaNO_2$. After five minutes, 300 μL of 10% $AlCl_3$ and 2 mL of 1 M NaOH were added. Six minutes later, the solution was diluted to 10 mL with distilled water and mixed by inversion. Measurements were taken at 510 nm. Total flavonoids were quantified using a calibration curve with catechin over a concentration range (20, 40, 60, 80, and 100 μg/mL). Results were expressed as milligrams of catechin per kilogram of honey.

Antioxidant activity

The radical reduction method using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was performed. One gram of honey was dissolved in 1.0 mL of ethanol, creating 1×10^{-1} to 1×10^{-4} dilutions. Three 200-μL aliquots were taken from each dilution. Subsequently, 1.8 mL of a 0.003% DPPH solution were added to each aliquot, which was left in the dark for 30 minutes. Measurements were taken at 517 nm. Ascorbic acid ($C_6H_8O_6$) was used as a positive control and 100% ethanol was used as blank. The percentages of DPPH radical reduction were calculated using the following formula:

$$DPPH\ Reduction(\%) = 1 - \left(\left[sample\ absorbance\ /\ DPPH\ absorbance\right]\times 100\right)$$

The resulting curve was then subjected to a linear regression statistical analysis in Excel to determine the antioxidant activity of each sample in terms of its Median Effective Concentration (EC50).

Antibacterial activity

The antimicrobial activity bioassay was performed using the well diffusion method (Pimentel *et al.*, 2013) against suspensions of 1.5×10^8 CFU/mL of *Bacillus cereus* (ATCC 25923), *Staphylococcus aureus* (ATCC 4012), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 128), *Pseudomonas aeruginosa* (ATCC 27853), *Shigella flexneri* (ATCC 9748), and *Pseudomonas syringae* (ATCC 11043).

HPLC analysis of phenolic acids and flavonoids

Ten g of honey were dissolved in 100 mL of distilled water. The lipophilic fraction was separated from the aqueous phase with $(C_2H_5)_2O$ three times (v/v) (2:1, 1:1, 1:1) using liquid-liquid extraction. The organic solvent was filtered through a paper filter cone with Na_2SO_4. Finally, the solvent was evaporated using a rotary evaporator at 37 °C and 550 psi. The extracts were diluted to a 1% concentration with HPLC-grade acetonitrile and filtered through a 0.45 μm membrane.

High-performance liquid chromatography (HPLC) analyses were performed on a Waters Alliance™ e2695 separations module with a UV detector. A Luna® HPLC column (Phenomenex) was used to separate the extract compounds with a particle size of 5.0 μm, C18, 150×4.6 mm. A gradient elution was used with mixtures of (A) formic acid and (B) acetonitrile, starting the gradient with 98% of A; 100% of B at 70 min, and 98% of A at 72 min, with a flow rate of 0.5 μL. The profiles were detected at 255 nm.

Statistical analysis

The results of the physicochemical evaluations were expressed as mean values with standard deviations. Tukey's test was performed to compare means with a 0.05 significance level. The HPLC spectra were subjected to a chemometric analysis using a Principal Component Analysis (PCA), while Microsoft™ Excel 2010 and SIMCA 14.1 software were used to determine the hierarchical clustering. The data were preprocessed, adjusting the baseline to diminish or eliminate noise contribution. Additionally, the average of the spectra was determined.

RESULTS AND DISCUSSION

Degrees Brix and moisture

The percentage of °Brix in Melipona honey ranged from 76.5 to 77.25% g 100 g^{-1} without significant differences between the analyzed samples (Table 1). These results are similar to those reported by Moo-Huchin *et al.* (2015) for samples from the Yucatán Peninsula (72.8 to 77.3%) and very close to the data reported by Lage *et al.* (2012) for some Melipona honeys from Brazil (68.09 to 72.12%). In general, honeys produced by stingless bees have lower °Brix values than those produced by *A. mellifera* (≥75), because stingless bee honeys have a higher water content and lower total sugar percentage (Biluca, 2016). According to the European Union, honey must meet certain sugar composition criteria to be marketed as such —*e.g.*, the sum of fructose and glucose of flower honey should not be less than 60%. Therefore, a >60% sugar in honey is considered a parameter of honey authenticity (Belay *et al.*, 2013).

Vit *et al.* (2023) used Nuclear Magnetic Resonance (NMR) to determine 41 parameters in 20 honeys from three genera of stingless bees, reporting the presence of fructose, glucose, maltose, maltotriose, raffinose, and sucrose. Their study reported a variation in total sugar content of 25.07, 54.73, and 57.75 for *Geotrigona*, *Melipona*, and *Scaptotrigona*, respectively.

Moisture content determines the amount of water present in the product and is therefore a critical quality parameter of honey. It is associated with the degree of maturity of the hive, the botanical origin of the honey sample, extraction techniques, and storage conditions

(Belay, 2013). Typically, the moisture content of Melipona honey is higher (≤30%) than in honey produced by *Apis mellifera* (≤20%) (Ramón-Sierra, 2015). The analyzed samples recorded low moisture values ranging from 21.1% to 22.3%, which fall within the maximum moisture limit of 30% for Melipona honey (Vit *et al.*, 2004) (Table 1). The results of this study are consistent with other studies that have been carried out in the Yucatán Peninsula (Moo-Huchin *et al.*, 2015; Ramón-Sierra *et al.*, 2015).

Acidity

Total acidity (the sum of free acidity plus lactonic acidity) is a reference parameter indicative of the fermentation state of honey. According to the bibliography, this value is highly variable within the group, as acidity corresponds to the organic acids in honey, which vary according to the floral composition and bee species (Lage *et al.*, 2012; Souza *et al.*, 2006). The honey samples evaluated in this study were acidic (pH of 3.67e±0.02 to 4.06a±0.02) (Table 1). The pH values are consistent with those reported by Dardón and Enríquez (2008) and Fonte *et al.* (2013) for *M. beecheii*. However, the samples evaluated in this study showed highly variable total acidity, ranging from 55.83±1.61 to 77.35±7.86 meq kg^{-1}. Since the average value is not representative, the parameter should be referred to as a range of variation between minimum and maximum values. According to the guidelines proposed for the genus Melipona by Vit *et al.* (2004), the maximum permissible total acidity should be 70.00 meq kg^{-1}, although some of the samples exceed this value. Flora composition may be the cause of the abovementioned variation in acidity values.

Determination of HMF content and diastase activity

Hydroxymethylfurfural (HMF) and diastase are the parameters most frequently used for the evaluation of honey freshness. The former is produced by the degradation of fructose and inadequate treatments of honey, with a nearly negligible presence in fresh honey, while the latter is added to honey by the bee and its activity decreases in old or heated honeys (Oddo *et al.*, 1999). Although the use of diastase as an indicator of honey quality has been questioned (White, 1994), it is still used as a reference and was evaluated in this research.

The HMF values of the honey samples ranged from 2.87 mg kg^{-1} to 12.39 mg kg^{-1} (Table 1). These values are lower than those previously reported by Moo-Huchin *et al.* (2015), who recorded 4 to 45.5 mg kg^{-1} HMF values in honey samples collected in the

Table 1. Physicochemical parameters of *Melipona beecheii* honeys from Hopelchén, Campeche, Mexico.

Collection month	Sample	Brix %	Moisture (g/100 g)	pH	Total acidity meq/kg	HMF (mg/Kg)	Diastase activity (°Gothe)
July	M1	77.00	21.20	$3.67^{c}\pm0.02$	$70.30^{ab}\pm1.79$	$12.39^{a}\pm3.01$	$6.50^{b}\pm1.50$
	M2	77.25	21.20	$4.06^{a}\pm0.02$	$55.86^{b}\pm1.14$	$6.51^{ab}\pm0.02$	12.00^{a}
	M3	76.50	22.30	$3.96^{bc}\pm0.01$	$77.34^{a}\pm5.57$	$5.61^{ab}\pm0.72$	12.00^{a}
	M4	77.00	21.20	$4.00^{ab}\pm0.02$	$56.16^{b}\pm1.19$	$2.87^{b}\pm0.26$	8.00^{b}
	M5	77.00	21.20	$3.91^{c}\pm0.00$	$76.90^{a}\pm1.60$	$4.64^{b}\pm0.95$	5.00^{b}
March	Mixture (M6)	77.00	21.20	$3.79^{d}\pm0.01$	$72.02^{a}\pm1.44$	$6.59^{ab}\pm0.09$	8.00^{b}

Different letters in the same column indicate significant differences (ANOVA, $p<0.05$).

Yucatán Peninsula. For their part, Silva *et al.* (2013) reported that 10.80 to 15.76 mg kg^{-1} HMF values in nine honey samples of *Melipona subnitida* produced in Brazil. Complying with a low HMF content is important, because it guarantees the freshness of the honey purchased by consumers.

The HMF content recorded in this study suggests that the honey samples of *M. beecheii* collected in Hopelchén had been recently harvested.

Diastase is the enzyme responsible for converting starch into dextrins and sugars. The diastase number (DN) expresses, in Gothe units, the diastatic activity of honey as the number of mL of a 1% starch solution hydrolyzed by the enzyme in 1.0 g of honey. Vit *et al.* (2004) pointed out that Melipona honey exhibits low diastase activity and has a minimum diastase index on the Gothe scale (3.0). The diastase activity measurements in the samples ranged from 5.0 to 12.0 Gothe units. Therefore, the obtained data confirm that the evaluated honey was fresh, had been recently harvested, and was stored under appropriate conditions.

Color intensity

The color intensity of honey is a qualitative parameter that indicates the presence of pigments —which are in turn associated with the presence of antioxidants, such as carotenoids and flavonoids (Moniruzzaman *et al.*, 2013). The color intensity values of the honey ranged from 102 to 320 mAU (Table 2). Color has been related to the antioxidant activity of honey, with a higher content of phenolic compounds being reported in darker honeys (Moniruzzaman *et al.*, 2013).

Phenolic compounds and antioxidant activity

The total content of phenolic compounds and antioxidant activity values are presented in Table 2. The lowest total phenolic content (22.9 mg EAG kg^{-1}) was found in sample M4. Sample M3 showed the highest phenolic content value (37.33 mg EAG kg^{-1}). Samples M1 and M3 had some of the highest color intensity values, as well as a high phenolic content. However, a low correlation was recorded between these two variables ($R^2=-0.272$), similar to the correlation between color and flavonoid content ($R^2=-0.113$) and antioxidant activity ($R^2=0.304$). These results differ from those described by Can *et al.* (2015) (16.02 to 120.04 mg GAE 100 g^{-1}) and da Silva *et al.* (2016) (17.0 to 66.0 mg GAE g^{-1}).

Table 2. Color intensity, total phenolic and flavonoids contents, and half maximal effective concentration (EC50) of *Melipona beecheii* honey.

Collection month	Sample	Color intensity (mUA)	Total phenolic mgEAG/Kg	Flavonoids mg Cat/Kg	EC50
July	M1	$320^{a}\pm2.2$	33.41	0.058	1.26
	M2	$111^{c}\pm5.1$	25.15	0.071	0.64
	M3	$302^{a}\pm16.5$	37.33	0.077	0.66
	M4	$102^{c}\pm11.8$	22.9	0.054	0.86
	M5	$127^{bc}\pm2.5$	28.91	0.089	0.57
March	Mixture (M6)	$162^{b}\pm9.8$	23.01	0.085	0.61

The flavonoid content in the honey ranged from 0.058 to 0.088 mg catechin kg^{-1}; these results were similar to the findings of Muñoz *et al.* (2007), who reported a low flavonoid content (0.014-13.8 mg 100 g^{-1}) in a honey sample from Chile. Moniruzzaman *et al.* (2014) and other authors indicate that the geographical origin of honey affects its phenolic composition and flavonoid concentration, which in turn impact its antioxidant activity.

The antioxidant capacity of honey was determined by the DPPH assay. An EC50 between 1.261 and 0.57 mg mL^{-1} was obtained; however, these values were lower than those reported by Vit *et al.* (2009), Moniruzzaman *et al.* (2014), and other authors. These results may be caused by the low color intensity, low phenolic content, and low flavonoid content, which affected the antioxidant activity.

Antibacterial activity

A traditional and widely spread belief about the medicinal properties of Melipona honey pertains to its antibacterial properties. Packaging *M. beecheii* honey in eye drop dispensers is not uncommon. Various authors have demonstrated the inhibitory activity of different methods against some bacterial species, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*; however, the variations among the methodologies prevented the comparison of the results (Miorin, 2003; Campeau and Patel, 2014). The well diffusion method (Pimentel *et al.*, 2013) used in this work to determine the antibacterial activity of honey was performed against six human pathogens and one phytopathogenic bacterial species (Table 3).

All samples inhibited *S. aureus* and *P. syringae*. In the case of *P. aeruginosa*, sample M3 did not show inhibitory activity. The greatest inhibitory effect of the honey was observed against *S. aureus*, impacting growth in all evaluated dilutions and recording the highest inhibition values (0.50-1.5 cm). These results are similar to the findings of Zamora and Arias (2011), who reported that the analyzed honey samples exhibited greater antibacterial activity against *S. aureus*.

Honey had very similar inhibitory results against both *P. aeruginosa* and *P. syringae*. Inhibition halos were reported only in the first dilutions (75% and 50%), with lower inhibition values than those obtained for *S. aureus*. Although the growth of *E. coli* and *S. flexneri* was not totally inhibited, a qualitative decrease in growth was observed in the samples, as the cells grew poorly on the culture medium. This effect was observed in all samples against *E. coli*, while, in the case of *S. flexneri*, the decrease in bacterial growth was observed in samples M3 to M6.

Honey did not impact *B. subtilis* and *B. cereus* at all, as no inhibition was observed in any of the evaluated dilutions. This phenomenon had been previously reported by Vermeulen *et al.* (2005) and Blaser *et al.* (2007), who mentioned not only the susceptibility of *S. aureus* to Melipona honey, but also that the main inhibitory effect on growth was found in Gram-negative bacteria.

In this regard, antibacterial validation should be conducted with reference strains deposited in international collections. Since the antagonism evaluation methodologies are diverse, the bodies that validate official protocols should specify the validation method.

Table 3. Antibacterial activity of honey.

Collection month	Sample	Dilution %	Area of inhibition (cm)						
			Bacillus cereus	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Pseudomona aeruginosa***	***Shigella flexneri***	***Pseudomonas syringae***
July	M1	75	0.00	0.50	0.00	*	0.55	0.00	0.50
		50		0.50		*	0.40		*
		25		0.50		*	0.20		*
		10		0.40		0.00	0.10		0.00
	M2	75	0.00	0.60	0.00	*	0.75	0.00	0.50
		50		0.60		*	0.55		0.40
		25		0.60		0.00	0.40		0.00
		10		0.30		0.00	0.00		0.00
	M3	75	0.00	0.85	*	*	*	*	0.85
		50		0.80	0.00	*	*		0.65
		25		0.50	0.00	*	*		0.00
		10		0.00	0.00	*	*		0.00
	M4	75	0.00	1.50	0.00	*	0.80	*	1.50
		50		1.50		*	0.80		0.70
		25		1.00		*	0.00		0.70
		10		0.70		*	0.00		0.00
		75	0.00	1.30	0.00	*	0.80	*	0.80
		50		0.90		*	0.70		0.40
		25		0.90		*	0.70		0.00
	M5	10		0.60		*	0		0.00
March	Mixture (M6)	75	0.00	1.50	0.00	*	0.50	*	0.50
		50		1.50		*	0.00		0.50
		25		1.40		*	0.00		0.00
		10		0.80		*	0.00		0.00

The area of inhibition (cm) is shown after a 24-h incubation.
(*) Decreased concentration of bacterial growth.

Chemometric Analysis of Chromatographic Profiles by HPLC

Before performing chemometric analyses, a series of pretreatments had to be conducted on the raw chromatographic profiles, since baseline variations and retention time negatively affect the analysis results. The airPLS function was used to correct baseline deviations.

A principal component analysis (PCA) was applied to analyze the chromatographic data from the six honey samples, in order to understand similarities in the distribution and abundance of the chromatographic peaks.

The first principal component (PC1) was strongly associated with the variables of samples M5 and M6, accounting for 60% of the variance. The second principal component (PC2) explained 20% of the variance and was mainly associated with the variables of samples M1, M2, M3, and M4. The cumulative variance of these principal components amounted to 80% (Figure 1).

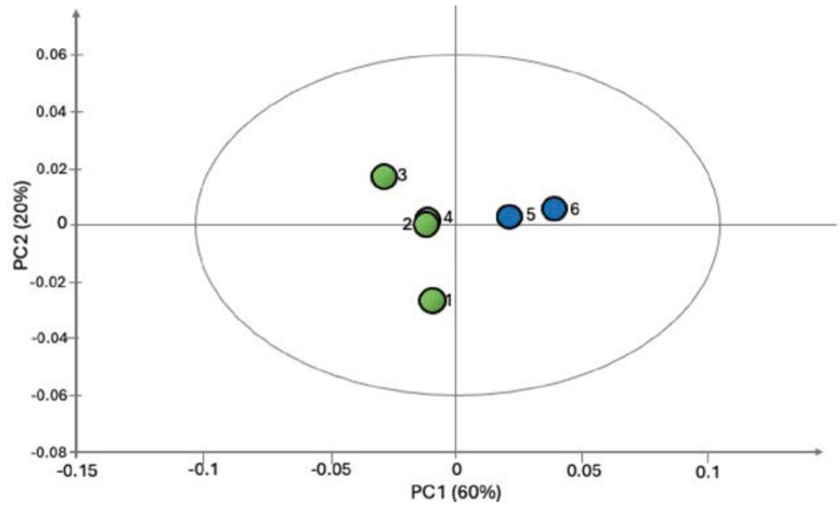


Figure 1. Result of the Principal Component Analysis (PCA) applied to the six honey samples.

A loading plot analysis was performed to determine the components related to the previous clustering. This type of analysis indicates the influence of a given component on the different chromatographic profiles. PC1 is the component at t_R 30 min, which influences the separation from the other samples, with higher concentrations in M5 and M6. The slight separation between them is attributed to the component at t_R 17.1 min, which is more abundant in M5. In contrast, the component at t_R 22.6 min influences grouping (b) and the component at t_R 29.0 min is estimated to be involved in the separation observed for sample M3 within its group. Meanwhile, the component at t_R 11.6 min separates sample M1 within PC2. Finally, the components at t_R 29.0, 24.7, and 23.7 min are correlated variables —*i.e.*, they are shared by all samples in PC2 (Figure 2). Another way to determine the similarity of the chromatographic profiles was the use of a hierarchical clustering analysis, calculating and comparing the similarity between samples. The resulting dendrogram enables the visualization of the distances between samples, forming groups based on their similarity.

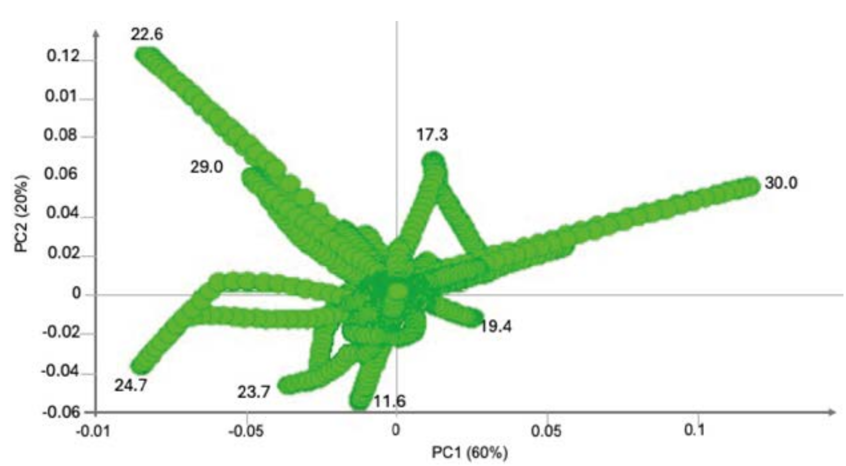
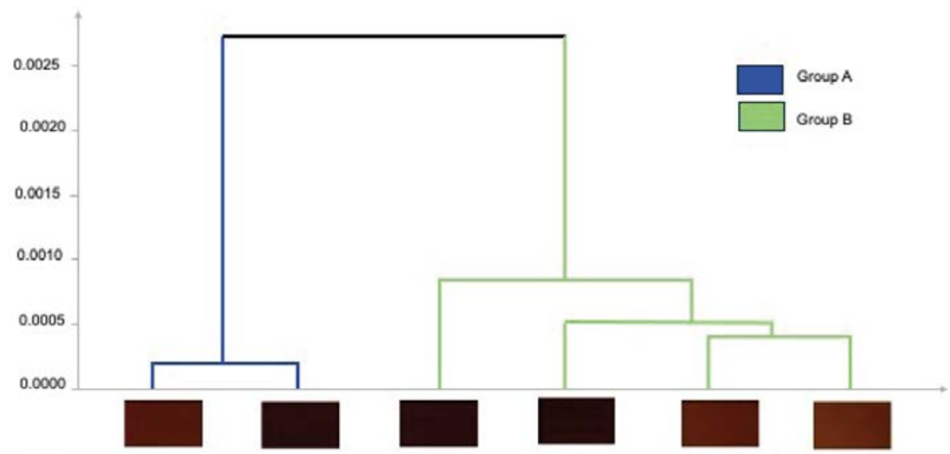


Figure 2. Principal Component Analysis of the resulting chromatographic profiles.

The dendrogram obtained in this study consisted of two groups: A (M5 and M6) and B (M1, which shares greater similarity with M3, and to a lesser extent with M2 and M4). This classification was correlated with the color intensity and phenolic content of the honeys. Group A comprised samples with intermediate levels of phenolic compounds and color intensity, while Group B divides the samples into those with higher and lower phenolic content and color intensity, respectively (Figure 3).



Sample number	M5	M6	M1	M3	M2	M4
Color intensity (mAU)	127	162	320	302	111	102
Total phenolic Content (mgEAG/g)	28.9	23.0	33.4	37.3	25.1	22.9

Figure 3. Color intensity of six honey samples with the same geographical origin.

Although no compound was identified, the principal component analysis indicates a variability among the small number of samples analyzed from Campeche, despite the proximity of collection sites. This result suggests the need to characterize honey according to its geographic region.

CONCLUSIONS

The advancements shown can contribute to the establishment of a legal and standardization framework (NOM: Official Mexican Standard) for Melipona honey. Some methodologies, such as the determination of other compounds by HPLC, are not currently applicable, given the lack of sufficient data about the diversity of the components of this type of honey. Its incorporation is, therefore, a pending matter. The therapeutic, antimicrobial, and antioxidant biological characteristic of Melipona honey are confirmed. The chemometric analysis of the chromatographic profiles (in this case, for *M. beecheii*) confirms the diverse botanical origins that can be found in the same geographic location.

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Colegio de Postgraduados

Revealed comparative advantage and competitiveness of Mexican mango exports

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ABSTRACT

Objective: The analysis of competitiveness of Mexican mango in the global market from 1994 to 2020 was conducted.

Design/methodology/approach: The revealed comparative advantage index (RCAI) and the normalized revealed comparative advantage index (NRCAI) were calculated, with the aim of understanding the exporting specialization of Mexico and thus determine the presence of comparative advantages in exports.

Results: The average values obtained for the USA market were 1.09 (RCAI) and 0.04 (NRCAI), and for the Canadian market 6.33 (RCAI) and 0.69 (NRCAI).

Conclusion: The export sector of Mexican mango is competitive and has comparative advantages because the indices are positive.

Keywords: Comparative advantage, competitiveness indices, production, market.

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INTRODUCTION

Developing countries produce 90% of tropical fruits and they come mainly from producers with small surfaces that destine part of their production to the global market (FAO, 2022). Therefore, they are beneficial for the agricultural sector because of the income and currencies generated through exports, highlighting their social and economic importance.

The main exporting countries of mango are Mexico, Thailand, Brazil, the Netherlands and Peru, which together contribute 67.01% of the global exports. India is the main producer and sixth exporter. Peru is the eighteenth producer and fifth exporter. China and Indonesia are the second and third producers, but they do not stand out among the main exporters, in contrast with the Netherlands, which is not a producer yet is positioned as the fourth exporter because it is a re-exporting country (FAOSTAT, 2022).

The previous data reflect that the global trade patterns are changing, which is why commercial interaction is definitive to obtain more advantages (Valencia et al., 2017). This implies that the countries diversify their efforts to obtain a broad margin of participation in the markets where competition between countries and economic sectors is the constant (Gómez, 2006).

Image by Kang-Rui LENG at Pixabay

Presently, international trade is progressively more intense, particularly in foods of high economic and social impact (Maya *et al.*, 2011), which is why the study of competitiveness is fundamental to determine the presence of comparative advantages that allow the sustainability of the population linked to the agro-exporting activity (De Pablo and Giacinti, 2012).

The concept of competitiveness is expressed as the ability of a country to conquer, maintain or increase its participation in international markets (Hilasaca, 2014). This can be described as a specific characteristic of the products that give them advantages in the global market, and their capacity to participate will depend to a great extent on their level of competitiveness in international trade (Contreras, 2000).

The comparative advantage is an important concept to explain commercial patterns; it establishes that each country should be specialized in the products whose relative costs are lower than in the rest of the countries (Pat *et al.*, 2009). However, in a model based on the differences of available resources, it is not possible to observe the relative costs, which is why Balassa (1965) establishes that it is not necessary to include all the factors that impact the comparative advantage, since it can be revealed through the observed commercial patterns (Hassan and Ahmad, 2018). The comparative advantage of observed data is called revealed comparative advantage and its calculation allows measuring the level of competitiveness of a country or a product in specific markets, during specific periods (Caamal *et al.*, 2017). The objective was to analyze the competitiveness of Mexican mango in the global market from 1994 to 2020, through the calculation of the revealed comparative advantage index (RCAI) and the normalized revealed comparative advantage index (NRCAI) to determine the presence of comparative advantages in exports. The working hypothesis suggests that the export sector of Mexican mango is competitive and has comparative advantages because the indices are positive.

MATERIALS AND METHODS

The database from the Statistical Division of the Food and Agriculture of the United Nations (FAO-FAOSTAT) was consulted to analyze and generate information.

Measuring competitiveness

The competitiveness of a product can be measured in the global market through a revealed comparative advantage index, with the convenience that it can be estimated using international trade statistics (Avendaño and Acosta, 2009). These indices allow obtaining a good approach to the exporting performance of countries in a specific product (Torres, 2009).

Balassa's revealed comparative advantage index

The revealed comparative advantage index (RCAI) allows understanding the exporting specialization of a country, calculated as a quotient between the participation of a product in the exports of a country and the participation of this same product in global exports (Caamal *et al.*, 2017). The procedure for the calculation is the following:

$$RCAI_{kij} = \frac{X_{kij}/XT_{ij}}{X_{kiw}/XT_{iw}}$$

Where: $RCAI_{kij}$=Is the revealed comparative advantage index for product *k* of country *i* toward country *j*; X_{kij}=Exports of product *k* of country *i* toward country *j*; XT_{ij}=Total exports from country *i* to country *j*; X_{kiw}=Exports of product *k* carried out by country *i* toward the world (*w*); XT_{iw}=Total exports of country i toward the world (*w*).

The RCAI reflects greater competitiveness as there is higher value obtained and vice versa; the following values can be obtained: 1) RCAI>0, there is comparative advantage for the country or product reflecting that it is competitive in international markets, and 2) RCAI<0, there is comparative disadvantage for the country or product reflecting that it is not competitive in the international market (Contreras *et al.*, 2019).

Normalized revealed comparative advantage index (NRCAI)

The Normalized Revealed Comparative Advantage Index (NRCAI) measures the degree of deviation of the real exports of a country based on its level of comparative advantage in terms of its relative scale regarding the global export market, since it takes into consideration the size of the countries and emerges as an alternative measurement capable of revealing the reach of the comparative advantage that a country has in a basic product more accurately and consistently than other indices. This allows making the results obtained in its calculation symmetrical, taking only values between −1 and 1 (Yu *et al.*, 2009); the following formula is suggested for its calculation:

$$NRCAI = \frac{RCAI - 1}{RCAI + 1}$$

Where: *RCAI*=Revealed comparative advantage index; the values of *NRCAI* are located in 3 ranges: between +0.33 and +1, there is comparative advantage for the country in the product's exports; between −1 and −0.33, there is disadvantage for the country in the product's exports; and between −0.33 and +0.33, there is a trend toward intra-product trade (Durán and Álvarez, 2011).

With the use of indicators of revealed comparative advantage (RCAI and NRCAI), the magnitude of the comparative advantage that a country has in a specific product can be explained; in addition, they allow performing comparisons of comparative advantages between goods based on their value obtained throughout the years and their markets (Yildiz and Mete, 2019).

Export coefficient

The export coefficient (EC) reflects the relationship that is established between the exports value and the production value during a period of time. It measures the percentage of production that is exported; a higher export coefficient means that a higher proportion of the production is exported, and a lower export coefficient means that a lower proportion of the production is exported (Caamal *et al.*, 2017). The procedure for calculation is:

$$EC_{ij} = \left(\frac{X_{ij}}{Q_{ij}}\right) * 100$$

Where: EC_{ij}=Export coefficient of product i from country j; X_{ij}=Exports of product i from country j; Q_{ij}=Total production of product i from country j.

Export specialization coefficient

The export specialization coefficient (ESC) allows measuring the degree to which a country has a comparative advantage, classifying it as an exporter of the product, and relating the exports with the apparent national consumption (ANC); if the ESC is higher than zero, it is considered a net exporting country and competitive at the national level in the product (Caamal *et al.*, 2018). For its calculation, the following formula is used:

$$ESC_{ij} = \left(\frac{X_{ij}}{Q_{ij} + M_{ij} + X_{ij}}\right) * 100$$

Where: ESC_{ij}=Export specialization coefficient; X_{ij}=Exports of product i from country j; M_{ij}=Imports of product i from country j; Q_{ij}=Domestic production of product i from country j.

RESULTS AND DISCUSSION

Variables and indicators of competitiveness

The export coefficient (EC) indicates the percentage of the total production of a country that is destined to exports; for the case of Mexico, during the period of analysis (1994-2020), it presents an average of 14.63%; this result does not differ much from that obtained by Caamal *et al.* (2018), since in their analysis they obtained an export coefficient of 17% for the 2005-2016 period. This explains that although it is not such a high proportion of the production, it indicates the importance of the increase in exports because its behavior presents a positive trend, reflecting that mango is a viable product for the global market (FAOSTAT, 2022).

The export specialization coefficient (ESC) indicates the degree of market penetration of a country according to its exports and apparent consumption. Figure 1 shows that during the period of analysis the index shows a growing trend, averaging around 17.22% and indicating that Mexico has the capacity to be a competitive country in mango exports (FAOSTAT, 2022).

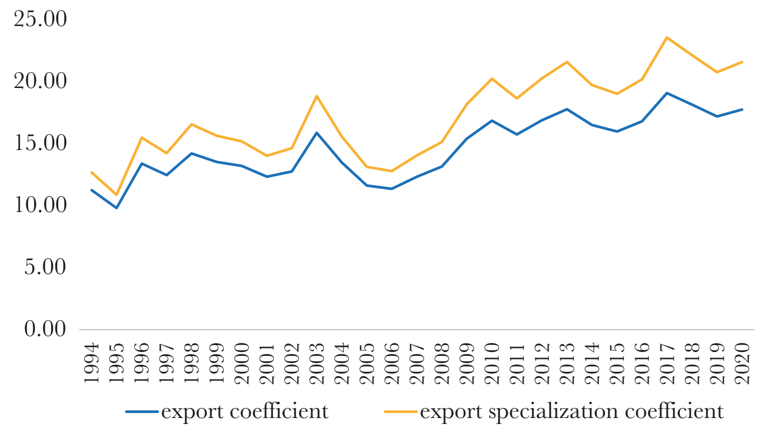

Figure 1. Behavior of the export coefficient and the export specialization coefficient.
Source: Prepared by the authors with data from FAOSTAT, 2022.

Distribution and behavior of exports

The main destinations of exports are the United States of America (USA) and Canada (CAN), with an average market participation percentage of 87.01% and 9.76%, respectively (Table 1). The data indicate that a large part of the exports are concentrated in the United States market, because of the comparative and competitive advantages that Mexico has compared to other countries (FAOSTAT, 2022).

The volume of Mexican mango exports toward the main export destinations has been increasing. Exports to the United States averaged an annual growth rate of 5.05% and a total annual growth rate of 4.83%. Canada presented an annual growth rate of 5.30% and a total annual growth rate of 4.85% (FAOSTAT, 2022).

Balassa's revealed comparative advantage index

The United States of America is the main mango consumer in the world, so it represents an excellent export destination for the Mexican product; the values of RCAI obtained

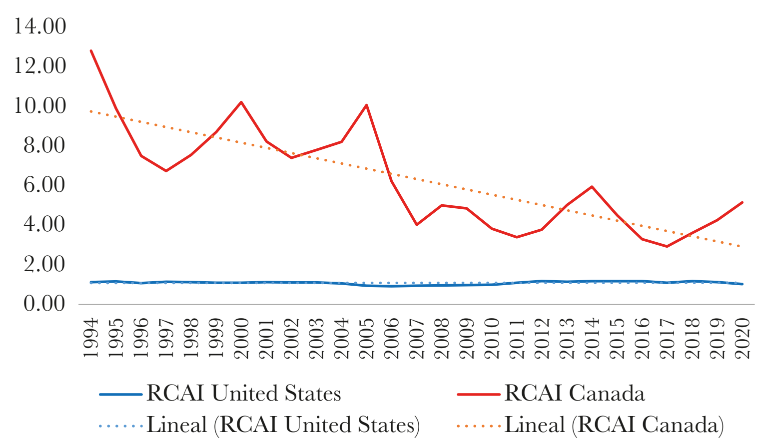

Figure 2. Behavior of the RCAI in the United States and Canada.
Source: Prepared by the authors with data of FAOSTAT, 2022.

Table 1. Mango exports from Mexico (tons).

Year	World Exports	Exports to United States	%	Exports to Canada	%
1994	125,777	109,237	86.85	11,988	9.53
1995	131,720	114,648	87.04	10,478	7.95
1996	159,416	140,059	87.86	11,552	7.25
1997	187,112	165,470	88.43	12,626	6.75
1998	209,393	180,133	86.03	18,434	8.80
1999	203,985	178,042	87.28	18,975	9.30
2000	205,940	180,644	87.72	20,107	9.76
2001	194,541	172,135	88.48	18,617	9.57
2002	194,590	166,728	85.68	18,494	9.50
2003	216,315	190,397	88.02	21,356	9.87
2004	211,936	186,163	87.84	22,369	10.55
2005	195,210	169,984	87.08	21,113	10.82
2006	232,383	201,450	86.69	24,817	10.68
2007	236,005	202,498	85.80	25,488	10.80
2008	226,084	194,914	86.21	24,051	10.64
2009	232,643	199,122	85.59	26,798	11.52
2010	275,367	238,753	86.70	27,907	10.13
2011	287,772	245,608	85.35	33,166	11.53
2012	297,295	257,469	86.60	30,771	10.35
2013	338,169	295,135	87.27	36,256	10.72
2014	289,646	251,433	86.81	32,858	11.34
2015	331,149	290,812	87.82	33,512	10.12
2016	369,316	329,016	89.09	32,962	8.93
2017	435,816	353,973	81.22	38,732	8.89
2018	395,537	349,076	88.25	37,959	9.60
2019	412,453	368,120	89.25	36,827	8.93
2020	421,637	372,240	88.28	41,118	9.75
Growth rate (%)	5.01	5.05		5.30	
Average annual growth rate (%)	4.76	4.83		4.85	

Source: Prepared by the authors with data from FAOSTAT, 2022.

show a constant behavior with low variation, an average index of 1.09 was obtained. Canada is the second export destination of Mexican mango; the Canadian market shows a clear comparative advantage because it presents higher RCAI values than the unit with an average of 6.33; the variation in this market is very noticeable with a decreasing trend (FAOSTAT, 2022). This reflects that Mexican mango is a product that presents revealed comparative advantage (RCA) in the main export markets, indicating that it is a competitive product in the global market.

Normalized revealed comparative advantage index

During the period of analysis, average values of 0.04 and 0.69 were obtained for the United States of America and Canada, respectively, showing a positive trend for the USA market and a slightly negative trend for the Canadian market (Figure 3). This reflects that Mexico presents comparative advantages in mango and therefore it is increasing its competitiveness, thus maintaining its position in international markets, since during the period analyzed it has remained as the main exporter and as the fourth producer of mango globally (FAOSTAT, 2022).

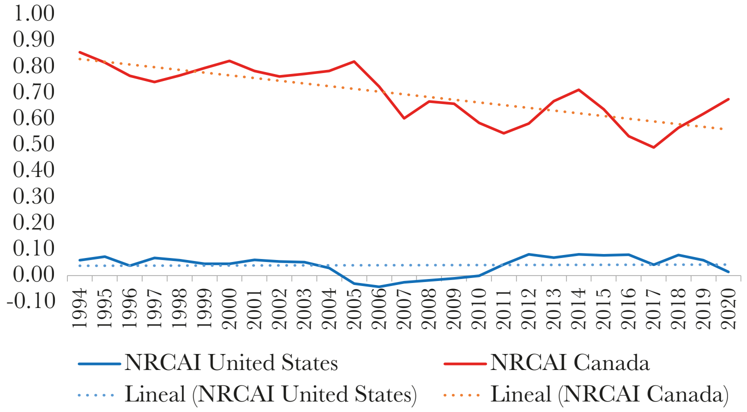

Figure 3. Behavior of the NRCAI of the United States and Canada.
Source: Prepared by the authors with data from FAOSTAT, 2022.

CONCLUSIONS

Mexico has been consolidated as a leading country at the global level, positioned as fourth producer and as the main exporter of mango; its main commercial partners are the United States of America and Canada, which together hoard 96.77% of total exports. In addition, an export coefficient of 14.63% and an export specialization coefficient of 17.22% are seen, reflecting that mango is an exportable product and that Mexico is a competitive country in the global market. The RCAI obtained are higher than zero, reflecting that Mexican mango is competitive in global markets, mainly in the United States market because it presents higher stability and a positive trend in contrast with the Canadian market which, despite presenting very high values of RCAI, is a market that presents very noticeable variations and a negative trend.

The average NRCAI of the United States market is found between −0.33 and +0.33 indicating a trend toward intra-product trade; however, the trend is positive so there are comparative advantages compared to the Canadian market which, despite being within the range of +0-33 and +1, presents a slight negative trend. However, since Mexico presents a NRCAI of +0 in the entire period analyzed in both markets, the exporting specialization of Mexico in mango toward these export destinations can be identified.

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Tanniferous trees used for gastrointestinal nematode control in small ruminants in tropical zones

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ABSTRACT

The objective of this review is to present an overview of the potential of tanniferous trees for the control of gastrointestinal nematodes in sheep in the tropics. A systematic review of scientific articles in various databases was carried out on the potential of tropical tanniferous trees, their effect on the control of gastrointestinal parasites and the improvement of weight gain, a range of 16 years (2006-2022) was considered in the publications consulting a total of 150, of which 46 were related to the topic for the selection of information, a total of 31 scientific articles were obtained, which were considered. Table 1 shows that foliage extracts of species considered tanniferous such as *L. leucocephala* cause inhibition of the hatching of eggs and on the infective larva L3 of *H. contortus*. The bibliographic evidence consulted allows us to elucidate that the use of tropical trees with forage potential and high contents of condensed tannins in their tissues can be successfully used for the control of gastrointestinal nematodes *in vitro* and *in vivo*. The largest percentage of those consulted used tannin-bearing plant extracts that have been shown to have anthelmintic properties and high nutrient content, making them a viable and economical alternative for feeding small ruminants in production systems in tropical areas due to their consumption preference and their high nutrient content.

Keywords: Secondary metabolites, food potential, tanniferous trees.

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INTRODUCTION

The diet of ruminants in tropical livestock production systems depends essentially on grasses (Poaceae) which present a seasonal production of biomass; this particularity suggests the need to develop strategies for sustainable feeding through the use of local forage resources with feed production throughout the year. In these agroclimatic regions there is a broad diversity of tropical tanniferous plants (TTP), such as tzalam (*Lysiloma lastisiliquum* L.), huaxin (*Leucaena leucocephala*), chimay (*Acacia pennatula*), jabín (*Piscidia pisipula* L.), carob (*Ceratonia siliqua*), and pixoy (*Guazuma ulmifolia*), among others. The need to identify and take advantage of these resources in every region emerges, to decrease the dependency on inputs and technologies that reduce the profitability in livestock systems (SIAP, 2017). In addition, these tanniferous plants are promising to control gastrointestinal nematodes (GIN) in ruminants (Hoste *et al.*, 2015), when 3-6% of condensed tannins is included in ruminants' supplementation. This is especially important because the infestation by gastrointestinal nematodes is a limitation for development in tropical livestock production, where the economic losses are reflected in high rates of mortality, morbidity, high costs for their control, and reduction of reproductive and productive parameters (Almada-Arturo, 2015)3.

Condensed tannins (CT) are flavonoids that act as principal pigments in many seeds, and they are present in the plant tissues of some forage plants and function as a defense method against insects and herbivores, and an improvement in animal production has been seen due to the diversity in the chemical properties they have (Torres-Acosta *et al.*, 2008). Authors such as Provenza (2006) state that ruminants manage to better satisfy their nutritional needs and regulate the intake of toxins when the diet includes a diversity of natural ingredients, in contrast with diets based on commercial feed. One of the characteristics of tannins is their astringency, caused when the tannins join the saliva proteins and adhere to the mucosa membranes of the animal's mouth and form complexes between the tannins and saliva glycoproteins which, as consequence, increase the salivation and decrease the palatability of the forage (Jon Lasa *et al.*, 2010). Based on this, the study conducted an analysis and updating of the potential of tanniferous tree species for the control of gastrointestinal nematodes of sheep in the tropics.

MATERIALS AND METHODS

A systematic review of scientific articles and publications was carried out with databases such as Refseek, Scielo, Dialnet, Redalyc and Google Scholar on the potential of tropical tanniferous trees and control of gastrointestinal parasites, in addition to variables of improvement in weight gain in sheep. Information consultation was done using the keywords: "taninos condensados" + "nematodos gastrointestinales", "plantas taniníferas" + "ovinos en pastoreo" + "*Haemonchus contortus*" + "zona tropical", "leguminosas ricas en taninos" + "ganancia de peso" + "rumiantes", "metabolitos secundarios" + "potencial forrajero", "concentración" + "taninos" + "en la inclusión" + "en ovinos". A range of 16 years (2006-2022) in the publications was considered, and similarly titles in Spanish and English published within the period were considered.

The number of publications consulted was 150, of which 46 were related to the effect of secondary metabolites of tanniferous plants in small ruminants. Abstracts and complete documents were considered, resulting in 31 scientific articles, whose information was organized into two sub-themes to ease their analysis and discussion.

Sub-theme 1: The diet in production systems of small ruminant meat in tropical zones

In tropical zones, sheep and goat production systems are not very competitive compared to temperate zones because the grasses used present high fiber content and low nutritional quality, especially in regions with acid soils of low fertility and long periods of drought (Tiemann *et al.*, 2008), which negatively affect the productive and reproductive parameters of animals. Grasslands in tropical zones in Mexico are a natural resource in livestock production systems (LPS) considered the sustenance of greatest importance for grazing animals. A large part of ruminants in the country depend on this resource as main source of nutrients, although the plants that are most abundant in the grasslands have high nutritional value, it is considered as a limitation for their consumption due to the high content of secondary compounds (Perevolotzky *et al.*, 2006).

The most frequent technological innovations in the diet of small ruminants in the tropics are ensilage of corn stubble, starter feed for lambs (creep feeding), integral diets for the fattening stage, and diets for females in gestation stage and lactation, and also inclusion of urea in traditional diets (Rodriguez-Castillo 2017). The ruminants can satisfy the needs for nutrients and regulate the intake of secondary compounds when they are offered a variety of foods. This fundamental variety is obtained from the TTP that can present beneficial effects on animal health and nutrition at low doses and appropriate mixtures; the variety of foods allows animals to express their dietary preferences, which at the same time improve their wellbeing.

Sub-theme 2: Effect of the inclusion of condensed tannins on forage trees and shrubs on GIN decrease and weight gain in sheep

Tree and shrub species with forage potential in the tropics are characterized by the high concentrations of condensed tannins (CT) with concentrations between 2 and 4% in dry base, and these parameters are optimal to obtain physiological benefits in ruminants (Tiemann et al., 2008). They also present a high protein content (14 to 28%) with fiber contents below 40% which allow a higher voluntary consumption and digestibility obtaining increases of 50% or more in the productive yields, such as weight gain, in comparison to tropical grass species (Poaceae). The tanniferous species contributed to the sustainability of the LPS by controlling erosion and improving the physical and biological conditions of the soil, and the biomolecules that it contains are considered responsible for reducing the levels of gastrointestinal parasites and increasing animal production, particularly in young small ruminants (Rey-Obando *et al.*, 2011).

The sheep kept in grazing are exposed to different diseases; two of the main conditions are gastrointestinal parasite diseases and gas formation in the rumen (Márquez-Lara and Suarez-Lodoño, 2008). After being ingested, the infected larvae are unsheathed in the digestive tract and shed twice until they are pre-adults, where they move freely on the surface of the gastric mucosa and mature sexually; then they copulate and the females begin to lay eggs, thus concluding the cycle (Cepeda-Martinez, 2017; Sepúlveda-Jimenéz *et al.*, 2018).

The effects caused by the consumption of tanniferous plants on the populations of GIN can be classified into direct ones with the interactions they have on the physiological functions of the GIN, and indirect ones that constitute the improvement in protein absorption. It has been shown that a higher assimilation of proteins is associated with the improvement in immunity of the host (Hoste *et al.*, 2012). The action mechanism of tannins on *H. contortus* larvae (L3) prevents the development of its evolutionary cycle and in adult nematodes, tannins join the mouth and reproductive tract of parasites (as a result of the affinity of tannins for the proline-rich proteins in the nematode cuticle) (Torres-Acosta, 2008). The response of animals to the intake of CT depends on their concentration in the plants, since plants with concentrations between 5% and 10% of the DM reduce the consumption and digestibility of the forage, while the concentrations between 2% and 4%

of the DM favor the intestinal absorption of proteins due to the decrease of proteolysis by the ruminal microflora.

Studies carried out by Cristel and Suárez (2006) have shown that the consumption of fodders with medium to high CT contents by parasitized sheep resulted in a reduction of the egg count per gram of feces (EPG) and adult parasites. In a coffee shop test carried out by Torres Acosta (2008), the animals had a consumption preference for TTP that contain a large variety of secondary metabolites such as *Acacia pennatula*, *Lisyloma latisiliquum*, *Piscidia piscipula* and *Leucaena leucocephala* instead of *Brosimum alicastrum* that has good digestibility and scarce amount of tannins. Bonilla-Valverde (2017) evaluated a diet formulated with corn grain, soy flour and corn straw with inclusion of tannin extract from *Schinopsis balansae* and *Castanea sativa* with concentrations of 0.15, 0.30 and 0.45% in sheep fed at free access, showing that the daily weight gain and feed conversion improved with the inclusion of 0.15 and 0.30% of tannin extract in the diet.

Other authors such as Martínez-Martínez (2018) included 4% of DM from commercial SilvaFeed® CT in the diet of sheep with consumption of 1200 g d^{-1} of DM and this did not affect the productive variables such as daily weight gain and feed conversion. Maldonado-Peralta (2018) evaluated the fresh forage of *Guazuma ulmifolia* fed to sheep in grazing with *Cynodon nlemfuensis* and supplemented with 0, 25 and 50% of *G. ulmifolia* in proportion to the requirement of DM and obtained a higher consumption of DM in 20% with the treatment of 50% of *G. ulmifolia* improving the feed conversion.

Research carried out by Rodríguez Fernández *et al*. (2013) showed that the design of silvopastoril arrangements with fresh forage of *Guazuma ulmifolia*, *Leucaena leucocephala* and *Crescentia cujete* in grazing goats with grasslands of the guinea grass *Panicum maximum* cv. Tanzania and arrangements based on *G. ulmifolia*, *C. cujete* and *L. leucocephala*, influenced in a higher weight gain when *G. ulmifolia* and *C. cujete* were combined with 22.5 and *L. leucocephala* with 33.6 g day, while with Poaceae (formerly grasses), the weight gain was 13.2 g day. It has been established that young or lactating male lambs fed with species from the Fabaceae family (formerly legumes) increase their weight because their organism responds favorably to the supplementary additions of protein in the diet; in contrast, the same does not happen in adult animals where the amino acids are not a limitation for their performance (Márquez-Lara and Suarez-Lodoño, 2008).

Table 1 shows that the forage extracts from species considered tanniferous such as *L. leucocephala* cause inhibition of egg eclosion (López-Rodriguez 2022; Rivero-Pérez, 2018) and on the infectious larvae L3 of *H. contortus* (López-Rodriguez 2022; Rivero-Pérez, 2018; Castañeda-Ramirez, 2017). Méndez-Ortiz (2019), Sandoval GV (2019), G.I. Ortiz-Ocampo (2016), C. Martínez-Ortíz-de-Montellano (2010) and Felix Heckendorn (2007) tested different plant extracts *in vivo* such as *Gymnopodium floribundum*, *L. leucocephala*, *Schinopsis balansae*, *Cichorium intybus*, *Lotus corniculatus*, *Onobrychis viciifolia* and *Lysiloma latisiliquum* where they agreed in the favorable results of every case with reductions of H. contortus hpg, except for Ortiz-Ocampo (2016) with Coffea arabica extract that did not reduce the hpg value of the nematode.

Table 1. Effect of condensed tannins on gastrointestinal nematodes of sheep.

Reference	Plant species	Parasite	Method of use	Livestock species	Condition of the experiment	Find
López-Rodriguez (2022)	*Leucaena leucocephala*	*H. contortus*	hydroalcoholic extract of foliage	Sheep	*In vitro* with concentrations of 100, 90, 80, 70, 60 and 50 mg mL^{-1}	Hydroalcoholic extract of *L. leucocephala* showed 71% inhibition of egg hatching at 100 mg mL^{-1}.
Nora Antonio-Irineo (2021)	*Gliricidia sepium*, *Leucaena leucocephala*, *Guazuma ulmifolia* and *Bursera simaruba*	*H. contortus*, *Trichostrongylus* spp., Oesophagostomum spp., Cooperia spp., and Nematodirus spp.	Aqueous extracts	Sheep	*In vitro* with three concentrations 0.75, 1.00 and 1.25 mL	Extracts of *Leucaena leucocephala*, *Gliricidia sepium* at doses of 1.25 mL had a higher ovicidal activity.
Méndez-Ortiz (2019)	*Gymnopodium floribundum*	*H. contortus*	Sheet flour	Sheep	*In vivo*, feed with 20, 30 and 40% flour	Leaf meal of *G. floribundum* was 40% in the diet reduced the load of female *H. contortus* worms.
Sandoval GV (2019)	*Schinopsis balansae*	*H. contortus* and *Trichostrongylus*	Aqueous extraction of the heartwood of the Quebracho tree	Goats and sheep	*In vivo*, 500 g of ground corn plus the daily addition of 25 g of TC.	The hpg was low and similar in the first three samplings, the hpg of the control decreased being lower than the group that consumed tannins, the treatment represented a dose without anthelmintic effect.
Rivero-Pérez (2018)	*Leucaena leucocephala*	*H. contortus*	Hydroalcoholic pod extract	Sheep	*In vitro* with concentrations 50, 25, 12.5 and 6.25 mg/mL	The extract had an effect on the inhibition of the hatching of field nematode eggs and on the infective L3 larva.
Castañeda-Ramírez (2017)	*Acacia collinsii*, *Lysiloma latisiliquum*, *Havardia albicans*, *Senegalia gaumeri*, *Mimosa bahamensis*, *P. piscipula*, *Acacia pennatula*, *Gymnopodium floribundum*, *L. leucocephala* and *Bunchosia swartziana*.	*H. contortus*	Methanol Extracts:Water	Sheep and goats	*In vitro*, egg hatching and L3 establishment were tested.	No effect was found on the hatching of *H. contortus* eggs, but it did on L3 establishment.
G.I. Ortiz-Ocampo (2016)	*Acacia Pennatula* and *Coffea arabica*	*H. contortus*	Acetone extract:water	Sheep	*In vitro*, inhibition of larval unsheathing was tested	*H. contortus* tolerated *A. pennatula* extract at concentrations of 150 and 300 μg extract/mL PBS and *C. arabica* extract reduced unsheathing from 150 μg extract/mL.

Table 1. continues...

Reference	Plant species	Parasite	Method of use	Livestock species	Condition of the experiment	Find
G.I. Ortiz-Ocampo (2016)	*Coffea arabica*	*H. contortus*	Acetone extracts:water from the precast by-product	Sheep	*In vivo* with the inclusion of 10% of the precast by-product extract	*C. arabica* extract did not reduce the value of hpg *H. contortus*.
C. Martínez-Ortíz-de-Montellano (2010)	*Lysiloma latisiliquum*	*H. contortus*	Fresh foliage	Sheep	*In vivo*, it was consumed *ad libitum*	*L. latisiliquum* directly influences the biology of adult *H. contortus* by affecting nematode size and female fecundity.
JA Calderón-Quintal *et al.* 2010	*A. pennatula*, *L. latisiliquum*, *Piscidia piscipula* and *Leucaena leucocephala*	*Haemonchus contortus* strains CENID-INIFAP and UNAM	Acetone extracts:water from foliage	Sheep	*In vitro*, inhibition of larval migration was tested	*A. pennatula* and *L. latisiliquum* inhibited larval migration in two strains of *H. contortus*, the CENID-INIFAP strain. *A. pennatula*, *L. latisiliquum* and *P. piscipula* had an effect on the UNAM strain.
Abdul Jabbar (2007)	Commercial CT*	*H. contortus*	TC Commercial Extract	Sheep	*In vivo*. Diets with 2% and 3% TC	HPGs had a gradual reduction, differing significantly on days 60-120 in both diets.
Felix Heckendorn (2007)	*Cichorium intybus*, *Lotus corniculatus*, *Onobrychis viciifolia*.	*H. contortus* and *Cooperia curticei*	Fresh forage	Sheep	*In vivo*	There was a significant reduction of 89, 63 and % hpg of *H. contortus* with *C. intybusachicory*, *L. corniculatus* and *O. viciifolia*. No anthelmintic effect was found against *C. curticei*.
Abdul Jabbar (2007)	*Chenopodium album* and *Caesalpinia crista*	*H. contortus*	Raw aqueous methanolic extract of *Chenopodium album* foliage and seeds of *Caesalpinia crista*	Sheep	*In vitro*, inhibition of egg hatching was tested	*C. crista* (0.134 mg/mL) is more potent in egg hatching than *C. album* (0.449 mg/mL).
Abdul Jabbar (2007)	*Chenopodium album* and *Caesalpinia crista*	*H. contortus*	Crude aqueous methanolic extract	Sheep	*In vivo*	The maximum reduction in hpg of *H. contortus* was observed with *C. crista* (93.9%). In 93.9 and 82.2% with and *C. album* at 3.0 g/kg, respectively.

*Commercial CT prepared from commercially available tannin {Kenya source, used in the textile industry and which contains 1.19% g CT/kg DM according to what was determined by the reagent Butanol-HCl method (Porter *et al.*, 1986)}.

The bibliographical evidence consulted allows elucidating that the use of tropical trees with forage potential and high content of condensed tannins in their tissues can be successfully used to control gastrointestinal nematodes in vitro and in vivo, primarily against *H. contortus*. It seems that the antagonistic effect on any of the stages of the physiological process of gastrointestinal nematodes is associated to the type and concentration of condensed tannins in the plants studied, as well as the application method. However, experimentation under conditions of conventional productive management of tropical regions is necessary to discern the anthelminthic potential of tanniferous species, and to develop a practical and inexpensive method to facilitate the adoption by producers.

CONCLUSIONS

In the highest potential of studies consulted, extracts from tropical tanniferous plants were used that proved to have anthelmintic properties and high nutrient content. Because of this, the suggestion is to evaluate the effect of consumption of these plants fresh or in flours on the incidence of GIN in ruminants. The use of tanniferous plants is a viable and economic alternative for the diet of small ruminants in the production systems of tropical zones due to their consumption preference and high nutrient content.

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Plant diversity and uses in family gardens, a case study

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ABSTRACT

Objective: To characterize the current condition of home gardens in the Ejido Nueva Esperanza 1st Section, Palenque, Chiapas.

Methodology: A semi-structured interview was conducted with 43 families to determine socioeconomic conditions, garden characteristics, floristic composition, and their uses (satisfaction). Gardens were categorized by size (small, medium, and large) and location (center, intermediate, and periphery of the community). Data were analyzed descriptively to obtain frequencies, percentages, and averages. Additionally, Shannon-Wiener (H') and Simpson (S) diversity indices were calculated according to size and location.

Results: Families were found to be below the poverty line, with agriculture as their primary activity. A total of 3549 individuals were recorded, grouped into 46 botanical families, 82 genera, and 89 species. Native species were the most dominant at 52%. There were 33 tree species, 33 herbaceous species, 15 shrubs, 5 rosettes, and 3 arborescent species. Families reported using plants for fruit, medicinal purposes, ornamentation, horticulture, timber, condiments, fuel (firewood), and medicinal-horticultural purposes, primarily for self-consumption. The highest number of individuals recorded were fruit trees, with 28 species. Small ($H'=2.8$), intermediate ($H'=2.6$), and peripheral ($H'=2.6$) gardens showed higher diversity similar to Fisher's alpha.

Implications: This study highlights the importance of floristic composition and plant species diversity in home gardens concerning the uses attributed by families.

Conclusion: The diversity and composition of plant species in home gardens are determined by their location and size, as well as the value of use that the family attaches to them.

Keywords: Family agriculture; wealth; floristic composition; plant use; family economy.

INTRODUCTION

The biodiversity present in home gardens is determined by traditional knowledge and the value of use that families attribute to plant and animal species over time (Watson and Eyzaguirre, 2002; Mariaca, 2012; Reyes-Betanzos and Álvarez-Ávila, 2017).

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Home gardens are ancestral production systems highly adaptive, located around the house where the family has recreated from generation to generation (Mariaca, 2012), assembled within a logic reflecting the structure and social dynamics not only of the household but also of the community (Lope-Alzina *et al.*, 2018). Ecological, agronomic, cultural, and social processes occur within these gardens, considering cultural parameters related to their floristic composition (García de Miguel, 2000), biodiversity conservation, food production for family consumption, and generating monetary income from their products or by-products (Mariaca, 2012).

Home garden production is one of the oldest land-use activities. Globally, it has been highlighted as a strategy for achieving the Millennium Development Goals and subsequent Sustainable Development Goals (Montemayor *et al.*, 2007; Krishnamurthy *et al.*, 2017).

The home garden as a traditional agroecosystem provides various fulfillments with use value and potential benefits (Dussel, 2014), especially those related to food, health, economic income, rituals, fuel, and ornaments to provide an aesthetic vision in family gathering spaces and social relationships that contribute to maintaining their quality and enjoyment (González, 2008; Cahuich-Campos, 2012; Olvera-Hernández, 2017). They also contribute to generating direct and indirect economic income (Pulido *et al.*, 2008) with minimal investment (Toledo *et al.*, 2008). Additionally, they are considered *in situ* biodiversity reservoirs as they contribute to the care, conservation, distribution, and domestication of useful species (Pulido *et al.*, 2008; Escobar and Estrada, 2015; Salazar-Barrientos *et al.*, 2015; Bautista-García *et al.*, 2016; Castañeda-Navarrete, 2021).

Despite global recognition and high ratings in productive and service functions, home gardens have not been given concrete importance as an inclusive development strategy (Krishnamurthy *et al.*, 2017). Many initiatives, programs, and promoted projects have failed by not taking into account local culture and realities (Cano Contreras, 2015), leading to the possible extinction of gardens as sustainable and highly productive systems in their various dimensions (González, 2007). Consequently, in many places, their productive condition and benefits do not contribute to reducing living costs or meeting family needs.

Due to the increasing urbanization in the study community from family growth, commercial activities, and the establishment of oil palm (*Elaeis guineensis* Jacq) and rubber (*Hevea brasiliensis* Muell Arg) crops, which are the primary income source for most families (Castellanos, 2018; Méndez y Mier y Terán, 2020), the role of gardens as productive units is being relegated and undervalued. Therefore, understanding floristic richness allows us to document diversity, plant uses, and benefits, as well as changes in management priorities (Pulido-Salas, 2017). Consequently, it is necessary to "untangle" the implicit complexity of home gardens and understand their intimate relationship with the biological, cultural and social environments (Mariaca, 2012; Lope-Alzina *et al.*, 2018).

Therefore, this research aimed to characterize the current condition of home gardens in the Ejido Nueva Esperanza 1st Section to understand the diversity, floristic composition, and use value as satisfiers of plant species in family gardens in the Ejido Nueva Esperanza 1st Section, Palenque, Chiapas.

MATERIALS AND METHODS

Study area location

The research was conducted in the Ejido Nueva Esperanza 1st Section, Palenque, Chiapas, located at Km. 96 of Federal Highway 186 Villahermosa-Escárcega, between geographical coordinates Longitude: 92.188056 and Latitude: 17.701944, at an average altitude of 30 m. The climate is warm humid with rain all year round and warm humid with abundant summer rains (INEGI, 2020).

Sample size

The equation proposed by Linch *et al.* (1974) was used to determine the sample size of the total registered households:

$$n = \frac{NZ^2 p(1-p)}{Nd2 + Z^2 p(1-p)}$$

Where: n=sample size, N=number of households with gardens in the study area, Z=value of a normal distribution $Z\alpha/2$ (1.96) for a 95% confidence level, p=probability of success (0.95), d=sampling error (0.05).

Based on the calculated sample size, 43 semi-structured interviews were randomly conducted with household heads from May to November 2021. The interviews were conducted informally under the interviewees' consent. The interview consisted of four sections to understand the families' sociodemographic situation, the current condition of the garden, identification of plant species found in the gardens, and their satisfiers.

Sociodemographic situation

The sociodemographic situation of the surveyed families was described by considering the following factors: name, gender, number of family members, educational level, productive activities, occupation, family income, and access to basic services.

Current condition of home gardens

In order to understand the current condition of the gardens, the names given by the owners, as well as the activities and family participation in the gardens, were researched.

Classification of gardens

Due to the non-uniform surface areas, gardens were grouped by size: small (120 to 425 m^2), medium (425 to 800 m^2), and large (800 to 2500 m^2), following the methodologies of Van der Wal and Bongers (2012) and Agustina *et al.* (2019). Similarly, based on their location in the community, they were classified as center, intermediate, and periphery (García de Miguel, 2004).

Composition of plant species

In each garden, plants were recorded with their common name, afterwards their scientific name was registered and verified on the websites: https://www.tropicos.org (Tropicos), *http://www.theplantlist.org/* (*The Plant List* (TPL), and some records were corroborated on the consultation platform Enciclovida: *https://enciclovida.mx/* (CONABIO, 2022) and *https://www.naturalista.mx/* (Naturalista, 2022). Additionally, the origin of the plants and their growth habit were consulted (Flores, 2012; Villaseñor, 2016).

Diversity of plant species in home gardens

In order to determine the diversity of plant species in each size category, the following indices were used: Fisher's Alpha, Shannon-Wiener, and Simpson.

Fisher's Alpha Index (α) is an abundance model derived from a logarithmic series and only uses the number of species (S) and the total number of individuals (N). Its calculation is performed using the following equation:

$$S = \alpha \ln\left[1 + \left(N / \alpha\right)\right]$$

Where: S=number of species in the sample, and N=total number of individuals in the sample.

Shannon-Wiener Diversity Index (H'), based on the proportional abundance of species (Equation 2).

$$H' = \sum_{i=1}^{S} pi \ \ln\left(pi\right) \tag{2}$$

Where: S=number of species, pi=proportion of individuals of each species i, and ln=natural logarithm. Higher H''values indicate greater species diversity (Sánchez-Gutiérrez *et al.*, 2021).

Simpson's Index (S) measures the probability that two individuals randomly selected from each size category are of the same species (Equation 3).

$$S = \frac{1}{\sum \frac{ni\left(ni-1\right)}{N\left(N-1\right)}} \tag{3}$$

Where: ni=number of individuals in the i-th species, N=total number of individuals. Higher S values indicate lower dominance of one (or a group) species (Zarco *et al.*, 2010).

Uses of plant species in home gardens

Considering the plant species found in the gardens, their uses were identified according to the interests of the families and classified as: fruit, medicinal, ornamental, horticultural, timber, spices, fuel, spice-timber, and medicinal-vegetables.

The experimental data obtained were analyzed for frequency, percentages, and averages using Microsoft Excel 2013 software.

RESULTS AND DISCUSSION

Productive activities and family occupation

The productive activities of the families include agriculture (56%), commerce (11%), livestock (9%), professional services (4%), and government employment (2%). Agriculture is the predominant activity, with most families involved in oil palm (*Elaeis guineensis* Jacq) and rubber (*Hevea brasiliensis* Muell Arg) production, or working as laborers in these monocultures (Castellanos Navarrete, 2018; Méndez-Rodríguez and Giménez-Cacho, 2020). This focus on monocultures has reduced livestock activity in the region since 1998 (Castellanos Navarrete, 2018; Méndez-Rodríguez and Giménez-Cacho, 2020). Additionally, about 20% of the population engages in occasional activities such as blacksmithing, painting, beekeeping, mechanics, and other trades. The rural population is currently involved in various productive and commercial activities (Van der Wal *et al.*, 2011).

Family economic income

Family economic income ranges from $200.00 to $14,500.00 pesos per month, with an average of $3,234.00 pesos. Our results show that family incomes in the ejido do not exceed the extreme poverty line established by CONEVAL (2019). According to CONEVAL (2020), a family of four with an income below $13,133.30 pesos per month is considered to be in poverty. Furthermore, as of December 2021, CONEVAL established that rural families are considered to be in extreme poverty if they do not have incomes above $1,463.43 pesos per person (for the basic food basket) and in poverty if they do not have incomes above $2,784.70 pesos per person (for the food and non-food basket) (CONEVAL, 2021).

Basic services available to families

All surveyed families have piped water and electricity services, while 98% have sewage systems, 91% use LP gas, 79% have cell phone service, and 47% pay for private satellite television services. The recorded data exceed what was reported by CONAPO (2015), as basic services have been introduced, improved, and expanded in the past five years. However, in terms of health, education, and economic income, it is still categorized as a marginalized community.

Current condition of home gardens

Name, ages, and sizes of gardens

In the Ejido Nueva Esperanza 1st Section, the home garden is known as "solar", similar to what Vogl *et al.* (2002) reported in Palenque. In southeastern Mexico, it is called "patio" and "traspatio" (Mariaca, 2012; Chablé-Pascual *et al.*, 2015), and they are located in spaces adjacent to the houses (Pulido *et al.*, 2008; Mariaca, 2012).

The research recorded that 93% of families have their own "solar", most of which were inherited from parents to children and are considered the main resource (Cruz, 1990).

About 5% rent the house, and 2% lend it to a family member. Furthermore, 42% are ejido members, and 58% are residents.

The ages of the gardens vary. Gardens from 87 years old (since the ejido's founding) to one year old were recorded, similar to what Jiménez-Osornio *et al.* (2015) reported for the gardens in Tahdziu, Yucatan.

Additionally, gardens ranging from 120 to 2,500 m^2 in surface area were observed, with an average size of 749 m^2. The areas allocated for housing varied from 30 to 300 m^2, averaging 91 m^2. The spaces where plant and animal species were recorded ranged from 15 to 2,200 m^2, with an average of 658 m^2. These areas were divided into sections for chicken coops, pigsties, vegetable cultivation, medicinal plants, ornamental plants, and other uses. These findings are similar to what Vogl *et al.* (2002) reported in Palenque. Similarly, Sol (2012) noted that in Tabasco, garden sizes range from 3 m^2 in urban areas to 400 m^2 in rural areas. Pulido *et al.* (2008) mentioned that garden sizes in Latin America vary from 0.05 ha to 2.5 ha. The size of the gardens is related to the family's land dimensions (Pantoja, 2014), resulting in diverse garden sizes and types (Sol, 2012).

In the ejido studied, gardens measuring 0.25 ha (2500 m^2) were originally allocated to each ejido member at the time of the ejido's establishment. However, due to family growth, the garden sizes have decreased, and their organization has diversified, resulting in microenvironment mosaics that contribute to a spatial-temporal organization of biotic and abiotic components (Lope-Alzina *et al.*, 2018).

Family activities and participation in gardens

The management activities carried out in the gardens are: weeding (29%), watering (22%), permanent planting (8%), grafting (3%), pest and disease control (4%), pruning (3%), organic fertilization (11%), and chemical fertilization (3%). However, 17% stated that they do not perform any management in their gardens. These results are similar to what Reyes-Betanzos (2014) observed in Bandera de Juárez, Paso de Ovejas, Veracruz, and also align with their agricultural activity calendar. Agricultural activities are carried out according to lunar cycles (Castañeda-Guerrero, 2020). It was recorded that in 29 gardens, vegetables are cultivated, of which 16 have scattered plantings without care or management, and only six receive minimal attention.

Regarding the labor time families dedicate to the garden, they mentioned that it averages one hour per week, less than what Reyes-Betanzos (2017) reported in Bandera de Juárez, Veracruz, where families dedicated five hours a week. Maroyi (2009) indicated that families allocate an average of 1.6 hours daily to the garden in Hehema, Zimbabwe, suggesting that the time families invest varies according to the size and whether production is for self-consumption or commerce (Torquebiau, 1992).

Regarding participation in the garden, women (66%) dedicate more time to tasks, deciding mainly what plants to sow and having more knowledge about their uses and management (Cruz, 2016; Pulido *et al.*, 2018). Men's participation is 34%, mainly performing these tasks in the evenings since they engage in their primary productive activities in the mornings. The activities they mostly perform in the garden are: weeding, fruit planting, pruning, fertilization, and pest and disease control.

The participation of girls and boys is low, at 21% and 24%, respectively. This may be because garden learning is not significantly promoted at home or school as part of their daily practices or cultural heritage from generations. The situation differs in indigenous communities, where children and youth are commonly involved in family agriculture (Gutiérrez-Sánchez, 2017). In Choles communities in Chiapas, children perceive backyard elements as part of their life experience (Ubiergo-Corvalan *et al.*, 2021). In Oaxaca, children and women play an important role in garden care and management (Manzanero-Medina *et al.*, 2018). This may be due to the idiosyncratic and cultural differences of mestizo families, as in our research community.

Floristic composition of plant species in gardens

A total of 3,279 plant individuals were recorded, grouped into 46 botanical families, 82 genera, and 89 species. These results were higher than those recorded in 66 gardens in Bandera de Juárez, Veracruz, by Reyes-Betanzos and Álvarez-Ávila (2017), who identified 75 plant species and 39 botanical families. However, Vogl *et al.* (2002) listed a total of 241 species in 30 households in two indigenous migrant localities in southeastern Palenque, Chiapas. Sol (1993) obtained 144 species in the Ejido Lindavista of the same municipality, and Flota-Bañuelos *et al.* (2016) reported 223 species in Campeche, Mexico. Similarly, Castañeda-Guerrero *et al.* (2020) listed 357 species belonging to 263 genera and 102 botanical families in Totonac gardens, in Puebla, Mexico. In Bangladesh, Kabir and Webb (2008) reported 419 species belonging to 109 botanical families. Additionally, Akinnifesi *et al.* (2010) obtained 186 plant species in urban and suburban gardens in Brazil, suggesting that the floristic composition of gardens is determined by species use and family culture.

Frequency and origin of plants

Among the recorded species, those with the highest frequency were: cilantro (*Coriandrum sativum* L.), chives (*Allium schoenoprasum* L.), and banana (*Musa*×*paradisiaca* L.). Native species were the most dominant with 46 species (52%), with the most common being: parsley (*Eryngium foetidum* L.), tomato (*Solanum lycopersicum* L.), and cacao (*Theobroma cacao* L.). Introduced species accounted for 43 species (48%), with the most dominant being: cilantro (*C. sativum*), chives (*A. schoenoprasum*), and banana (*M. paradisiaca*), as shown in Table 1. These results are similar to what Castañeda-Guerrero (2020) recorded in Caxhuacan, Puebla, where native species represented 58%, and introduced species 42%. The similarity in species origin percentages could be due to the utility value and importance families attribute to the plants present in the gardens. This aligns with the results of Pulido *et al.* (2008) in Latin America, where native species represent more than 50% of the plants in gardens.

Growth habit

A total of 33 tree species (37%) were recorded, used for fruit, medicinal purposes, fuel, and shade. Additionally, 33 herbaceous species were noted for their use in vegetables, medicinal purposes, and ornamentation, 15 shrub species for ornamental and medicinal uses, five rosettes, and three arborescent species. These results are similar to those obtained by Guzmán *et al.* (2012) in Ocuatitlán, Nacajuca, Tabasco, and Montañez-Escalante (2014) in Yucatan. In contrast, Guerra (2005) in Yaxcabá, Yucatan, reported a greater abundance of ornamental species.

Diversity of plant species in home gardens

Large home gardens recorded the highest specific richness (61), followed by small (51), and finally medium (47). However, small gardens have the highest diversity (H′=2.8), while medium and large have lower values (H′=1.9 and 2.7, respectively). Simpson's index indicated that small and large gardens have no dominant species and are more equitable (E=7) than medium gardens (E=0.5). Fisher's alpha index showed greater diversity in small gardens (13.0) (Table 2).

The greater richness in large gardens could be due to recording a higher number of unique species, as described by Van der Wal and Bongers (2012) in 61 gardens in Tabasco. However, large gardens recorded lower diversity, consistent with Martínez and Juan (2005), who determined that large gardens in 24 municipalities in southern Mexico have lower diversity than small gardens, similar to what Lok (1998) found in Costa Rica and Agustina *et al.* (2019) in Pujon, Malang Regency, East Java, Indonesia. This is mainly because small gardens cultivate more densely small species (mainly vegetables and ornamental) and establish many species in pots and containers as Martínez and Juan (2005) and Sol-Sánchez (2012) indicated.

Regarding garden location, the periphery has greater species richness (R=66) compared to gardens in the center (R=39). In terms of diversity, Fisher's alpha shows a higher value for peripheral gardens (13.8), while Shannon indicates that intermediate and peripheral gardens are equal (H′=2.6) and the center has a lower value (H′=2.3) (Table 2).

These results may be attributed to peripheral gardens being less fragmented than those in the center, where ornamental species predominate. Additionally, peripheral gardens border ejidal plots and are often used in conjunction. This aligns with García de

Table 1. Origin and density of plants in the 43 family gardens in the Ejido Nueva Esperanza 1st Section, Palenque, Chiapas.

Introduced	Number	%	Native	Number	%
Coriandrum sativum L.	1020	31.1	*Eryngium foetidum* L.	235	7.2
Allium schoenoprasum L.	360	11.0	*Solanum lycopersicum* L.	210	6.4
Musa×paradisiaca L.	287	0.9	*Theobroma cacao* L.	113	3.4
Aloe vera (L.) Burm. f.	80	8.8	*Tradescantia spathacea* Sw.	75	2.3
Nephelium lappaceum L.	52	2.4	*Annona muricata* L.	45	1.4
Hibiscus rosa-sinensis L.	35	1.6	*Capsicum annuum* L.	39	1.2
Euphorbia milii Des Moul.	31	1.1	*Carica papaya* L.	34	1.0
Brassica oleracea L.	30	0.9	*Zinnia violacea* Cav	30	0.9
Hibiscus sabdariffa L.	30	0.9	*Solanum torvum* Sw.	26	0.8
Ananas comosus (L.) Merr.	26	0.8	*Cedrela odorata* L.	21	0.6
Subtotal	1951	59.5	Subtotal	828	25.3
33 remaining especies	270	8.2	36 remaining especies	230	7.0
Total	2221	67.7	Total	1058	32.3

Table 2. Diversity of plant species by size and location of the family garden.

Indice	Orchard size (m)			Orchard location		
	small	medium	large	central	Intermediate	Periphery
Wealth	51	47	61	39	41	66
Number of individuals	645	1201	1433	1086	534	1659
Simpson	0.9	0.7	0.9	0.8	0.9	0.8
Shannon H′	2.8	1.9	2.7	2.3	2.6	2.6
Equity	0.7	0.5	0.7	0.6	0.7	0.6
Fisher's alpha	13.0	9.7	12.9	7.9	10.4	13.8

Miguel's (2004) research in the Yucatan Peninsula, which reported that peripheral gardens exhibit greater species richness and diversity, emphasizing the importance of considering families' cultural parameters. However, the richness and diversity of garden species are also influenced by families' preferences and interests, local conditions, and management practices.

Uses of plant species in home gardens

The plant species recorded in the gardens were grouped into the following: fruit, medicinal, ornamental, horticultural, timber, spices, fuel, spice-timber, and medicinal-vegetables (Table 3). Table 4 presents the main plant species found in the gardens, their reported uses by families, and the parts used.

Similar results were presented by Pulido *et al.* (2008) in their study on family gardens in Latin America, where they regrouped nine categories of use. Lower results were reported by Chablé-Pascual *et al.* (2015) in Chontalpa, Tabasco, where they identified three categories of use: food, medicinal, and ornamental. Ordoñez et al. (2018) showed higher results, recording 31 uses of plant species in the gardens of Oaxaca, with the main categories being food, ornamental, medicinal, construction, and small-scale sale.

Fruit trees are the main source of benefits for families, with 28 species (20.4%) reported, similar to what Castañeda-Guerrero (2020) found in Caxhuacan, Puebla. Spices and fuel reported the lowest benefits at 0.1%.

Table 3. Uses, richness, and percentage in the family gardens of the Ejido Nueva Esperanza 1st Section.

Satisfactories/uses	Species (number)	Density (number)	(%)
Fruit trees	28	725	20.4
Medicinal	19	329	9.3
Ornamental	18	163	4.6
Horticultural	13	2251	63.4
Timber	6	49	1.4
Season them	2	3	0.1
Fuels	1	4	0.1
Seasoning-timber	1	7	0.2
Medicinal-vegetables	1	18	0.5

Among the fruit trees, the most dominant species were banana (*M. paradisiaca* L.) with 287 individuals, cacao (*T. cacao*) with 113 individuals, and rambutan (*Nephelium lappaceum* L.) with 52 individuals. Medicinal plants included 19 species, with notable ones being aloe vera (*Aloe vera* L. Burm.f.) with 80 individuals, purple heart (*Tradescantia spathacea* Sw) with 75, guaco (*Mikania congesta* DC), and turkey berry (*Solanum torvum* Sw) with 26 individuals.

Ornamental plants included 18 species, with the most common being hibiscus (*Hibiscus rosa-sinensis* L.) with 53 individuals, crown of thorns (*Euphorbia milii* Des Moul.) with 31, and zinnia (*Zinnia violacea* Cav) with 30 individuals. Vegetables formed the fourth group of beneficial plants with 13 species and 1981 individuals, including cilantro (*C. sativum*) with 1020 individuals, chives (*A. schoenoprasum*) with 360, and parsley (*E. foetidum*) with 235 individuals. The dominance of these vegetable species is due to their daily use in cooking (Sol, 2012).

Although vegetables are mainly used for self-consumption, they also have economic value as they contribute to family well-being, as reported by Chi-Quej *et al.* (2014). Due to their easy adaptation, they can be grown in small pots, raised beds, or intensively. They do not require complex agronomic management, they grow quickly, and have high demand for commercialization among neighbors and family due to their frequent consumption. Despite the high dominance and use of vegetables, they were only present in 13 of the 43 gardens studied, indicating little interest in their production. This is similar to the study by Van der Wal *et al.* (2011) in Cárdenas, Tabasco, where there is a higher presence of fruit trees than vegetables.

Although the participants reported specific uses for plant species, come plants can have multiple uses (López-Armas, 2017). For example, papaya (*Carica papaya* L.) is reported primarily as a fruit, but families in the ejido also attribute other uses to it, such as medicinal (seeds), forage (leaves), and desserts (green fruits for sweets and preserves). Similarly, the banana (*M. paradisiaca*) is used for various purposes: medicinal (stalk, fruits, fresh leaves), ritual (stalks used to hold candles during wakes or rosaries on Day of the Dead), wrapping (fresh leaves for pozole or tamales), and covering (dried leaves used to cover seedbeds). All

Table 4. Most common plant species in home gardens, uses reported by families, and parts used.

Common name	Scientific name	Reported uses Part used	Part used
Papaya	*Carica papaya* L.	Fru	Fr
Tamarind	*Tamarindus indica* L	Fru	Fr
Mango	*Mangifera indica* L	Fru	Fr
Banana	*Musa×paradisiaca* L.	Fru	Fr
Cocoa	*Theobroma cacao* L.	Fru	Se
Orange	*Citrus sinensis* (L.) Osbeck	Fru	Fr
Soursop	*Annona muricata* L.	Fru	Fr
Annatto	*Bixa orellana* L.	Con, Mad	Se, Ra
Rambutan	*Nephelium lappaceum* L.	Fru	Fr
Lemon	*Citrus limon* (L.) Osbeck	Fru	Fr

Use (Fru: fruit, Mad: Wood, Con: condiments; use part (Fr: fruit, Se; seeds, Ra: Branches).

these uses of plant species provide families with food, health, security, comfort, and well-being, among other benefits, as classified by Mariaca (2012) in the gardens of Chiapas and Tabasco and Cahuich-Campos (2012) in Campeche.

In addition to the benefits provided by plant species, other benefits identified in the community's family gardens include recreation areas, family gatherings, workspaces, and rest areas for pack animals, as highlighted by Sol (2012) in Tabasco.

CONCLUSIONS

The size of the gardens is determined by the family's size, as the more children there are, the smaller the gardens. This condition is acceptable because, in certain rural areas, children tend to stay living close to their parents. Species diversity is variable, and floristic composition depends on the family's primary use or requirement. Small gardens were the most diverse, although with fewer individuals.

The diversity of satisfiers ranges from edible to medicinal uses. Other known uses were not reported but are known, such as toys made from flowers of Erythrina (Fabaceae) and bean pods (*Phaseolus* sp.). Although gardens generate products that support family economics, these incomes are minimal and below the extreme poverty line.

In general, the area's gardens show potential for short-cycle vegetable production; however, seed availability is scarce and only obtained in cities. This is because the population has lost the diversity of native seeds and relies on commercial varieties.

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Technique comparison to assess sperm DNA fragmentation in hair ram

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ABSTRACT
Objective: To evaluate 5 DNA fragmentation techniques in thawed sperm samples from hair ram subjected to fragmentation with H_2O_2.
Design/methodology/approach: Samples were 36 straws from 4 Blackbelly rams that were thawed, mixed (pool), diluted in PBS to a concentration of ' 30×10^6 sperm/mL and divided into three treatments: T0: sample without oxidant (considered as 0% damaged DNA), T100: sample incubated with 300 μM H_2O_2 for 24 hours (induction of DNA fragmentation (100%)). Subsequently, half of T0 and T100 were mixed to obtain a proportion of 50% sperm with fragmented DNA (T50). The samples were analyzed with different techniques: Aniline Blue (AB), Toluidine Blue (TB), Acridine Orange (AO), Chromomycin A3 (CMA3) and Sperm Chromatin Dispersion (SCD).
Results: In the linear regression, all the techniques presented a significance level of less than 5%, as well as a significant correlation ($r=0.962$, $P<0.01$). However, between treatments, it was observed that the AO technique (34.82±3.00%) at T50 and the AB technique (89.55±1.45%) at T100 were the least sensitive in detecting DNA damage compared to the other techniques.
Limitations on study/implications: New techniques are increasingly.
Findings/conclusions: The techniques that best evaluate the DFI of sperm in hair ram are CMA3, SCD and TB.

Keywords: Fluorescence, H_2O_2, Semen Quality, Blackbelly.

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INTRODUCTION

Currently, the fertility potential of the ram can be determined based on semen analysis, through a series of assessments, in which motility, morphology, metabolic activity and integrity of the sperm membranes stand out; however, these assessments are inefficient to predict fertility (Kamimura *et al.*, 2010; Alves *et al.*, 2015; Arbaiza-Barnechea and Cabrera-Villanueva, 2021). This is why the study of integrity of the sperm DNA has been considered an important index of fertility, since there is evidence of subfertility in males with normal values in routine parameters and with higher percentages of DNA fragmentation. Therefore, the assessment of the DNA Fragmentation Index (DFI) should be considered an important parameter to include in the traditional assessment in various animal species, since it stands out as a fertility indicator, a good predictor of embryo development and paternal genetic information (Ortega et al., 2010; Andraszek *et al.*, 2014; Czubaszek *et al.*, 2019; Huanca *et al.*, 2020; Ribas-Maynou *et al.*, 2021).

Image by Gerd Altmann at Pixabay

There are diverse methodologies that allow analyzing the sperm DNA fragmentation which allows predicting more exactly the male's fertility through direct methods to evaluate the amount of protamine or to measure the chromatin structure based on the different stains. These methods can be divided into two groups; the first includes the techniques that mark DNA cleavage because they incorporate molecules marked with fluorochromes on the cleavage ends, such as TUNEL (*terminal d-utp nick-end labeling*), SCD (*Sperm Chromatin Dispersion*) and ISNT (*in situ nick translation*), although many of these techniques are laborious and require high equipment accuracy, and the field conditions also do not allow performing complex techniques (Rui *et al.*, 2017). However, the SCD technique can determine sperm DNA fragmentation more accurately, since it is one of the simplest, fastest, most accurate, highly reproducible and inexpensive tests (Fernández *et al.* 2005, Carretero *et al.* 2012). Thus, a second group is found, where the techniques that are based on stains are included: Aniline Blue (AB), Toluidine Blue (TB), Acridine Orange (AO), and Chromomycin A3 (CMA3) (Kazerooni *et al.*, 2009). Therefore, the objective of this study was to evaluate the efficacy of five different techniques in the identification of sperm DNA damage in thawed samples from Blackbelly ram.

MATERIALS AND METHODS

Study area

The experiment was carried out in the Molecular Genetics Laboratory of the Instituto Tecnológico de Conkal, in the municipality of Conkal, Yucatán. Likewise, at the Instituto Nacional de Investigaciones Agrícolas y Pecuarias (INIFAP), located in the experimental field of Mocochá on Kilometer 25 of the former Mérida-Motul highway, located on 21° 06' 18" latitude North and 89° 27' 12" longitude West, with sub-humid tropical climate (Awo) and mean annual temperature of 26.5 °C, with total precipitation of 900 mm and 9 masl (INIFAP 2018).

Ram selection. Four male ram of the Blackbelly breed were used, with an average age of 2.0 ± 0.5 years and average live weight of 42.5 ± 2.9 kg.

Obtaining and processing semen. Through an artificial vagina and with the help of a ewe that served as mannequin, a total of 36 ejaculates were collected (9 ejaculates/ram) that complied with the following criteria: volume >0.5, mass motility >4 (scale 0-5), motility $>70\%$, and sperm concentration $>3{,}000 \times 10^6$ spermatozoa/mL. The semen was collected with a frequency of twice per week in the morning hours (8:00).

Sperm dilution. The ejaculates obtained were diluted with OPTIX-cell® (imv, L'Aîgle, France) at a final concentration of 400×10^6 spermatozoa/mL, to later be packaged in French straws (Minitüb®, Tiefenbach, Germany) of 0.25 mL.

Cooling. The straws were placed in a refrigerator at 5 °C for a period of four hours, to be frozen later in liquid nitrogen (LN2).

Semen freezing. Freezing of the samples was carried out by placing the straws four centimeters above the LN2 surface, for 10 minutes. Immediately after, the straws were submerged in LN2 and stored until their assessment.

Semen thawing. The defrosting procedure was carried out through immersion of the straws in a double water bath at 37 °C for 30 seconds.

DNA fragmentation induction with hydrogen peroxide (H_2O_2). The induction of DNA damage was conducted according to the technique proposed by Peris *et al.* (2007). For this purpose, four straws from different males were thawed, mixed (pool), diluted in PBS at a concentration of 30×10^6 spermatozoa/mL and divided into three treatments: T0: sample without oxidant (considered as 0% of damaged DNA); T100: sample incubated with 300 μM of H_2O_2 for 24 hours (induction of DNA fragmentation (100%)). Then, half of the T0 and T100 were mixed to obtain a proportion of 50% of spermatozoa with fragmented DNA (T50).

Technique analysis

Sperm chromatin dispersion (SCD): The SCD technique methodology was carried out based on Fernández *et al.* (2013) with some modification. Suspensions from each T0, T50 and T100 aliquot were taken in the amount of 20 μL from each sample, to mix with 40 μL agarose of low fusion point (Agarose, low gelling temperature, BioReagent, for molecular biology; Sigma-Aldrich®) at 1% in an Eppendorf tube (to obtain a final concentration of 0.7%) at 37 °C. Of the semen-agarose mixture, 30 μL were pipetted on a glass slide covered with standard agarose (Agarose, low EEO, BioReagent, for molecular biology; Sigma-Aldrich®) at 0.65% dry and covered with a clean glass slide cover and introduced in the refrigerator at 4 °C for seven minutes. After that time, the slides were taken out of the refrigerator and the slide covers were removed without altering the subjacent layers; then a lysis solution was used (0.4 M Tris [Sigma-Aldrich®], 50 mM EDTA [Baker®], 0.4 M DTT [Sigma-Aldrich®], 0.3% SDS [Sigma-Aldrich] and 1% Tritón X-100 [Promega®, Molecular biology grade], pH 7.5) adding, according to what was described by Gundogan *et al.* (2010) and Ribas-Maynou *et al.* (2021), 100 μg/mL of proteinase K (Thermo Scientific® 600 U/mL, 20 mg/mL) to the lysis buffer to pipette horizontally to the slides and leave the solution at room temperature for 120 minutes to extract membranes and proteins. After a five-minute wash in abundant distilled water, the preparations were dehydrated in sequential baths of 50, 70 and 100% (v/v) of ethanol (Hycel®) for a two-minute period each, then they were left to dry to later finally stain the cells with 10 μL of propidium iodine (Invitrogen®, Molecular Probes™) for fluorescence microscopy. A minimum of 200 spermatozoa were evaluated per slide. The spermatozoa were classified according to Peris-Frau *et al.* (2019): intact DNA (nuclei with small halos or none) and fragmented DNA (nuclei with large DNA dispersion halos).

Acridine orange (AO): The technique was applied according to what was described by Mohammadi and Soltani (2021) with some modifications. Seven microliters of the sample from each T0, T50 and T100 aliquot were deposited on a glass slide, left to dry in open air, and then fixed in a Carnoy solution (methanol [J.T. Baker®]-acetic acid [Meyer®] 3:1) during 30 minutes. Then, the smears were left to dry for 10 minutes, to later be stained for five minutes with an AO (Meyer®) solution recently prepared in the following way: the mother solution was prepared dissolving 0.05g of AO in 50 mL of distilled water and then storing it at 4 °C without access to light; the stain solution was prepared by mixing 10 mL of the mother solution, 40 mL of 0.1 M citric acid (Reasol®), and 2.5 mL of 0.3 M $Na_2HPO_4\cdot7H_2O$ (Wholer®). Then, the smears were submerged in water (10 times quickly in a container with distilled water), and they were left to dry to be read immediately at 100x with a fluorescence microscope, counting at least 200 spermatozoa, where the identification of spermatozoa with normal DNA structure (green fluorescence) and damaged single-chain DNA (orange fluorescence) was made.

Cromomycin A3 (CMA3): The methodology was used according to what was described by Roodbari *et al.* (2015) and Rahimizadeh *et al.* (2020) with some modifications. Seven microliters of sample from each T0, T50 and T100 aliquot were deposited and then fixed on a Carnoy solution (methanol-acetic acid 3:1) for 30 minutes at 4 °C. Then, the slides were incubated in the dark and a slide cover was placed on them for 20 minutes at room temperature with 100 μL of the CMA3 solution (Sigma-Aldrich®) (0.25 mg/mL in McIlvaine buffer (0.2 M Na_2HPO_4) (Sigma-Aldrich®), 0.1 M citric acid (Reasol®), and 10 mM of $MgCl_2$ (Sigma®), pH 7). A minimum of 200 spermatozoa were counted for each slide with the help of an epifluorescence microscope at 100x magnification, and they were distinguished among positive spermatozoa (spermatozoa stained in brilliant yellow with abnormal condensation of chromatin) and negative spermatozoa (stained in opaque yellow with normal condensation of chromatin).

Aniline blue (AB): Staining was done according to what was described by Oliveira *et al.* (2013) with some modifications. Seven microliters of sample of each T0, T50 and T100 aliquot were deposited on a glass slide, they were left to dry in the air and then fixed in a Carnoy solution (methanol-acetic acid 3:1) for 30 minutes at room temperature. After fixation they were stained for 25 minutes in an AB (Meyer®) aqueous solution at 5% dissolved in acetic acid (Meyer®) at 4% (pH 3.5) and then washed twice with distilled water; then they were left to dry and observed with a clear field microscope at magnification of 100x, and at least 200 cells per smear were evaluated. The spermatozoa that were not stained were considered normal, while those stained with dark blue color were considered as spermatozoa with chromatin faults.

Toluidine blue (TB): Staining was carried out according to what was described by Nava-Trujillo *et al.* (2011) and Carretero *et al.* (2020) with some modifications. Seven microliters of sample from each T0, T50 and T100 aliquot were deposited on a glass slide, left out to dry and then fixed in a Carnoy solution (methanol-acetic acid 3:1) for five minutes at room temperature. The smears were stained with TB (Research organics®) (0.05% in 10 mL of McIlvaine buffer, pH 4.0) for five minutes at room temperature and then they were washed three times with distilled water to later dry. The smears were observed with a clear field microscope with 100x magnification, and at least 200 cells were assessed per smear, which were classified as spermatozoa with normal chromatin, those stained with clear blue color; while those stained with dark or violet blue color were considered cells with damaged chromatin.

Statistical analysis. The techniques assessed on DNA fragmentation were analyzed with a general linear model with procedure (ANOVA) and Tukey's test at $P \leq 0.05$ was used to determine the statistical differences between techniques. In addition, an analysis was carried out with Pearson's correlation coefficient (r^2) to determine the association between techniques and a linear regression analysis with the expected percentages of spermatozoa that exhibited damage on the DNA. The data were subjected to a statistical analysis system with the Statistical Package for the Social Sciences IBM ® SPSS software, version 25.0.

RESULTS AND DISCUSSION

In the results between treatments, it was observed that the TB, SCD and CMA3 techniques were the ones that presented best sensitivity to detect DNA damage, while the AO technique (34.82±3.00%) in T50 and the AB technique (89.55±1.45%) in T100 were the ones that presented least sensitivity (Table 1).

On the other hand, a linear coefficient regression was observed between the percentages of spermatozoa induced to fragmentation and what is expected for all the techniques (Figure 1). Likewise, the values of the square roots were higher than 0.95 in all the techniques, thus showing a high acuteness and repeatability (Table 2).

The various techniques were compared (Figure 2), where a significant correlation was observed among the five techniques.

Lastly, all the techniques analyzed presented a significant correlation coefficient, higher than 0.962 (Table 3).

This study shows that the TB, SCD and CMA3 techniques were the ones that showed the best sensitivity to detect damage in the Blackbelly ram sperm DNA, compared to the AO and AB techniques. These results are like those reported by Chohan *et al.* (2006), who observed differences in the high levels of DNA damage of human spermatozoa, both fertile and infertile, evaluated with the SCD technique while no differences were observed with the AO technique. Likewise, Rahiminia *et al.* (2018) obtained similar results with the TB,

Table 1. Percentage of spermatozoa with DNA damage in the different treatments.

Treatments	TB	AO	CMA3	AB	SCD
T0	3.01±0.75 $^{a, C}$	2.68±0.62 $^{a, C}$	2.11±0.41 $^{a, C}$	3.36±0.65 $^{a, C}$	4.29±0.72 $^{a, C}$
T50	52.93±1.86 $^{a, B}$	34.82±3.00 $^{b, B}$	48.06±1.73 $^{a, B}$	49.64±1.43 $^{a, B}$	47.06±2.47 $^{a, B}$
T100	98.51±0.55 $^{a, A}$	99.55±0.25 $^{a, A}$	95.58±1.50 $^{a, A}$	89.55±1.45 $^{b, A}$	97.97±1.03 $^{a, A}$

T0: Sample without oxidant (considered as 0% damaged DNA); T100: sample incubated with 300 μM H_2O_2 for 24 hours (induction of DNA fragmentation (100%)). Subsequently, half of T0 and T100 were mixed to obtain a proportion of 50% sperm with fragmented DNA (T50). Toluidine Blue (TB); Acridine Orange (AO); Chromomycin A3 (CMA3); Aniline Blue (AB); Sperm Chromatin Dispersion (SCD). (^{ab}) Different letters in the same row show significant differences between techniques by groups. (^{AB}) Different letters in the same column show significant differences in the technique within the group. Level of significance for both letters ($P<0.001$ ***).

Table 2. Square roots and linear regression equation of validation to evaluate the integrity of DNA through Aniline Blue (AB); Toluidine Blue (TB); Acridine Orange (AO); Chromomycin A3 (CMA3) and Sperm Chromatin Dispersion (SCD). All the linear regressions presented a level of significance lower than 5%.

Test	Square root	Linear regression
Aniline Blue	$R^2=0.98$	y=4.42+0.68*x
Toluidine Blue	$R^2=0.95$	y=12.77+0.82*x
Acridine Orange	$R^2=0.99$	y= 3.73+0.96*x
Chromomycin A3	$R^2=1.00$	y=1.85+0.93*x
Sperm Chromatin Dispersion	$R^2=0.99$	y=2.93+0.94*x

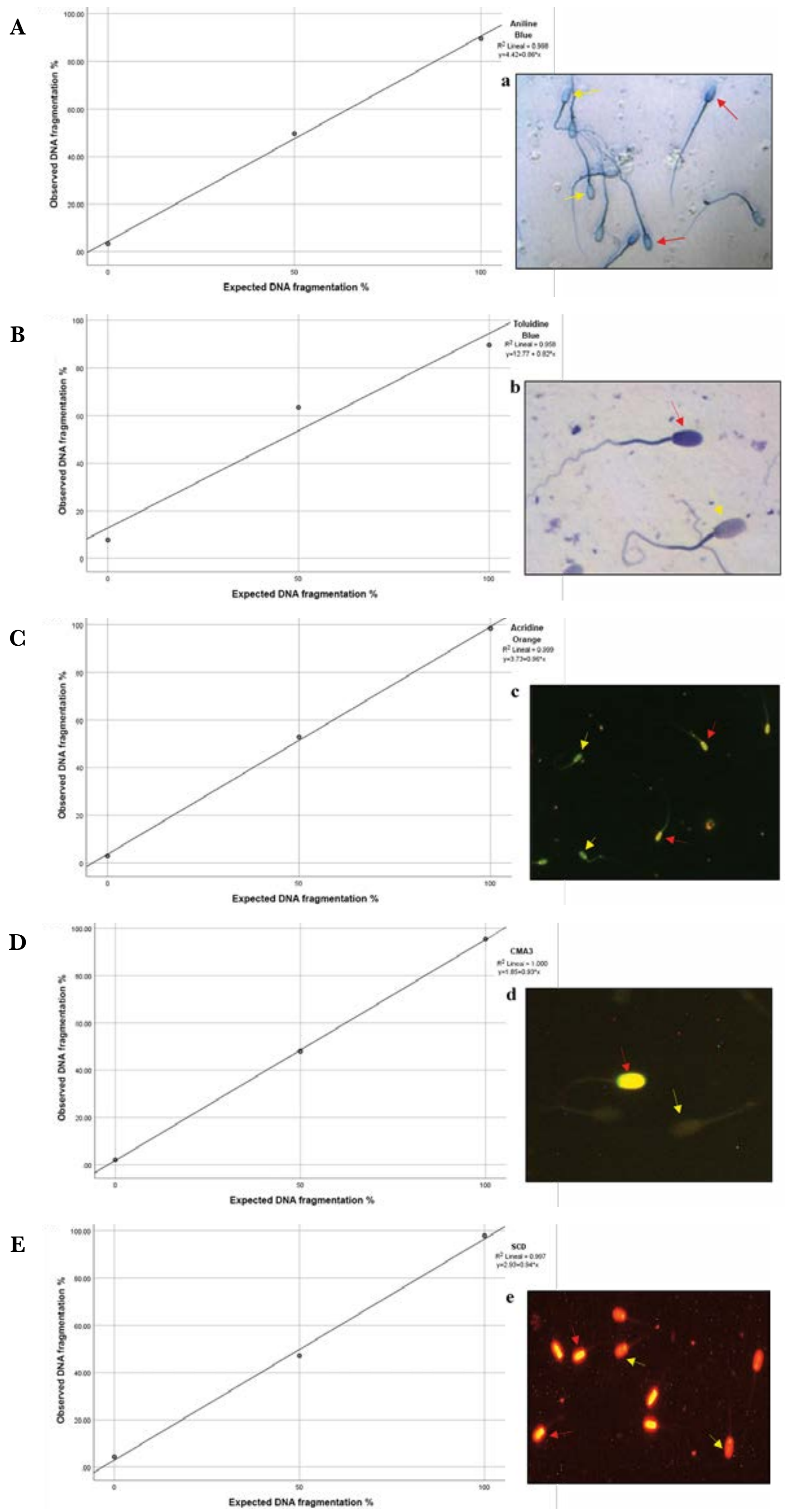

Figure 1. Linear regression analysis (A, B, C, D, E) and images (a, b, c, d, e) of DNA damaged spermatozoa among different treatments (T0, T50 and T100). The presence of DNA damaged spermatozoa was detected with (A, a) Aniline Blue; (B, b) Toluidine Blue; (C, c) Acridine Orange; (D, d) Chromomycin A3 and (E, e) Sperm Chromatin Dispersion. DNA damaged spermatozoa are marked with "b" and intact DNA spermatozoa with "a" in all images.

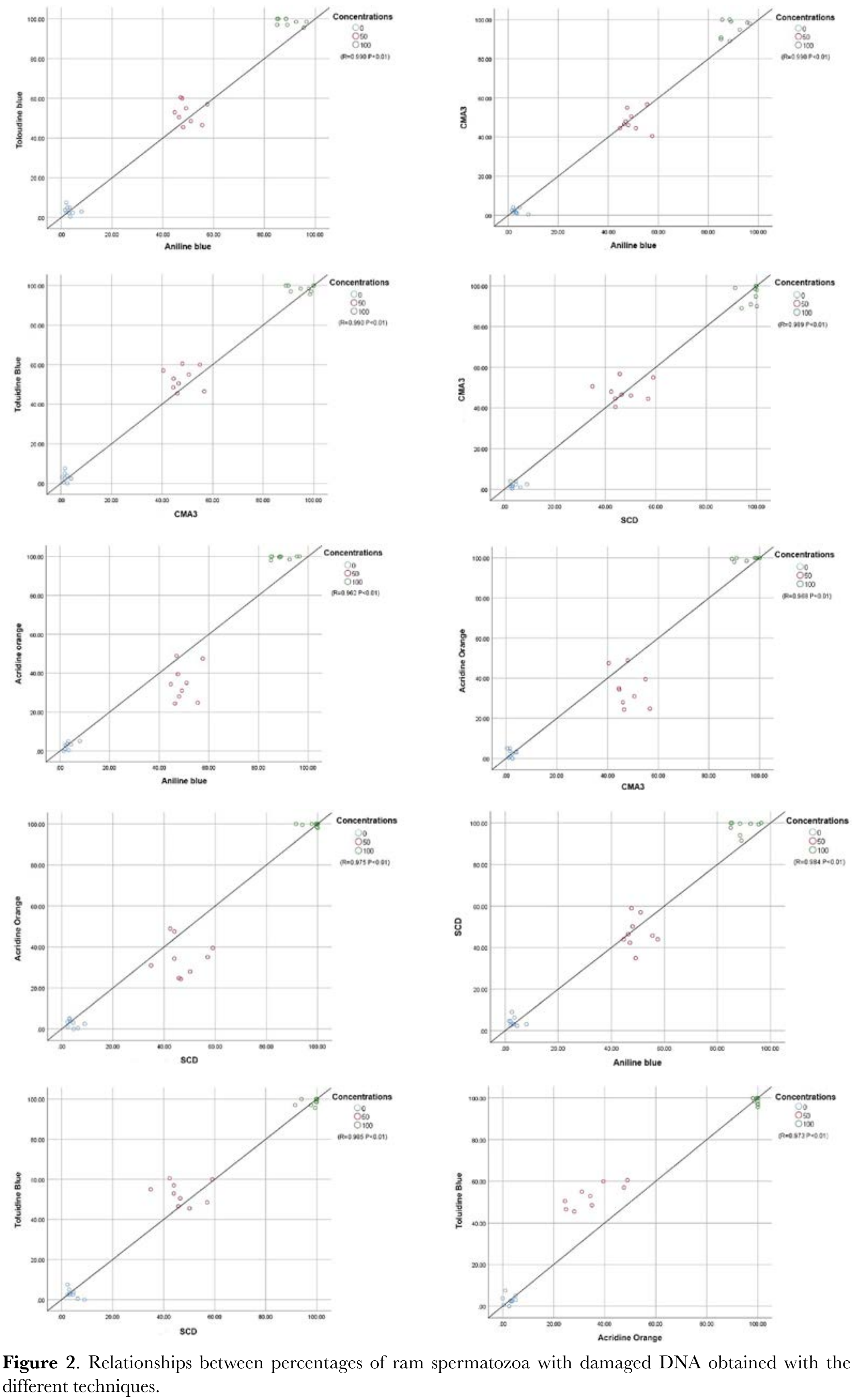

Figure 2. Relationships between percentages of ram spermatozoa with damaged DNA obtained with the different techniques.

Table 3. Correlation matrix of the techniques to detect sperm DNA fragmentation.

	TB	**AO**	**CMA3**	**AB**	**SCD**
TB	1				
AO	0.973**	1			
CMA3	0.991**	0.968**	1		
AB	0.990**	0.962**	0.990**	1	
SCD	0.986**	0.975**	0.989**	0.984**	1

Toluidine Blue (TB); Acridine Orange (AO); Chromomycin A3 (CMA3); Aniline Blue (AB); Sperm Chromatin Dispersion (SCD). **$P \leq 0.01$.

AO and CMA3 techniques in sperm samples from humans with oligoasthenoteratospermia and with normozoospermia. However, in contrast with this study's result, the AB technique was less sensitive than the TB and CMA. Likewise, it was seen that the incubation of Blackbelly ram spermatozoa with 300 μM of H_2O_2 for 24 hours of incubation at room temperature induces damage in the sperm DNA (T100). This result is similar to that reported by Peris *et al.* (2007) in thawed semen samples of Dorset breed ram, incubated with 300 μM of H_2O_2 during 24 hours. Similarly, Aitken *et al.* (1998) observed a significant increase in the DNA damage of human spermatozoa incubated with 200 μM of H_2O_2 during 2.5 hours, reaching 90% of fragmentation. It is well known that hydrogen peroxide (H_2O_2) has a negative impact on the chromatin of the spermatozoon, inducing double-chain breakdown (Kodoma *et al.*, 1997). The result presented in Table 2.3 is similar to what was found by Czubaszek *et al.* (2019), who obtained a correlation coefficient of 0.96 between the techniques CMA3-AB, although the techniques CMA3-AO (0.48) and AB-AO (0.45) differed from what was found in this study. However, Karimura *et al.* (2009) observed a correlation coefficient in ram of 0.89 between the techniques AB-TB, while in goats it was 0.35 respectively. These results are similar to those obtained in this study in ram. These differences can be because the chromatin of ram is the same than that of cattle and pigs, which presented a higher degree of DNA condensation. This is a result of the condensation around protamine 1 and 2, although in the species mentioned only protamine 1 is present in its chromatin, so although there is less efficiency of condensation in their DNA, there are stronger protamine-protamine unions from the high number of cysteine residues that allow stronger unions and, therefore, it is more difficult to achieve the decondensation of chromatin.

CONCLUSIONS

The techniques that best assess sperm DNA fragmentation of hair ram are CMA3, SCD and TB; however, the TB technique is more efficient and inexpensive since it does not require staining with fluorescence or sofisticated equipment for assessment.

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Methods to improve the fraction of non-degradable protein in the rumen: A review

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ABSTRACT
Objective: To investigate methods to reduce dietary protein degradability at the ruminal level and to analyze their effects on ruminal fermentation based on the analysis of available literature.
Design/methodology/approach: The protection of dietary protein leads to a lower degradation at the ruminal level and an increase in the supply and utilization of amino acids.
Limitations of the study/implications: The efficacy of the processing method depends on the ingredients used in the diet.
Conclusions: The use of physical, chemical, or combination treatments is justified on raw materials with high protein value and degradability; these efficiently protect the protein from ruminal degradation and provide a better supply to the small intestine.

Keywords: Protein, Cattle, Methods.

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INTRODUCTION

High-yield ruminants for meat production require diets with adequate nutrients to achieve the desired productive performance objectives (NASEM, 2016). Dietary protein is the most expensive nutrient. In the case of feedlot-finished ruminants, more rumen-undegradable protein (RUP) is needed to reach the required levels of metabolizable protein (MP) because microbial protein is insufficient to cover the needs of animals with high nitrogen (N) demand (Silva *et al.*, 2023). Protecting dietary protein to pass through the rumen intact and avoid degradation leads to increased supply and utilization of amino acids (AA) (Loregian *et al.*, 2023). It also contributes to reducing greenhouse gas emissions derived from the fermentation of certain AA (Palangi and Lackner, 2022). Therefore, it is necessary to integrate ingredients with significant levels of RUP into the diet so that ruminants can express their maximum potential and improve the profitability of production systems while reducing the negative impact on the environment (da Silva *et al.*, 2020; Valizadeh *et al.*, 2021). Nutrition researchers have suggested physical, chemical, or a combination of both methods to increase RUP in dietary protein ingredients, protecting it from degradation and fermentation in the rumen, thus optimizing its utilization (Shishir *et al.*, 2020; Roca-Fernández *et al.*, 2020; Rigon *et al.*, 2022). This review aimed to investigate methods to reduce the degradability of dietary protein at the ruminal level, increase the RUP fraction, and analyze their effects on ruminal fermentation based on the available literature.

MATERIALS Y METHODS

The literature search was performed on Google Scholar, Scopus, and Web of Science using the keywords "Rumen Undegradable Protein", resulting in 15,900 articles published from 1978 to 2024. However, studies performed *in vitro* and with unconventional diets or feeds were omitted. It can be observed that there is little data available regarding rumen degradability, especially in recent years, probably due to the problems arising from increasingly restrictive regulations on animal welfare.

Physical Methods

Various methods have been investigated for a long time to increase the RUP; however, thermal treatments in their different modalities are more effective than chemical treatments (Iommelli *et al.*, 2022).

Extrusion

Extrusion is a widely used technique for applications in human and animal food (Lillford, 2008; Zhang *et al.*, 2011). It consists of a thermomechanical physical process where the material is subjected to kneading, compression and cooking through a sudden increase in temperature and pressure for a short time. Heat transfer and pressure cause structural changes (Maillard reaction) in the feed ingredients, such as protein denaturation, fiber solubilization, and starch gelatinization (Solanas *et al.*, 2008).

Microwave

Microwave irradiation increases the temperature of the material with dielectric properties when the energy is absorbed and converted into heat. Its use shows advantages compared to other thermal treatments due to the reduced time required, which reduces energy and operating costs (Guzik *et al.*, 2021).

Roasting

This method is commonly used in oilseeds after solvent extraction. It consists of heating the material by conduction at a variable temperature ranging from 100 to 200 °C in a time range of 0 to 4 hours with or without pressure. Pressure roasting is carried out in horizontal or vertical cylinders similar to an autoclave; however, the pressure method has certain implications, such as increasing the processing time to increase and decrease the pressure, which leads to inaccuracy in times and temperatures (Poel *et al.*, 2005). Roasting favors the increase of the RUP and alters the sensory and functional properties, improving palatability, aroma, and color and reducing the antinutritional factors of the material (Haji-Mohammadi *et al.*, 2022).

Chemical Methods

Chemical treatments that increase RUP have varied, from tannins to alcohols, acids and aldehydes (Poel *et al.*, 2005).

Tannins

Tannins have been widely used and studied in ruminant diets and are generally classified into hydrolyzable tannins (HT) and condensed tannins (CT). Of these, only CT can form complexes with dietary protein when dosed at low levels in the diet, which promotes greater resistance to microbial degradation at the ruminal level, including crude protein (CP), dry matter (DM), organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF); ammonia (NH_3) concentration is also reduced and fecal nitrogen excretion (ENF) is increased (Orzuna-Orzuna *et al.*, 2021).

Formaldehyde

The application of formaldehyde (HCHO) decreases the activity of proteolytic microorganisms through a two-step process: 1) the formation of a methyl compound and 2) a slow condensation (Brand *et al.*, 2023) promoting the formation of methyl cross-links between HCHO and dietary protein under slightly acidic to neutral ruminal pH conditions, making the protein resistant to microbial degradation without affecting its digestibility in the small intestine (Firozi *et al.*, 2024). Among the implications of using HCHO is the high variability between foods since it depends on the solubility of the dietary protein and its possible residual effect on animal tissues and secretions (Wales *et al.*, 2010).

Malic Acid

It is an intermediate organic acid in the Krebs cycle in animal tissues and the succinate-propionate pathway of ruminal microorganisms (Ke *et al.*, 2018). Direct application in feed protects dietary protein from ruminal degradation and promotes denaturation and hydrolysis under acidic conditions of the abomasum for subsequent absorption in the small intestine (Thakur *et al.*, 2023).

Physical Methods To Increase The Rup Fraction On Ruminal Fermentation

Applying methodologies to increase the RUP alters the ruminal kinetics of CP (Rigon *et al.*, 2022). Heat treatment has a significant impact by reducing the degradation of dietary protein since it modifies the molecular structure, increasing the proportion of the β-lamina secondary structure and reducing the proportion of the α-helix structure in amide I, with a minor effect on amide II (Yan *et al.* 2014), these changes occur in the proportions of the secondary structures, where the different degradabilities of proteins at the ruminal level occur (Windt *et al.* 2022). The extrusion process at moderate temperatures (110 to 160 °C) makes DM, OM, and dietary protein more degradable without affecting the total volatile fatty acids (VFA) (Risyahadi *et al.*, 2023). Still, it decreases the branched VFA (isobutyrate and isovalerate) and acetate while increasing the proportion of butyrate (Amirteymoori *et al.*, 2021). These temperature ranges used for the extrusion process are insufficient to protect dietary protein (Orias *et al.*, 2002). Processing with longer time and temperature causes overprotection due to denaturation and the formation of cross-links between reducing sugars and AA (Berenti *et al.*, 2021). However, the optimal processing temperature and time depends on multiple factors such as moisture content, carbohydrate content and type, protein content, and presence of other compounds such as sulfites, and therefore, optimal heat treatment parameters vary from one dietary protein to another (Van Soest, 1994). On the other hand, dry or wet roasting of legume seeds decreases the proportion of α-helices concerning β-sheets, causing the dietary protein to increase its potential for post-ruminal digestion and absorption. However, roasting processing also affects the degradability of other nutrients, mainly starch (Espinosa *et al.*, 2024). In addition to structural changes, the thermal process improves nutritional quality by decreasing or altering antinutritional factors such as trypsin inhibitors, lecithin, phytic acid, tannins, etc., which generates better digestibility and availability of nutrients in the small intestine (Valizadeh *et al.*, 2021). Including heat-treated protein feeds in the ruminant diet decreases the NH_3 level at the ruminal level derived from the low degradation of dietary protein (Marques *et al.*, 2024). However, the excessive inclusion of protected protein causes a decrease in fiber degradability indirectly when it decreases microbial protein synthesis due to the reduced access to nitrogen necessary for its metabolism, mainly affecting cellulolytic bacteria (Chesini *et al.*, 2023).

Chemical Methods To Increase The Rup Fraction On Ruminal Fermentation

Chemical treatments such as the inclusion of tannins show inconsistent results on ruminal fermentation; certain authors (Krueger *et al.*, 2010) indicate that tannins, regardless of the source, do not affect ruminal variables in high-grain diets. In contrast, both Orzuna-Orzuna *et al.* (2021) and Berça *et al.* (2023) suggest that tannin supplementation in ruminant diets has a positive effect, increasing the molar proportion of propionate and butyrate while reducing the degradability of DM, OM, CP, NDF, ADF and NH_3 content. Including *Acacia mearnsii* derived tannins at increasing levels in steers-fed high forage diets caused a linear decrease in nitrogen compounds' apparent and true digestibility. In addition, it reduced the intake and degradability of OM, NDF and NH_3 content without affecting ruminal pH. However, non-fibrous carbohydrates (NFC) degradation was also affected (Orlandi *et al.*, 2015).

In contrast, another study (Ávila *et al.*, 2020) with the same tannin source (*Acacia mearnsii*) and with increasing levels of high grain diet for steers showed no effects on the intake and digestibility of DM, OM, NDF, ADF and NFC. However, the degradability of dietary protein was reduced without altering NH_3 concentration, ruminal pH decreased and VFA increased linearly with the inclusion of tannins. Therefore, the dose and source of tannins and the basal diet are important factors to assess the impact of tannins in ruminant diets. Treatment with xylose as a reducing sugar source reduces the degradability of dietary protein, increasing the RUP (Harstad and Prestløkken, 2000; Can and Yilmaz, 2002). The addition of xylose increases the total VFA compared to other thermal methods. However, there was an increase in the molar proportions of acetate butyrate (Khatibi *et al.*, 2019) and decreases in molars of valerate, isovalerate and isobutyrate without changing the NH_3 content (Ipharraguerre *et al.*, 2005); the degradability of DM, OM and NDF was not affected, but the degradability of CP was decreased (Abdollahzadeh *et al.*, 2021). Malic acid or its ionized form as malate did not affect DM and OM uptake and degradability; however, it increased the molar proportion of butyrate (Carro *et al.*, 2006), propionate (Foley *et al.*, 2009) and decreased acetate (Bharathidhasan, 2022). Carrasco *et al.* (2012) evaluated malic acid and sodium malic acid supplements in beef cattle; the acid form decreased the NH_3 level compared to its sodium form without altering VFA levels, indicating better protection of dietary protein.

CONCLUSIONS AND IMPLICATIONS

The effectiveness of the processing method depends on the feed on which it is used, and the decrease in protein degradability is due to the effect of protection, favoring performance in ruminant animals since the use of the nutrient is improved and the ruminal fermentation processes are optimized. The use of physical, chemical, or a combination of both treatments is justified on raw materials with high protein value and degradability. These effectively protect the protein from ruminal degradation and provide a greater protein supply to the intestine. Future research should consider a joint characterization of the methods and feeds to correlate animal productive responses better.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Profitability of biogas production as a source of energy for tequila distilleries from the anaerobic treatment of tequila vinasses

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ABSTRACT

Objective: To analyze the profitability of biogas production from tequila vinasses in an anaerobic packed bed reactor (PBR) plant to use biogas as substitute of heavy fuel (fuel oil) in boilers of the tequila industry.

Design/methodology/approach: Financial information for biogas production was gathered; the methodology with the approach of investment project evaluation was used for two reactor volumes, 7 m^3 and 10 m^3, and the profitability was determined through the following financial indicators: Net Present Value (NPV), Internal Rate of Return (IRR), Benefit-Cost Ratio (B/C R).

Results: Biogas production is profitable starting from a reactor's volume of 7 m^3 and it is more favorable when the process is scaled to 10 m^3. It was obtained for a volume of 7 m^3 and 10 m^3 at an updating rate of 12% (NPV $780 376.70 and $5 062 685.22), (B/C R $1.04 and $1.21), (IRR 14% and 26%).

Limitations on study/implications: The results are based on values reached in a PBR plant at laboratory scale with a capacity of 445 L, assuming that the values of yield and removal of the chemical demand for oxygen (CDO) are not modified when scaling the process to 7 m^3 and 10 m^3.

Findings/conclusions: Analysis of the results showed that the use of tequila vinasses to generate renewable energy for auto-consumption in an anaerobic PBR plant is profitable.

Keywords: Financial indicators, anaerobic digestion, AP digester, renewable energy, biofuel.

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INTRODUCTION

During agave (*Agave* spp.) distillation, contaminant residual waters are obtained called "tequila vinasses". Between 10 and 12 L of vinasses are generated per liter of tequila produced (García and León, 2018). This effluent is characterized by having low hydrogen potential (pH), high temperatures, high concentrations of biochemical demand of oxygen (BDO) and chemical demand of oxygen (CDO). The production of biogas is a sustainable process established for the simultaneous generation of renewable energy

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and the treatment of organic wastes (Angelidaki *et al.*, 2018). For this, it is necessary to find alternative ecological sources of fuels, which requires the design of an appropriate and affordable technology (Lorenzo *et al.*, 2015). Biogas consists primarily in methane (CH_4) in a range of 50 to 70% and carbon dioxide (CO_2) in 30 to 50% (Angelidaki *et al.*, 2018). The most common applications of biogas are the direct combustion for heat production and the generation of electric energy (Venegas *et al.*, 2019). To create economically efficient biodigestion systems, it is necessary to analyze the execution of the project that allows the construction of affordable facilities and a faster recovery of the investments (Cervi *et al.*, 2011); also, to establish economic parameters that show the conditions under which it operates, in order to ease managerial, economic and financial decision making (Nava, 2009). The objective of this study was to analyze the financial profitability of biogas production from tequila vinasses in an anaerobic packed bed reactor (PBR) plant for the use of biogas as substitute of fuel oil in boilers of the tequila industry.

MATERIALS AND METHODS

The biogas production system is constituted by a series of stages illustrated in Figure 1.

The PBR plant at laboratory scale (Arreola, 2018) that produces biogas from tequila vinasses is located in the Exact Sciences and Engineering university center at Universidad de Guadalajara, Mexico.

The necessary financial information for biogas production was gathered through surveys directed toward researchers in charge of the plant. The financial evaluation model

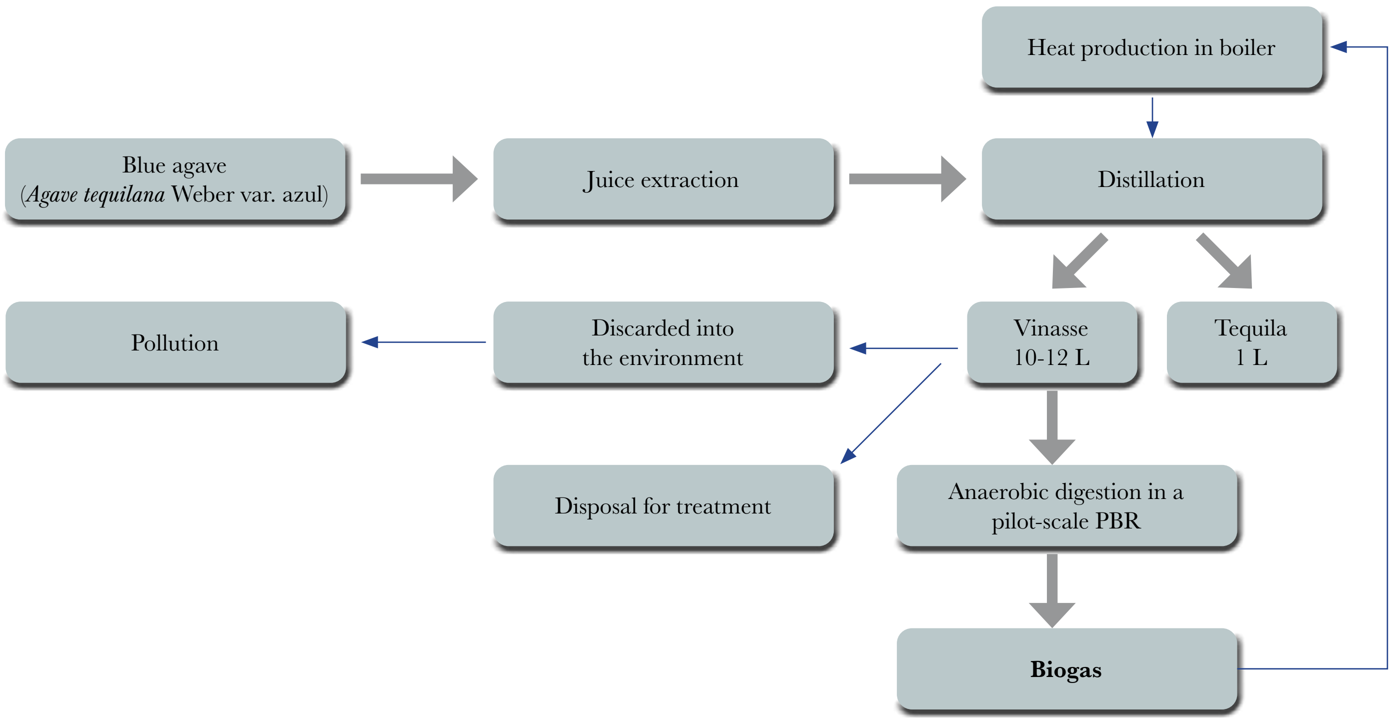

Figure 1. Production cycle of tequila, vinasse and biogas. Source: Prepared by the authors with information from interviews, 2021.

consisted in five stages: *estimation of total investment, production volumes, estimation of operations benefits and costs, calculation of the net cash flow, and financial indicators*. The economic values are expressed in current values for the year 2021 in Mexican pesos (MX). There is not a specific price for biogas in the market, so since fuel oil is the source of energy to substitute, the average annual price of fuel oil of $9.15/L MX from the year 2021 was taken as reference.

Conditions of biogas production: The production was estimated from the values reached in the PBR plant with a capacity of 445 L (base scenario), assuming that the values of yield and CDO removal are not modified when the process is scaled, 70% in biogas and raw vinasse of 60 kg/m^3. The plant operates with 75% of its capacity, with an operation period of 355 days per year. The profitability was evaluated in two scenarios, one with a reactor volume of 7 m^3 at which the project is profitable, and the second with a volume of 10 m^3 which represents the maximum volume of the reactor at which the production can be scaled based on the conditions of the system at laboratory scale. The technical data based on the reactor volume are presented in Table 1.

Investment and costs: The investment of the project was based on the working digester and the production process was scaled. The unitary costs contemplate the variable production costs (VPC) and the fixed costs (FPC), which are distributed monthly, whether there is production or not (Cisneros-López *et al.*, 2020). The annual investment, operation and maintenance costs were considered. In the variable costs, the following were included: cost of vinasse, assigned based on the cost generated for its production and harvest from the "agave" raw material $337.097/$m^3$; cost of electricity $1.35 K Wh (industrial fee in Jalisco) (CFE, 2021); drinking water $27.35 m^3 (fee from the zone where the plant is located) (SIAPA, 2021); and costs of chemicals $0.014/L of biogas. The fixed costs consider the following: cost of laboratory equipment, reagents for monitoring, office consumables, salaries, insurance, plant maintenance, payment necessary for monitoring and validation of the sale of CERs (carbon bonds), which is 20% of the sales of CERs each year, and five-year loan for the acquisition of the project's assets.

Financial evaluations and Profitability indicators: The methodology of project evaluation based on Baca (2013) was used, which calculates the profitability through the financial indicators NPV, IRR, B/C R and sensitivity analysis. Minimum Acceptable Rate of Return (MARR): the horizon of useful life of the project was fixed at 10 years at a MARR of 12%, which considers the bank rate from 2021 of 9% and 3% of risk.

Table 1. Technical data of the scenarios for biogas production.

	Scenario base	Scenario 1	Scenario 2
Reactor volume (m^3)	0.445	7	10
Water feeding flow (m^3)	0.099	1.55	2.2
Vinasse feeding flow (m^3)	0.049	0.77	1.1
Production of biogas (m^3)	0.95	14.94	21.3

Source: Prepared with data from the interviews, 2021.

Net Present Value (NPV): According to Baca (2013), the NPV formula is the following:

$$NPV = -I + \sum_{i=1}^{t} \frac{b_i - c_i}{(1+\partial)^t} \quad (1)$$

Where: *I*: initial investment; b_i benefit in time *i*; c_i cost in time *i*; ∂ rate of discount; *t* time or horizon of investment.

Internal Rate of Return (IRR): The IRR was determined based on Formula 2 from Weston and Bringham (1993) cited by Cisneros-López *et al.* (2020).

$$\sum_{i=0}^{n} \frac{CF_i}{(1+IRR)^n} \quad (2)$$

Where: *CF*: Net cash flow; *IRR*: Internal rate of return; *n*: number of periods; *i*: period.

Benefit-cost ratio (B/C R): It was calculated based on the formula that Cisneros-López *et al.* (2020) used.

$$\frac{B}{C} = \sum_{i=0}^{n} FI_i \,/\, \sum_{i=0}^{n} FS_i \quad (3)$$

Where: *B*: benefit; *C*: cost; *FI*: flow of income discounted; *FS*: flow of spending discounted; *n*: number of periods; *i*: period.

RESULTS AND DISCUSSION

The initial investment required for the project to work is \$4 429 817.78 for scenario one and \$4 545 118.75 for scenario two. Among investment costs, the cost of the plant stands out, which reaches \$2 146 464.80 and \$2 219 331.37, respectively.

Annual cost budget: The unitary cost of production for scenario one was \$0.19/L and for scenario two \$0.15L of biogas, which were obtained from adding the unitary variable cost of \$0.05 and \$0.04 L of biogas and the fixed unitary production cost of \$0.14 and \$0.011 L of biogas, respectively.

Annual production: In scenario one, an annual treatment is generated at 276 249.17 L of vinasses and 5 303 984 L of biogas is produced, and for scenario two 394 641.67 L and 7 577 120 L, respectively. In this evaluation, the benefit from the sale of CERs is taken into account, in agreement with Lorenzo *et al.* (2015) who mention that the bonds inject an extra cash entry. Vera *et al.* (2017) considers the benefits from the sale of CERs with a price of \$7.74/ton CO_2 eq/a (annual tons of carbon dioxide equivalent) for the year 2015, and a price of \$7.86/Ton CO_2 eq/a in this project.

Financial indicators: Both scenarios obtained a positive NPV, and the investment is recovered, as well as the opportunity cost of the resources. The most favorable scenario is two, as shown in Table 2.

In the B/C R, for each peso invested a benefit of $0.04 is obtained in scenario one and $0.21 in scenario two. The IRR in both scenarios was higher than the MARR of 12%, which indicates that the use of tequila vinasses such as biomass in a PBR plant to generate biogas becomes a competitive and profitable option, as is the case recorded by Lorenzo *et al.* (2015), Suárez-Hernández (2018) and Rostagno *et al.* (2020), who show the financial profitability of anaerobic digesters for biogas production from agroindustrial vinasses and residues, taking advantage of these contaminant residues and assigning them a value.

Authors like León *et al.* (2019) indicated that the digesters contribute a solution and alternative in residue management, through the use of available resources transforming them into biogas. Lorenzo *et al.* (2014), Alonso-Estrada *et al.* (2015) and López Velarde *et al.* (2019) mention that, because of the composition of vinasses, they are a promising substrate for the production of biogas and methane.

Table 2. Updated net cash flow of biogas production with tequila vinasses with a reactor volume of 7 m^3 and 10 m^3, in Mexican pesos 00/100 MX, with data from 2021.

Years/ concept	Scenario 1	Scenario 2
0	−4,736,624.88	−4,851,921.86
1	−752,888.96	−309,167.98
2	350,127.66	857,926.46
3	638,970.12	1,238,997.27
4	530,622.21	1,065,837.06
5	478,421.37	955,782.13
6	887,261.28	1,327,997.81
7	775,616.72	1,169,131.47
8	707,319.26	1,058,671.72
9	631,535.06	945,242.61
10	1,270,003.81	1,604,188.53
NPV	780,376.70	5,062,685.22
IRR	14%	26%
B/C	1.04	1.21

CONCLUSIONS

The results obtained show profitability of the project for biogas production from tequila vinasses, contributing to mitigate problems caused by vinasses (with the anaerobic treatment of residual waters), the use of fuel oil in tequila distilleries (with the generation of biogas for auto-consumption), and profitability (with the income obtained by the substitution of fuel oil, the sale of CERs and the cost reduction generated by the availability of vinasses). Biogas production from tequila vinasses with PBR digester was a profitable and competitive activity. The information from this study significantly helps to enrich the database of the treatment of tequila vinasses at laboratory scale and will help in decision making.

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Colegio de Postgraduados

Pre and postharvest treatments to reduce the chilling injury in *Lilium* stems

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ABSTRACT
Objective: To increase cold tolerance in floral stems of *Lilium* sp. 'Indian Summerset' stored for two and four weeks using pre- and postharvest treatments.
Design/Methodology/Approach: In the first phase, ascorbic acid (AA; 4 mM), glycine betaine (GB; 100 mM), and distilled water (T) were applied at preharvest and pre-conditioning was carried out at postharvest 0 °C for 24 h, then the steems were stored (6 °C; 85% RH). In the second phase, pulse solutions of AA, GB, or T were applied for 24 h after harvest and stored (7 °C; 92% RH) for two weeks. The variables evaluated were: fresh weight, solution absorption, foliage yellowing, floral opening, flower color, and vase life (VL).
Results: Cold storage causes leaves yellowing, deformation, flower color fading, and reduces VL.
Findings/Conclusions: Preharvest treatments did not have a significant effect on cold tolerance in lilies. In the second phase, the AA pulse solution delayed floral opening and maintained the flowers color, while GB only reduced the leaves yellowing.

Keywords: *Lilium* sp., ascorbic acid, glycine betaine, pre-conditioning, cold damage scales.

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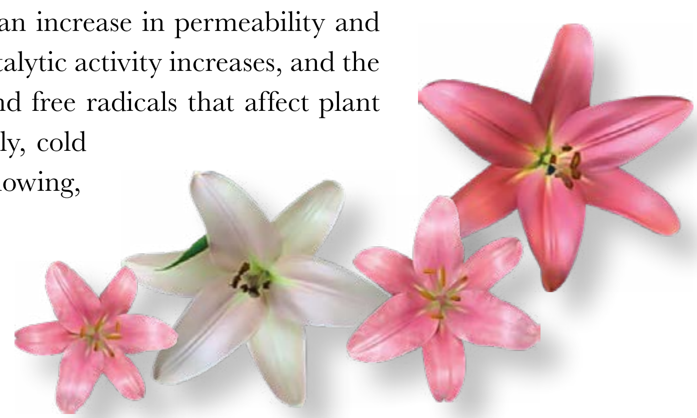
INTRODUCTION

Plants of the genus *Lilium* are primarily divided into *Longiflorum* hybrids, with white, large and aromatic flowers; Asian hybrids, which are great in variety of tepal colors and their resistance to pathogens; and the Oriental hybrids, which have a late floral opening with large and aromatic flowers (Dhiman *et al.*, 2022). To satisfy the demand on specific dates or to transport them, growers store the stems at low temperatures to delay senescence and increase the shelf life of the flowers (Cavalcante *et al.*, 2021).

However, in lilies, it has been reported that storage at temperatures close to 5 °C for more than two weeks causes "chilling injury". This physiological disorder begins with the peroxidation of fatty acids in the cell membrane, so its liquid-crystalline state changes to a solid-gel, causing an increase in permeability and electrolyte leakage. Consequently, catalytic activity increases, and the accumulation of toxic metabolites and free radicals that affect plant tissue begins (Wu *et al.*, 2024). Finally, cold damage in lilies causes leaves yellowing, tepals deformation, and buds abscission (Wei *et al.*, 2018; Darras, 2020).



To increase the tolerance to chilling injury in plant tissues, physical and chemical treatments can be applied. Among the physical ones is the pre-conditioning, which consists of subjecting the tissue to a stress event for short periods of time at temperatures close to 0 °C (Wu *et al.*, 2024). This event produces stress that generates the biosynthesis of hormones and heat shock proteins with the consequent accumulation of antioxidants (Li *et al.*, 2020). This technique is applied in the postharvest handling of fruits such as tomatoes (Zhou *et al.*, 2014) and citrus (Strano *et al.*, 2022). On the other hand, within chemical treatments, there is the application of ascorbic acid (AA) and osmolytes such as glycine betaine (GB) (Wu *et al.*, 2024). AA is an antioxidant able to eliminate radicals such as superoxide and hydroxyl; it decreases lipid peroxidation, protect the selective permeability of the cell membrane system and is a cofactor of ascorbate peroxidase (APX) that reduces H_2O_2 to H_2O (Celi *et al.*, 2023). In tomato plants, pretreatment with AA (0.5 mM) increased the tolerance to freezing stress (4 °C for 7 h), turgor and chlorophyll content in leaves; decreased electrolyte leakage (from 10.5 to 10%), and H_2O_2 content (from 90 to 70 nmol g^{-1} fresh weight) (Elkelish *et al.*, 2020). In anthurium 'Fire Glow', the postharvest application of AA (2-4 mM) decreased chilling injury on the spathe, presenting less darkening and electrolyte leakage (from 63 to 47%) and the activity of enzymes phospholipase and lipoxygenase compared to the control. It also increased the activity of antioxidant enzymes such as APX and catalase (CAT) (Mohammadi *et al.*, 2023).

Glycine betaine (GB) helps to stabilize proteins and activate antioxidant enzymes in stress situations (Fedotova, 2019). In banana (*Musa acuminata* 'Giant-Dwarf'), the preharvest application of GB (100 mM) reduced chilling injury caused by storage at 10 °C (6 h) by delaying the darkening of the epidermis for 8 d, it was observed lower polyphenol oxidase activity and lower electrolyte leakage; it also decreased the ethylene production and delayed the proteins and chlorophyll degradation (Rodríguez-Zapata *et al.*, 2015). In peach fruits, immersion in GB (100 Mm, 10 min) before cold storage (0 °C, 35 d) increased phenylalanine ammonia lyase activity and flavonoid content, reducing chilling injury and delaying senescence (Wang *et al.*, 2019). However, studies regarding the increasing of the cold tolerance in cut flowers are limited, therefore the aim of this work was to know the efficiency of the pre and postharvest application of AA and GB and the effect of pre-conditioning in floral stems of *Lilium* sp. 'Indian Summerset' stored at low temperatures.

MATERIALS AND METHODS

Lilium 'Indian Summerset' stems cultivated in a commercial orchard were used. The experiment was divided into two experiments. The first experiment preharvest applications of AA (4 mM), GB (100 mM), and distilled water (control 2, T2) were performed. Four preharvest sprays (10 mL per stem) were applied to the floral buds at 90, 95, 100, and 105 days after bulb sowing and the next day (day 106) the stems were harvested. Then 30 floral stems were selected, ten for each treatment (each stem was considered a replicate). On the other hand, another 10 stems were taken to carry out the pre-conditioning treatment (0 °C, 24 h). Finally, the stems were transported to the cold room to store in water for two and four weeks (6 °C and 85% RH).

In the second experiment, stems of lilies with the first bud flower with turning color were harvest and transported to the laboratory, then were trimmed and place in a vase with one of the following solutions: AA (4 mM), GB (100 mM), and distilled water (T2) for 24 h, and subsequently the solutions were replaced with tap water, and the stored at 7 °C and 92% RH during two weeks. In this phase, other floral stems were left at room temperature (control 1, T1) to corroborate their potential for vase life. For each treatment, 16 stems were used (each stem was considered a replicate).

After cold storage, the stems were placed in glass bottles with 250 mL of tap water and trimmed to 60 cm length, removing 20 cm of leaves from the stem base. The bottles were placed in a room at 21 °C and 76% RH with 12 h light and 12 h darkness. The variables evaluated were the following:

- Visual appearance of the flower stems. Photographs of flower stems were taken during vase life (VL) with a 12 MP iPhone® XR wide-angle camera to identify the symptoms of chilling injury.
- Changes in fresh weight (%). The stems were weighed before and after the cold storage period. When placed in vases, they were weighed daily with a digital scale (Esnova® SE-2000, with 0.1 g precision), and weight changes were calculated based on the difference between the initial and final weight.
- Water uptake (mL/ tallo). The solution consumption by the flower stems was calculated based on the difference in weight of the solution daily.
- Leaves color. A scale from green (1) to yellow (6) was established and measured daily in the leaves during VL.
- Flowers color. Using a precision colorimeter NR20XE (Shenzhen ThreeNH Technology, China), the L*, a*, and b* values of the flowers were obtained to calculate the Hue° angle and the chroma value.
- Floral opening (%). The total number of flower buds was counted when the stems were harvested and after storage the opening percentage was calculated.
- Vase life. After storage, the days in which the flower stems remained without showing symptoms of senescence such as wilting, petals abscision, or leaves yellowing were counted. The end of vase life was considered when 50% of the flowers were wilted and most of the leaves showed chlorosis.

Data analysis

The experimental design was completely randomized. In the first phase were used 5 replicates per treatment per cold storage period, and in the second one, 16 replicates. The data were analyzed with ANOVA and significant differences were determined with the Tukey test (α=0.05); where appropriate, nonparametric tests were used.

RESULTS AND DISCUSSION

First experiment: Preharvest treatments and pre-conditioning

No significant differences were found in the variables evaluated after two or four weeks of storage (6 °C and 85% RH) with preharvest treatments (AA, GB) and pre-conditioning

(0 °C for 24 h) and the control in *Lilium* 'Indian Summerset' to increase the tolerance to chilling injury. These results contradict what was reported in gerbera, where pre (1 mM) and postharvest (5 mM) applications of spermine and aminobutyric acid showed that the preharvest treatment was more effective in prolong the VL (Mohammadi *et al.*, 2020). In peonies, preharvest silicon applications were more effective in improving stem quality than the postharvest applications (Song *et al.*, 2021).

It is possible that the lack of effectiveness of these compounds was because foliar spraying requires higher concentrations of the compound. For example, Mohammadi *et al.* (2023) applied AA (0-4 mM) to anthurium stems 'Fire Glow' by foliar spray and pulse solution before storing at 4 °C. With foliar spraying, the greatest increase in VL (25 d) was achieved with 4 mM, while with the pulse solution, it was achieved with 2 mM (27 d), control stems last only 18 d.

Also it is possible that lilies come from a bulb that functions as organ of reserve, being the main source of nutrients during the stem growth and development (De Hertogh, 1992). Due to the relevance of the bulbs in *Lilium* plants, it would be important to verify that preharvest treatments would be applied directly to the bulb and not to the plant, just as has been reported for some growth retardants such as paclobutrazol that have resulted in high effectiveness (Torres-Pio *et al.*, 2021; Rios-Florida *et al.*, 2022).

In *Lilium* 'Indian Summerset', storage at 6 °C and 85% HR for two weeks caused leaves yellowing, flower buds deformation and abscission also to color degradation in the flowers as a symptom of chilling injury. Regardless of the treatment and the storage period at 6 °C, at least six color tones were observed in the flowers and four levels of deformation (Figure 1).

Second experiment: Application of pulse solutions

Pulse solutions with AA or GB decreased the loss of flower color caused by cold storage. After cold storage no differences in the color tone of the flowers (Hue° value), were recorded

Figure 1. Color degradation (A) and deformation (B) of the tepals in flowers of *Lilium* 'Indian Summerset' caused by storage at 6 °C and 85% RH. The scale goes from 1 (mild damage) to 4-5 (severe damage). T1: unstored stems.

but the color intensity (Chroma value) was affected. The treatment that had values similar to the control (T1) was AA, while the greatest color loss was observed with GB (Figure 2A). At the beginning of the VL, the flowers did not show petals deformation until day five; flowers with GB treatment showed a heterogeneous pattern in the color and waviness of the tepal margin (Figure 2B and C).

During cold storage ethylene biosynthesis increases and accelerates senescence provoking color fading (Nergi and Arndt, 2023). Ethylene activates transcription factors that induce the expression of genes that encode enzymes that degrade pigments such as peroxidases (Dias *et al*., 2021). On the other hand, it is normal that during floral opening the cell wall of the epidermal cells in the tepals undergoes modifications in its structure and shape. However, a stress situation, such as cold storage, causes water loss and, therefore, loss of turgor, which makes deformation and changes in the shape of the cells (Zhang *et al*., 2021).

Fresh weight (%): During cold storage, the stems of *Lilium* 'Indian Summerset' increased their fresh weight above 100%, compared to the initial value. During the VL, no significant differences were found in the stored stems, but they had greater weight gain than the unstored stems (T1). Furthermore, fresh weight began to decline on day five with T1, on day three in T2, and on day two in AA and GB treatments, after cold storage. This weight loss is important as it marks the point at which senescence is noticeable (Figure 3).

Woltering and Paillart (2018) stored rose stems for two days at 6 °C and for 28 days at 0.5 °C, after storage the stems were placed in water or in hydroxyquinoline citrate solution (8-HQC, 150 mg L^{-1}). Regardless of the solution, cold storage decreased fresh weight gain compared to unstored stems. Despite this, the use of 8-HQC improved the quality of the stems, since, being a compound with an acidic pH, it prevents the growth of microorganisms at the base of the stem and maintains the water conductivity through the xylem, resulting in greater weight gain compared to water alone.

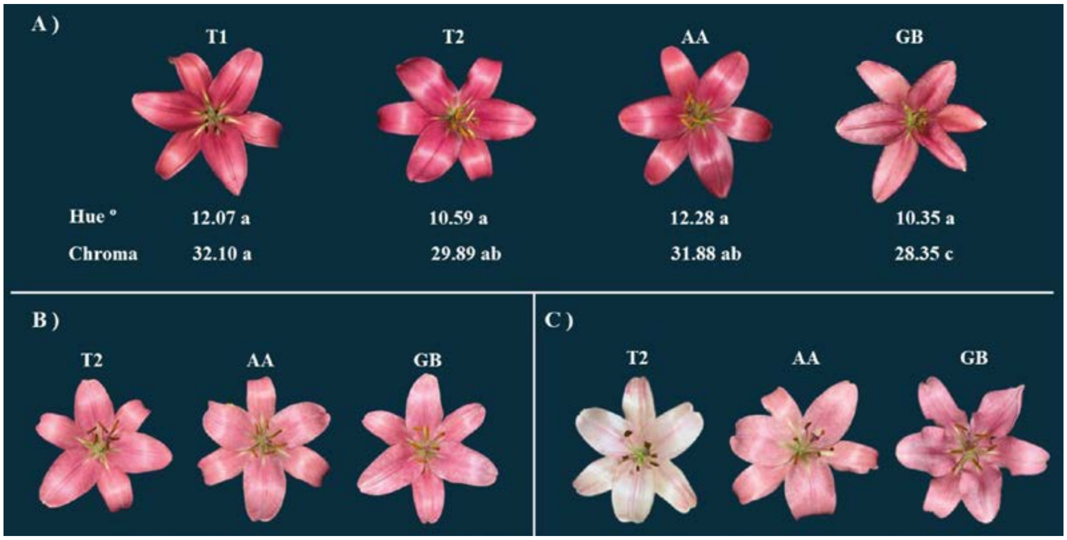


Figure 2. Color of *Lilium* 'Indian Summerset' flowers after storage at 7 °C and 92% RH for two weeks (A) and appearance of the best (B) and worst flowers (C) of each treatment on day five of the vase life. T1: unstored stems, T2: cold storage, AA: ascorbic acid (4 mM); GB: glycine betaine (100 mM).

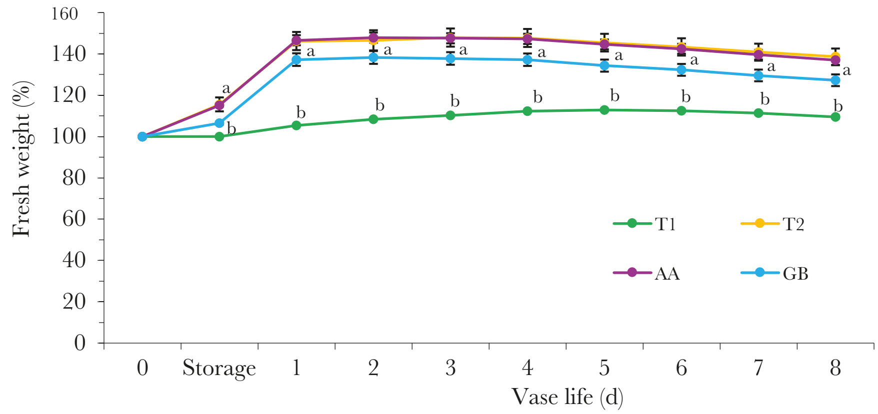


Figura 3. Changes in fresh weight of *Lilium* 'Indian Summerset' stems after two weeks of storage at 7 °C and during vase life. T1: unstored stems, T2: control (cold storage), AA: ascorbic acid (4 mM), GB: glycine betaine (100 mM).

Water uptake: *Lilium* 'Indian Summerset' stems stored for two weeks reduced their ability to absorb water. This is because cold storage affects the functionality of the stomata in the leaves. In rose, storage for 28 d at 0.5 °C affected the conductance of the stomata because when the stems were placed at room temperature, transpiration was lower than the unstored stems (Woltering and Paillart, 2018). Something similar occurred in Heliconia where cold storage (13 °C and 84% RH) decreased water consumption from 0.066 to 0.034 mL g^{-1} compared to the stems stored at 20 °C (Carrera-Alvarado *et al.*, 2021).

In *Lilium* "Indian Summerset" it was observed that, during the first three days of VL, no significant differences were found in water consumption. Starting on day four, the stems without cold storage had the highest water absorption, followed by the stems with AA, T2, and GB treatments (Table 1).

For an adequate water flow through the xylem in the floral stems, it is common to use biocidal and low pH compounds to control microbial growth and maintain water conductivity (Arriaga *et al.*, 2020). Ascorbic acid lowers pH and inhibits the oxidation of phenolic compounds and ROS generation, thereby reduces vascular obstruction of stems and increases water absorption (Chen *et al.*, 2024). This explains why the stems with AA treatment (4 mM) absorbed more water during their VL (Table 1).

Leaves color: In general, the leaves of *Lilium* 'Indian Summerset' presented six levels of yellowing during VL. Both treatments (AA and GB) for 24 h decreased and delayed leaves yellowing compared to T2 (Table 1 and Figure 4).

It has been reported that monodihydroascorbate, derived from the oxidation of AA, is an electron carrier in photosystem II. Furthermore, AA maintains the content of photosynthetic pigments, membrane stability, and water potential, regulates ROS, and promotes the antioxidant defense system (Celi *et al.*, 2023). For its part, GB stabilizes the structure and activity of proteins that participate in photosynthesis (Fedotova, 2019).

For this reason, both AA and GB are compounds that maintain the integrity of the pigments. For example, in tomato seedlings, treatment with AA (0.5 mM) increased their cold tolerance (4 °C for 7 h) and maintained chlorophyll concentration (Elkelish *et al.*,

Table 1. Vase life, floral opening, cumulative vase solution consumption, and leaves yellowing scale of *Lilium* 'Indian Summerset' stems stored at 7 °C for two weeks.

	Vase life (d)	Floral opening (%)		Uptake water (mL stem^{-1})		Yellowing scale	
		A	B	4 d	8 d	4 d	8 d
T1	12.13 a*	---	42.57 b	75.56 a	127.26 a	1.00 c	2.00 ab
T2	8.56 b	38.55 ab	50.47 ab	63.02 b	97.80 bc	1.83 a	2.29 a
AA	8.75 b	31.20 b	52.98 a	62.50 b	99.50 b	1.13 b	1.46 b
GB	8.37 b	43.44 a	55.98 a	58.19 b	85.69 c	1.2 b	1.45 b

[z] Different letters indicate significant differences from the Tukey test with α of 0.05. T1, control without cold storage; T2, control with cold storage; AA, ascorbic acid (4 mM); GB, glycine betaine (100 mM); A, upon exiting the two-week cold storage period; B, to the day of the vase's life when the fresh weight began to decline.

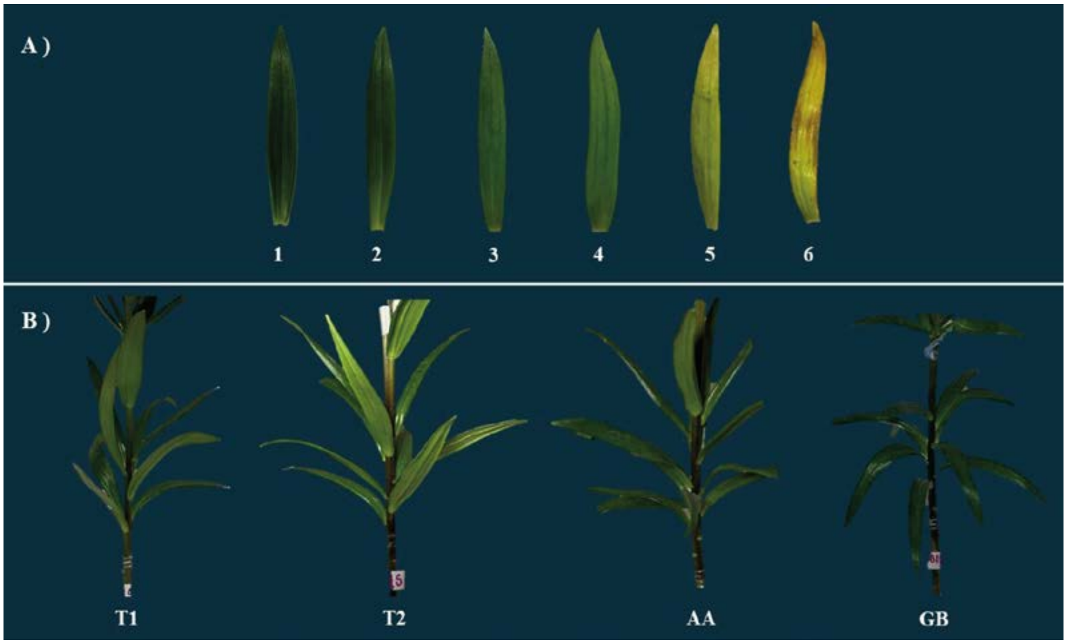


Figure 4. Scale of leaves yellowing on flower stems of *Lilium* 'Indian Summerset' after two weeks of storage at 7 °C (A) and appearance of leaves on day zero of vase life. T1: unstored stems, T2: control (cold storage), AA: ascorbic acid (4 mM), GB: glycine betaine (100 mM).

2020). In *Dalbergia odorifera* seedlings, GB spraying (10-50 mM) maintained the greenery and chlorophyll content (Cisse *et al.*, 2021).

Floral opening and vase life: Refrigerated storage aims to delay the floral opening of the buds and prolong their vase life (Cavalcante *et al.*, 2021). In *Lilium* 'Indian Summerset', storage at 7 °C for two weeks delayed flower opening. While the T1 stems reached their maximum floral opening on day seven and last 14 days, the stored stems reached their maximum floral opening on the second day after storage and had six additional days of VL (total 20 days). The GB pulse solution accelerated floral opening during cold storage (43%) while with AA it decreased (31%) compared to the control (T2) which had 38%

opening upon leaving the storage period. Finally, no significant differences were found in the duration of VL between treatments (Table 1).

Arabia *et al.* (2024) report that AA is an important antioxidant that controls the cellular redox state since it reduces free radicals and is a cofactor of enzymes such as APX. It interacts with ethylene, influencing processes such as senescence. Therefore, the exogenous application of AA can help to tolerate chilling injury disorders and increase the storage life of the product. In lilies, our results showed that AA delayed floral opening and yellowing of the leaves.

CONCLUSIONS

Cold storage of floral stems of *Lilium* cv. 'Indian Summerset' for two and four weeks accelerates the leaves yellowing, and provoked color fading and deformation of the flowers, and reduces the vase life. Preharvest treatments with AA and GB and pre-conditioning at 0 °C did not increased the tolerance to chilling injury in the stems. The postharvest application of AA through pulse solution for 24 h decreased chilling injury, maintain the tepals color, reduce the leaves yellowing and prevent the flower deformation. Studies around AA mode of action are necessary to increase the cold storage length in cut flowers.

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Colegio de Postgraduados

Production and commercialization of flowers in the municipality of Texcoco, Estado de México

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ABSTRACT

Objective: To characterize the production and commercialization of flowers in the municipality of Texcoco, Estado de México.

Design/methodology/approach: A total of n=78 floriculturists from 11 different localities were interviewed and asked about general characteristics of the production unit, and for each person their level of innovation was also identified with the aim of constructing a typology of floriculturists.

Results: Four groups of floriculturists were found, which were differentiated ($P<0.10$) by their level of infrastructure, innovation, number of family members that work in the production unit and number of clients which they have, in addition to gender.

Limitations on study/implications: The type of sample used does not allow generalizing the results found. In addition, due to the diversity of flowers that are produced in the zone, it is difficult to homogenize the innovations and level of innovation, so an approach is presented.

Findings/conclusions: The production and commercialization of flowers in the municipality of Texcoco is carried out by traditional floriculturists. The groups identified mainly have infrastructure directed toward production, so the highest levels of innovation are found in technology, with the opportunity area of commercial and organizational innovations.

Keywords: cluster, local floriculture production, floriculture market, technological innovation.

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INTRODUCTION

Most of the ecosystems in the world can be found in Mexico, owing to its geographic location, complex topography and wide variety of soils [1]. There are agroclimatic conditions to produce a large variety of flowers, such as cempasúchil (*Tagetes erecta*), chrysanthemum (*Chrysanthemum* spp.), lilies (*Lilium* spp.) and daisies (*Bellis perennis*) [2]. For the years 2013-2022, chrysanthemum stood out in national production of grosses (14 dozens of floral stems), with an annual average of 10 million and a mean annual growth rate (MAGR) of 1.9%, with cultivation in the states of Mexico and Puebla standing out, by order of importance, with 99% of the grosses.

In the municipality of Texcoco, the climates that predominate

are sub-humid temperate with lower moisture rainfall and monthly temperature means between 18°-20°, and sub-humid temperate with summer rains of average moisture, with mean monthly temperatures of 12°-14° [3]. This contributes to the development of the floriculture activity in the region through protected horticulture in a peri-urban environment, near large market zones and research centers [4]. Few studies approach the profitability of the floriculture activity in the municipality of Texcoco. The investment project of the floriculture business in the locality of Santa Catarina del Monte, Texcoco, was analyzed in 2013, and it was found that the investment project for chrysanthemum cut flower is viable, both in a technical and financial way for small-scale and medium-scale producers [5]. However, in 2015, the studies pointed out that the floriculture potential from Texcoco has not been developed as it should have, and that there are aspects regarding its production and commercialization that are unknown [6]. Therefore, it became necessary to perform this study about the production and commercialization of flowers in the municipality of Texcoco, with the objective of analyzing, characterizing and proposing actions to improve flower production.

MATERIALS AND METHODS

The research was carried out in the municipality of Texcoco, Estado de México. Semi-structured interviews were conducted during the months of June and July 2023, with a total of 78 floriculturists from 11 different localities (Figure 1), selected from directed sampling based on a register of floriculturists provided by the Ministry of the Farmland in the municipality.

Variables were used such as age, education, gender, marital status, years in the activity, percentage of income that comes from the activity, surface and infrastructure for production, type of property, number of people and family members that work in the production unit (PU), main flowers produced, number of clients, client reach, and reasons for commercialization. For the infrastructure, a level was determined, considering what is presented in Table 1. In addition, floriculturists were consulted about whether they adopted a set of 18 specific innovations in production, which were categorized into three types: i) technological innovations, ii) commercial innovations, and iii) organizational innovations (Table 2). Both for the calculation of level of innovation and level of infrastructure, the formula for the Innovation Adoption Index was used [7]. The variables described were scaled to later perform a cluster analysis with the Ward method. Finally, Scheffé means

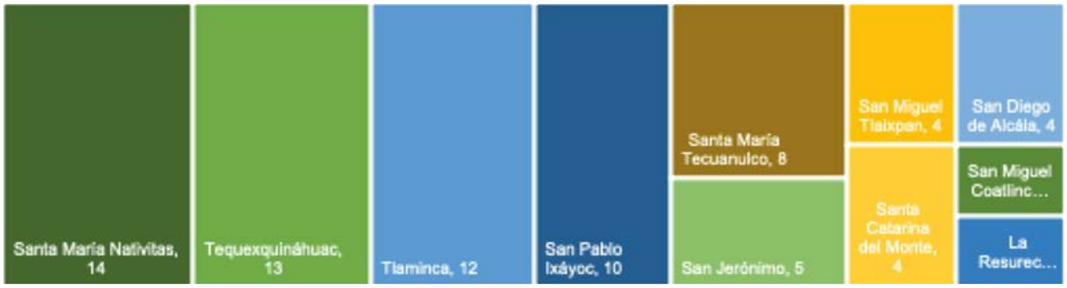

Figure 1. Universe of study. Source: Prepared by the authors with field information (2023).

Table 1. Infrastructure of the PU.

Infrastructure type
1. Water
2. Drain
3. Electricity
4. Internet at the Plot
5. Bank account
6. Cold storage rooms
7. Motorized cultivator
8. Own vehicle
9. Web sites
10. Online sales
11. Access to the bank by Internet
12. Electronic Invoice
13. Mother plant production area
14. Parihuela
15. Sprinkler pump

Table 2. Catalog of innovations.

Technology innovations
1. Soil analysis
2. Use of methyl bromide
3. Use of hydrogen peroxide
4. Padding
5. Fertirrigation
6. Crop rotations
7. Use of biological activators
8. Use of timer
9. Micronutrients
10. Composting
11. Soil improvers: agricultural lime and/or dolomitic lime and/or worm castings and/or liquid humus.
12. Mycorrhiza and azoospirilum
13. pH regulation for fertilization
Commercial innovations
14. Common purchases and/or sales
15. Sales by contract
Organizational innovations
16. Use of agricultural insurance
17. Use of credit
Technical-productive and administrative records

Source: Prepared by the authors.

difference tests were carried out for the quantitative variables analyzed in each group with a significance level of 10%. The analyses described were carried out through the use of the statistical package R.

RESULTS AND DISCUSSION

In general, 90% of the interview respondents are men and the rest women. Of them, 65% were married, 19% single, 8% living in civil union, and the rest are separated, widows or single mothers or fathers. They are on average 48 years old, with 11.8 years of education on average, and 20.8 years of experience in floriculture activity on average. In addition, 68% of them are owners of the land they farm, 26% rent it, and 6% of them work in a property on loan. The production of 19 different flower species was found, of which 29% of the floriculturists are devoted to the production of chrysanthemum, 14% to the production of sunflower (*Helianthus annuus*), 10% to the production of daisies, and 10% to the production of roses (*Rosa chinensis*), which as a whole represent 64%; the rest (36%) produces eleonora (*Chrysanthemum morifolium*), campana (*Moluccella laevis*), oriental (*Lilium candidum*), tulip (*Tulipa gesneriana*), geranium (*Geranium* spp.), African lily (*Agapanthus africanus*), aster matsumoto (*Callistephus chinensis*), among the most mentioned by floriculturists. On the other hand, of the total of interview respondents, for 46% of them, this activity represents economic income of 31 to 50%; for 22% it represents income between 51-70%; for 19% it represents more than 70% of their income; and only for 13% it represents between 0-30% of their economic income.

However, the characteristics of floriculturists and their production units (PUs) analyzed allowed grouping them into four groups (Figure 2).

Regarding the general characteristics of the PU and the floriculturist by groups, they are presented in Table 3, which shows the variables that present a significant statistical difference ($P<0.10$): number of family members who work, level of infrastructure, and number of clients of the PU.

Floriculturists from Group 1 represent 33% of the sample analyzed; 100% of them are men, the main flowers that they produce are chrysanthemum and daisies, and they are the ones that present the highest levels of infrastructure. Of them, 100% have the utilities of water and electricity, although the water used for production does not have adequate management, since 77% of them use the hose to irrigate; 86% have their own vehicle; 61% present production area of the mother plant; and 69% have rototiller, while 93% have dolly or aspersion pump. This allows inferring that due to the infrastructure they have, this fosters for the floriculturists to also be the ones that show the highest level of innovation (Table 3). Specifically, this infrastructure is a support to carry out the technological innovations, and in addition, this could also happen given that 69% of them are owners of their PUs. In reference to the number of family members who work in the PU, they are also the floriculturists that present a higher number of family members, the same as the number of clients. This group is characterized by its reach in the market, which is met with 23% of the floriculturists locally and regionally (Central de Abastos de Iztapalapa or Mercado de Jamaica), and the floriculturists refer as reasons for sale the fact that they value the quality of their product, offer a better price, or pay quickly and effectively.

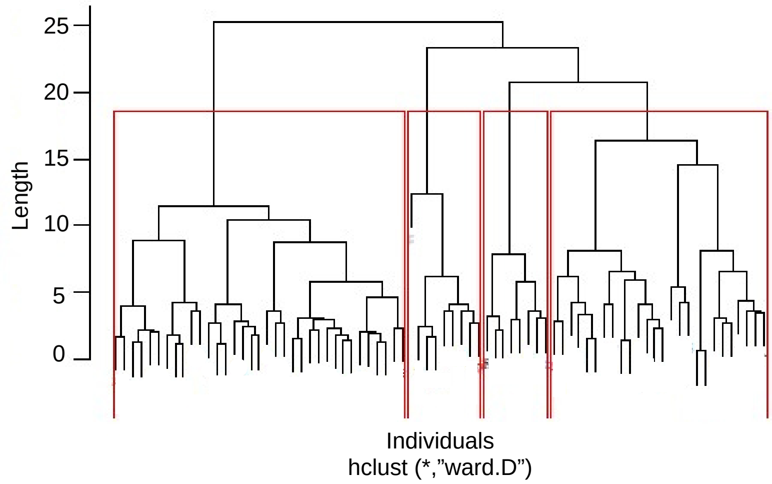

Figure 2. Resulting groups from the analysis.
Source: Prepared by authors with field information (2023).

Table 3. General characteristics of the PU and the floriculturist by groups.

Variable (units)	Group				
	1	2	3	4	Total
	n=26	n=35	n=9	n=8	N=78
Farmer age (years)	46.4 [a]	49.7 [a]	47.9 [a]	44.1 [a]	47.8
Farmer educatin (years)	12 [a]	10.8 [a]	15 [a]	11.8 [a]	11.8
Activity experience (years)	20.3 [a]	21.6 [a]	24.1 [a]	15.8 [a]	20.8
Number of people permanently working on the plot	4.6 [a]	2.7 [a]	2.1 [a]	2.1 [a]	3
Number of family members working on the plot	3.04 [a]	1.23 [b]	1.44 [ab]	1.38 [b]	2
Area (m^2)	2098.5 [a]	4118.4 [a]	2288.9 [a]	1706.4 [a]	2986.6
Infrastructure level (%)	48.2 [a]	32.4 [b]	35.3 [ab]	28.7 [b]	37.6
Number of customers	1.69 [a]	1.06 [b]	1.11 [b]	1.13 [b]	1.28

*Values with different letter in the same line are statistically different ($P<0.10$).
Source: Prepared by the authors.

Group 2 of floriculturists covers the highest percentage of the sample studied (44%), and 100% of them are men. They produce mainly sunflower, cempasúchil, campana and chrysanthemum. This group presents the second best level of infrastructure in their production unit; 89% have access to water and 77% to electricity, and also 66% have their own vehicle. Of the floriculturists, 40% have a production area of mother plant, 63% have a dolly or spraying pump. However, although they have the basic infrastructure for production, they are the ones that presented the lowest level of innovation, which is probably because in this group it is more common for the type of property to be rented or loaned.

Group 3 involves 12% of the sample, 100% of the floriculturists are men and they produce mainly chrysanthemum and daisy. Regarding their level of infrastructure, 100% have water service, 89% electricity, 78% vehicle, 56% have a production area for mother plant, 89% have a spraying pump or dolly, and for 66% the PU is their property while for the rest it is rented.

Group 4 covers 10% of the sample, and this group is characterized by being made up solely by women who produce mainly roses, aster matsumoto and African lily. Of them, 100% have water, 63% electricity, only 38% have a vehicle of their own, and 50% have dolly or spraying pump; 75% of them own the PU. From this group, it should be highlighted that although there was no statistical difference, they are the ones that have least activity in floriculture, since they present 8.3 years of experience less than the floriculturists from Group 3, who were the ones that presented the most experience on average (Table 3). It should also be highlighted that they are the ones that have least infrastructure to carry out the activity, and that no statistically significant difference was found; however, they present a higher level of innovation than floriculturists from Group 3, and even from the organizational point of view, and statistically, they have a higher level of innovation than floriculturists from Group 2 (Table 4).

Regarding the commercialization channels of Groups 2, 3 and 4, most of them satisfy the local market; that is, whether they sell directly in the street markets of their locality, in flower shops or in established markets in Texcoco such as the San Antonio Market or Texcoflor Market. They carry out these sales mainly because the clients purchase wholesale, or simply due to family tradition. The market that these groups of producers are focused on could be attributable to the low level of adoption of commercial and organizational innovations, which is related to the infrastructure that they have for this purpose (Figure 3). This applies even for floriculturists from Group 4 who were the ones with highest levels of adoption (Table 4).

This allows inferring that the production units analyzed are of traditional type; that is, although there are differences between them, they are devoted to the production of seasonal products, and for most of these, this type of production is not priority since they obtain income from other activities, and in addition their products are considered a commodity for there is not really anything that differentiates them from their competition, so the price they are paid is the one present in the market [4].

Table 4. Adoption of innovations among groups (%).

Innovation Adoption Index	Group				
	1	2	3	4	Total
Technology	42.3 [a]	21.5 [b]	29.1 [ab]	31.7 [ab]	30.4
Commercial	21.2 [a]	11.4 [a]	33.3 [a]	25.0 [a]	18.6
Organizational	6.4 [a]	1.9 [a]	0 [a]	8.3 [a]	3.8
General	34.0 [a]	17.1 [b]	24.7 [ab]	27.1 [ab]	24.6

* Values with different letter in the same line are statistically different ($P<0.10$).
Source: Prepared by the authors.

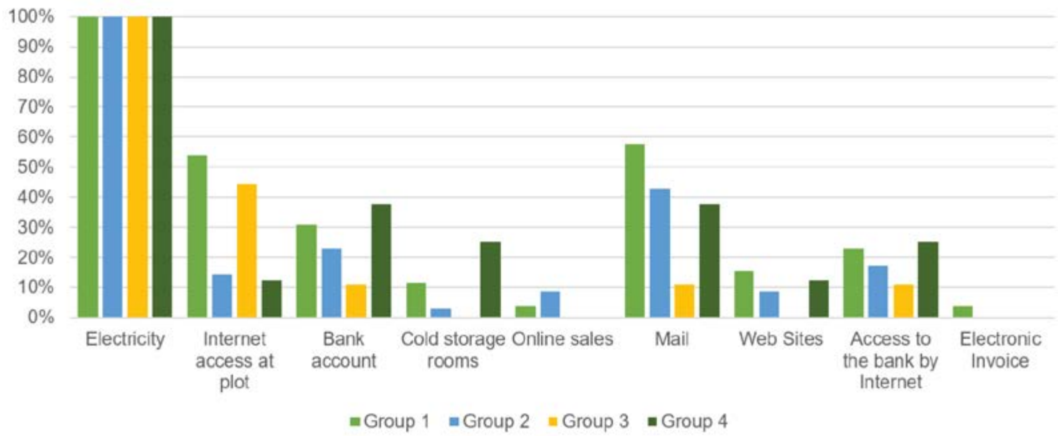

Figure 3. Infrastructure related to commercial and organizational innovations or to improve the commercialization channels.
Source: Prepared by the authors.

CONCLUSIONS

Four groups of floriculturists were found, which were statistically differentiated due to their level of infrastructure, the number of family members who work in the PU, and the number of clients they have. The levels of adoption are generally low; that is, they are adopting between 3 and 6 innovations out of 18 possible, depending on the group. Technological innovations are the group of innovations most frequently adopted, then the commercial and finally the organizational. It was found that there is a relationship between the level of infrastructure and the level of innovation. The level of infrastructure that the floriculturists have is focused on production, water, electricity, a vehicle of their own, and a dolly, among others. However, they do not have infrastructure that allows them to improve their commercialization and their organization; that is, access to internet, invoice emission, bank account, webpage, or email. Therefore, this study evidences that infrastructure is required in order to innovate. With the aforementioned, the groups found are characterized by being traditional businesses.

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Colegio de Postgraduados

Avifauna associated to a home garden in Valladolid, Yucatan, Mexico

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ABSTRACT

Objective: To identify the birds associated to a home garden in Valladolid, Yucatan, Mexico, and to understand which ones use cultivated plants as food.

Methodology: As part of the project "Fauna associated to cultivated plants in the south-southeast region of Mexico", 36 field visits were carried out, for six hours per day (6:00-10:00 and 16:00-18:00 hrs.), in the period from March 2022 to April 2023, in a home garden in Valladolid, Yucatan. The birds were watched with binoculars, photographed with digital cameras, and identified with field guides.

Results: A total of 67 bird species were observed, which belonged to 14 orders and 28 families. Of the species, 14 are migratory, three endemic, and four subject to special protection in Mexico. In addition, 21 bird species were found feeding off 22 species of cultivated plants. The most frequently used plant resource by the birds were fruits (16), followed by nectar (5) and tender leaves (1). The most consumed plants species by the birds were *Manilkara zapota* (L.) P. Royen, *Carica papaya* L. and *Spondias purpurea* L., which were used by three bird species each. The bird that consumed more cultivated plants was the Golden-fronted Woodpecker (*Melanerpes aurifrons* Wagler) that fed off five species.

Limitations on study: It was not possible to identify two birds at the species level: *Contopus* sp. and *Myiarchus* sp.

Conclusions: This study contributes to the knowledge of birds associated to home gardens and constitutes the basis for future studies on bird-plant trophic interactions in Mexico.

Keywords: Birds, home garden, Valladolid, Yucatán, Mexico.

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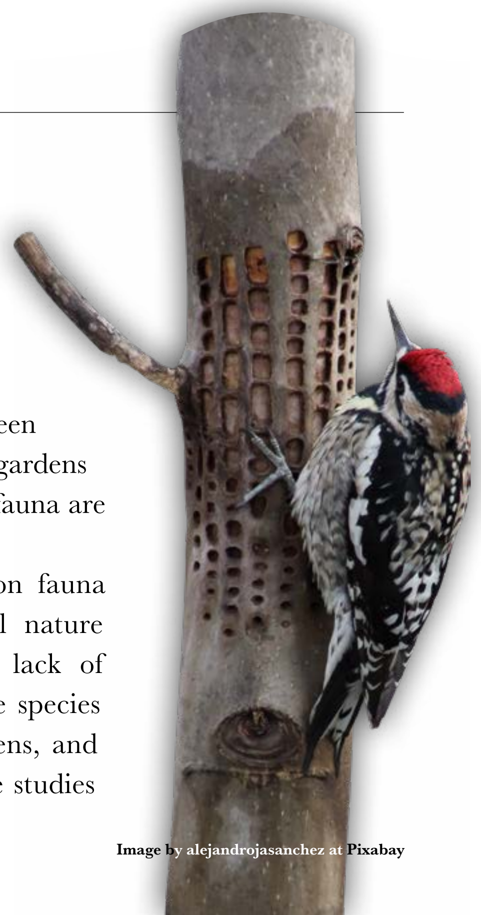
INTRODUCTION

Home gardens are complex agroforestry systems, of sociocultural and environmental importance, which combine agricultural and livestock production with conservation of flora and fauna (Guadarrama-Martínez and Chávez-Mejía, 2023). In the southeast of Mexico many studies have been conducted about the floristic composition of home gardens (Castañeda-Navarrete *et al.*, 2018), yet studies on wild fauna are scarce (Mariaca-Méndez, 2012).

In the Yucatan Peninsula, most of the studies on fauna associated to home gardens are of ethnobiological nature (Barranco-Vera *et al.*, 2023); however, there is a lack of studies with direct methods to allow determining the species of the different groups of fauna present in the gardens, and the way in which they relate to the vegetation. These studies



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are particularly important for birds, because they can find sites for nesting, refuge and feeding in home gardens (Sánchez-Soto, 2018). In addition, with studies of this nature, potentially damaging species for some crops can be detected (Sánchez-Soto, 2016). Therefore, the objective was to identify the species of wild birds associated with a home garden in Valladolid, Yucatan, Mexico, and to understand which could use cultivated plants as a feeding resource.

MATERIALS AND METHODS

Study area

The study was conducted in a home garden of 0.5 ha, approximately 50 years old, located in the city of Valladolid, Yucatan, Mexico (20° 41' 22" N and 88° 12' 6" W), 400 m away from the Central Park, 15 m away from the "San Juan" sports center, and 60 m away from the private school "Centro de Enseñanza Siglo XXI" in that city. It neighbors a portion of secondary vegetation of approximately 70 m^2 and another of 2.0 ha, separated by an avenue. The climate is predominantly warm subhumid with summer rains and the mean annual temperature is 25.8 °C (Orellana-Lanza *et al*., 2010). The geomorphology of the area is constituted by an ondulating plain of carstic nature (Bautista-Zúñiga *et al*., 2010), which favors water infiltration forming caves inside the garden. The soil is Luvisol and Cambisol (García-Gil *et al*., 2010) and the original vegetation was constituted by medium sub-deciduous forest (Flores-Guido *et al*., 2010).

The garden was made up by native and exotic plants. The timber-yielding native trees included chechén (*Metopium brownie* (Jacq.) Urb.), chaká (*Bursera simaruba* (L.) Sarg.), cedar (*Cedrela odorata* L.), maya nut (*Brosimum alicastrum* Sw.), and dyer's mulberry (*Maclura tinctoria* (L.) D. Don ex Steud). The native fruit trees, chirimoya (*Annona reticulata* L.), saramuyo (*Annona squamosa* L.), sapodilla (*Manilkara zapota* (L.) P. Royen), caimito (*Chrysophyllum mexicanum* Brandegee ex Standl), plum (*Spondias purpurea* L.), guava (*Psidium guajava* L.), soursop (*Annona muricata* L.), and papaya (*Carica papaya* L.). The exotic fruit trees, bitter orange (*Citrus aurantium* L.), sweet orange (*Citrus sinensis* (L.) Osbeck), Persian lime (*Citrus latifolia* Tanaka Ex Q. Jiménez), mandarin (*Citrus reticulata* Blanco), coconut (*Cocos nucifera* L.), banana (*Musa paradisiaca* L.), tamarind (*Tamarindo indica* L.), and mango (*Mangifera indica* L.). The native ornamental, sabal (*Sabal yapa* C. Wright ex Becc.), yellow oleander (*Cascabela thevetia* (L.) Lippold) and wax mallow (*Malvaviscus arboreous* Cav.); and the exotic, African tulip tree (*Spathodea campanulata* P. Beauv.), bouganvillea (*Bougainvillea glabra* Choisy) and neem (*Azadirachta indica* A. Juss.).

Edible, medicinal and honey horticultural species included chili (*Capsicum annum* L.), epasote (*Dysphania ambrosioides* (L.) Mosyakin & Clemants), momo (*Piper auritum* Kunth), purple maguey (*Tradescantia spathacea* Sw.) and palo de caja (*Allophylus cominia* (L.) Sw.).

In general, in the garden there were one to four plants per species, except for bitter orange of which there were around 10 plants.

Bird registration

Registration of birds associated to the home garden was carried out from April 2022 to March 2023, avoiding the days with strong rainfall or winds (Bibby *et al*., 2002). During this period, 36 daily visits were made, of six hours per day (6:00-10:00 and 16:00-18:00 hrs.), representing 216 h of observations. In each visit, linear visits were made that covered the entire garden.

The birds were observed with binoculars (Bushnell 10×50) and photographed with a digital camera (Canon EOS Rebel T7 with 75-300mm lens). For identification of the species, the field guides by Howell and Webb (1995), Peterson and Chalif (1989), and Van Perlo (2006) were used. The taxonomic nomenclature and the common names were taken from the study by Berlanga-García *et al.* (2019). Each species was assigned a category of habitat preference and trophic guild based on Howell and Webb (1995) and Arriaga (2008).

RESULTS AND DISCUSSION

During the study period, 67 bird species were recorded associated to the garden, from 28 families and 14 orders. Of these, 53 are resident and 14 are migratory; 4 species are subject to special protection, 3 are endemic, and 2 exotic (Table 1). The number of species recorded is equivalent to 15% of the total birds reported for the state of Yucatan (Chablé-Santos and Pasos-Enríquez, 2010).

This number is considerable compared to the 22 species reported by Montañez-Escalante *et al.* (2012), the 11 species mentioned by Heredia-Campos (2020), and the 4 mentioned by Barranco-Vera *et al.* (2023) in ethnobiological studies carried out in different home gardens of the state of Yucatan, since this study was focused especially on this group of vertebrates through direct observations. In this sense, this number is similar to the number of species (70) registered by Sánchez-Soto (2018) in a home garden located in a suburban area in the state of Tabasco, also located in the southeast of Mexico.

The families with the highest number of species are Icteridae with 9, Tyrannidae with 6, Parulidae with 5, and Columbidae with 5 (Figure 1), which are equivalent to 22% of the total species from these families reported for Yucatan (Chablé-Santos and Pasos-Enríquez, 2010). Likewise, most of the species were forest generalist (FG) (64%); that is, they tolerate conditions of habitat disturbance (Howell and Webb, 1995; Arriaga, 2008), followed by open area specialists (OA) (31%) which require open or semi-open habitats such as forest edges, clearings and even towns and urban areas (Howell and Webb, 1995; Arriaga, 2008), and to a lesser degree there were forest specialists (FS) which are particularly more sensitive to fragmentation and naturally more difficult to observe (Turner, 1996; Arriaga, 2008) (Figure 2).

It is possible that the abundance of FG and OA species is due to the heterogeneity of the habitat (Arriaga, 2008), composed by the urban matrix, the patches of secondary vegetation, and the home garden; therefore, this agroforestry system seems to provide connectivity, refuge and food to the FG and OA species, although it lacks adequate resources for FS species that are more sensitive and which require portions of landscape that are not altered by human activity (Barret *et al.*, 1994).

The predominant food guild was arboreal insectivore/frugivore with 15 species (22%), followed by sallying/sweeping insectivore with 9 species (13%), and foliage-gleaning insectivore with 8 species (12%) (Figure 3).

Table 1. Birds recorded in a home garden in Valladolid, Yucatan, Mexico.

Order and Family	Scientific name	Common name	H	TG	S
Galliformes					
Cracidae	*Ortalis vetula* (Wagler, 1830)	Plain Chachalaca	FG	AF	R
Columbiformes					
Columbidae	*Columba livia* (Gmelin, 1789)[1]	Rock Pigeon	OA	GR	R
	Streptopelia decaocto (Frivaldszky, 1838)[1]	Eurasian Collared-Dove	OA	GR	R
	Columbina passerina (Linnaeus, 1758)	Common Ground Dove	OA	GR	R
	Columbina talpacoti (Temminck, 1810)	Ruddy Ground Dove	OA	GR	R
	Zenaida asiatica (Linnaeus, 1758)	White-winged Dove	FG	GR	RM
Cuculiformes					
Cuculidae	*Crotophaga sulcirostris* (Swainson, 1827)	Groove-billed Ani	OA	TI	R
	Piaya cayana (Linnaeus, 1766)	Squirrel Cuckoo	FG	FI	R
Nyctibiiformes					
Nyctibiidae	*Nyctibius jamaicensis* (Gmelin, 1789)	Northern Potoo	FG	SwI	R
Apodiformes					
Apodidae	*Chaetura vauxi* (Townsend, 1839)	Vaux's Swift	OA	SwI	R
Trochilidae	*Cynanthus canivetii* (Lesson, 1832)	Canivet's Emerald	FG	NI	R
	Amazilia rutila (DeLattre, 1842)	Cinnamon Hummingbird	FG	NI	R
Cathartiformes					
Cathartidae	*Coragyps atratus* (Bechstein, 1793)	Black Vulture	OA	SC	R
	Cathartes aura (Linnaeus, 1758)	Turkey Vulture	OA	SC	R
Accipitriformes					
Accipitridae	*Rupornis magnirostris* (Gmelin, 1788)	Roadside Hawk	OA	R	R
	Buteo albonotatus (Kaup, 1847)	Zone-tailed Hawk	OA	R	M
Strigiformes					
Tytonidae	*Tyto alba* (Scopoli, 1769)	Barn Owl	FG	R	R
Strigidae	*Glaucidium brasilianum* (Gmelin JF, 1788)	Ferruginous Pygmy-Owl	FG	R	R
Trogoniformes					
Trogonidae	*Trogon melanocephalus* (Gould, 1836)	Black-headed Trogon	FG	AF	R
Coraciiformes					
Momotidae	*Momotus lessonii* (Lesson, 1842)	Lesson's Motmot	FG	AIF	R
	Eumomota superciliosa (Sandbach, 1837)	Turquoise-browed Motmot	FG	AIF	R
Piciformes					
Picidae	*Melanerpes pygmaeus* (Ridgway, 1885)[2]	Yucatan Woodpecker	FG	BI	R
	Melanerpes aurifrons (Wagler, 1829)	Golden-fronted Woodpecker	FG	BI	R
	Dryocopus linaeatus (Linnaeus, 1766)	Lineated Woodpecker	FG	SI	R
Falconiformes					
Falconidae	*Falco sparverius* (Linnaeus, 1766)	American Kestrel	OA	R	M
	Falco rufigularis (Daudin, 1800)	Bat Falcon	FG	R	R
Psittaciformes					
Psittacidae	*Eupsittula nana* (Vigors, 1830)[3, 4]	Olive-throated Parakeet	FG	AF	R
	Amazona albifrons (Sparrman, 1788)[3]	White-fronted Parrot	FG	AF	R

Table 1. Continues...

Order and Family	Scientific name	Common name	H	TG	S
Passeriformes					
Tityridae	*Tityra semifasciata* (Spix, 1825)	Masked Tityra	FG	SI	R
	Pachyramphus aglaiae (Lafresnaye, 1839)	Rose-throated Becard	FS	SI	R
Tyrannidae	*Myiarchus* sp. (Cabanis, 1844)	Flycatcher	FG	SI	R
	Pitangus sulphuratus (Linnaeus, 1766)	Great Kiskadee	FG	SI	R
	Megarynchus pitangua (Linnaeus, 1766)	Boat-billed Flycatcher	FG	SI	R
	Myiozetetes similis (Spix, 1825)	Social Flycatcher	FG	SI	R
	Tyrannus melancholicus (Vieillot, 1819)	Tropical Kingbird	FG	SI	R
	Contopus sp. (Cabanis, 1855)	Pewee	FG	SI	R
Vireonidae	*Vireo griseus* (Boddaert, 1783)	White-eyed Vireo	FG	FI	M
	Vireo pallens (Salvin, 1863)[3]	Mangrove Vireo	FG	UI	R
Corvidae	*Cyanocorax yncas* (Boddaert, 1783)	Green Jay	FG	AIF	R
	Cyanocorax yucatanicus (Dubois, 1875)[2]	Yucatan Jay	FG	AIF	R
Troglodytidae	*Troglodytes aedon* (Vieillot, 1809)	House Wren	FG	UI	R
Polioptilidae	*Polioptila caerulea* (Linnaeus, 1766)	Blue-gray Gnatcatcher	FG	FI	R
Turdidae	*Turdus grayi* (Bonaparte, 1838)	Clay-colored Thrush	FG	TI	R
Mimidae	*Dumetella carolinensis* (Linnaeus, 1766)	Gray Catbird	OA	UI	M
	Mimus gilvus (Vieillot, 1808)	Tropical Mockingbird	FG	FI	R
Fringillidae	*Euphonia affinis* (Lesson, 1842)	Scrub Euphonia	FG	AF	R
Icteridae	*Amblycercus holosericeus* (Deppe, 1830)	Yellow-billed Cacique	FG	AIF	R
	Icterus spurius (Linnaeus, 1758)	Orchard Oriole	FG	AIF	M
	Icterus cucullatus (Swainson, 1827)	Hooded Oriole	FG	AIF	R
	Icterus auratus (Bonaparte, 1850)[2]	Orange Oriole	FG	AIF	R
	Icterus gularis (Wagler, 1829)	Altamira Oriole	FG	AIF	R
	Icterus galbula (Linnaeus, 1758)	Baltimore Oriole	OA	AIF	M
	Molothrus aeneus (Wagler, 1829)	Bronzed Cowbird	OA	TI	R
	Dives dives (Deppe, 1830)	Melodious Blackbird	OA	AIF	R
	Quiscalus mexicanus (JF Gmelin, 1788)	Great-tailed Grackle	OA	O	R
Parulidae	*Mniotilta varia* (Linnaeus, 1766)	Black-and-white Warbler	FG	AIF	M
	Setophaga petechia (Linnaeus, 1766)	Yellow Warbler	OA	FI	MR
	Setophaga coronata (Linnaeus, 1766)	Yellow-rumped Warbler	OA	FI	M
	Setophaga dominica (Linnaeus, 1766)	Yellow-throated Warbler	OA	FI	M
	Setophaga virens (Gmelin, 1789)	Black-throated Green Warbler	OA	FI	M
Cardinalidae	*Piranga rubra* (Linneo, 1758)	Summer Tanager	OA	AIF	M
	Habia fuscicauda (Cabanis, 1861)	Red-throated Ant-Tanager	FS	UI	R
	Passerina caerulea (Linneo, 1758)	Blue Grosbeak	OA	GR	M
Thraupidae	*Thraupis episcopus* (Linnaeus, 1766)	Blue-gray Tanager	FG	AF	R
	Thraupis abbas (Deppe, 1830)	Yellow-winged Tanager	FG	AF	R
	Eucometis penicillata (Spix, 1825)[3]	Gray-headed Tanager	FS	AIF	R
	Saltator grandis (Deppe, 1830)	Black-headed Saltator	FG	AIF	R

Abbreviations. H: Habitat Preference (FG: Forest Generalist, OA: Open Area Specialist, FS: Forest Specialist). G: Guilds (AF: Arboreal Frugivore, GR: Granivore, TI: Terrestrial Insectivore, FI: Foliage-Gleaning Insectivores, SwI: Sweeping Insectivore, NI: Nectarivore/Insectivore, SC: Scavenger, R: Raptor, AIF: Arboreal Insectivore/Frugivore, SI: Sallying/Sweeping Insectivore, BI: Bark-Gleaning Insectivore, UI: Undergrowth Insectivore, O: Omnivore). S: Status (R: Resident, M: Migratory) (Howell y Webb, 1995: Arriaga, 2008). [1]Exotic, [2]Endemic (Berlanga et al., 2019), [3]Subject to Special Protection (SEMARNAT, 2010), [4]Near Threatened (UICN, 2020).

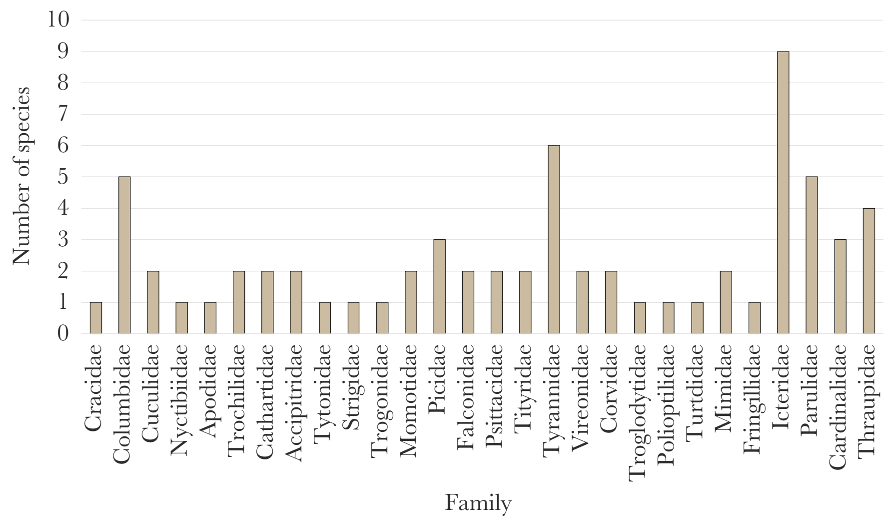

Figure 1. Species composition by family of a bird community in a home garden in Valladolid, Yucatan, Mexico.

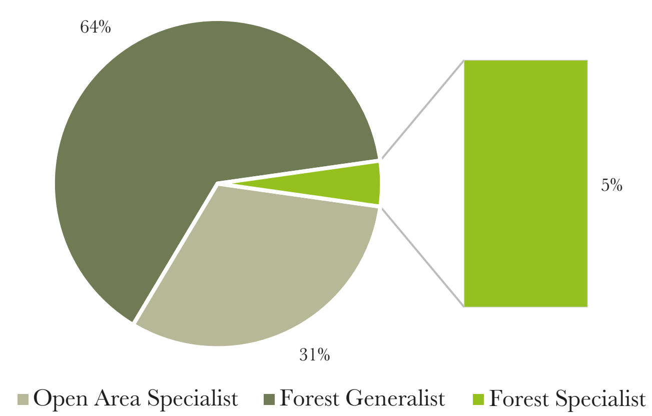

Figure 2. Proportion of habitat preference in a bird community in a home garden in Valladolid, Yucatan, Mexico.

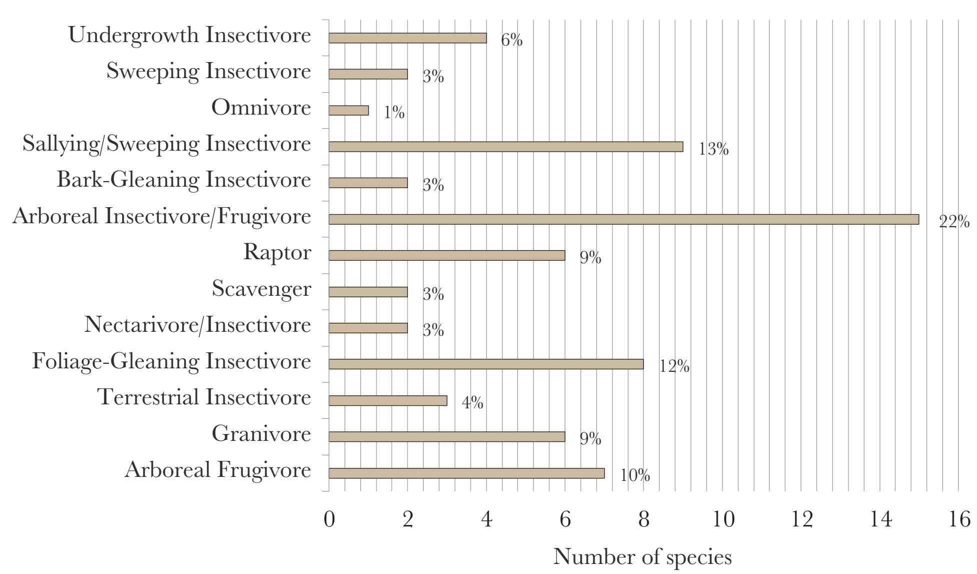

Figure 3. Proportion of food guilds in a bird community of a home garden in Valladolid, Yucatan, Mexico.

It is likely that the abundance of these categories of insectivores is because the diversity of trees in the garden regulates the entry of light and generates microclimates that allow the diversification of arthropods (Basset *et al.*, 1992; Van Der Wal *et al.*, 2019).

In total, 21 bird species were seen feeding off 22 cultivated plants (Figure 4). The most frequently used plant resource used by the birds was fruits (16), followed by nectar (5) and tender leaves (1). The most consumed plants by the birds were sapodilla (*M. zapota*), papaya (*C. papaya*) and plum (*S. purpurea*), consumed by 3 bird species each (Figure 4). These native fruit trees are considered "structural" species in home gardens in Yucatan, since together with other woody plant species, they provide the most plant cover and give structure to these agroecosystems (Montañez-Escalante *et al.*, 2012).

The bird that consumed the most cultivated plants was the Golden-fronted Woodpecker (*Melanerpes aurifrons* Wagler), which was observed feeding off five fruit tree species: chirimoya (*A. reticulata*), saramuyo (*A. squamosa*), papaya (*C. papaya*), tamarind (*T. indica*) and sweet orange (*C. sinensis*). The latter was consumed 11 times during the study period (Figure 5), so the bird is considered a pest by the garden owners. The Golden-fronted Woodpecker (*M. aurifrons*) has been observed feeding off other crops in the southeast of Mexico, such as cacao (*Theobroma cacao* L.) where losses of up to 5% have been reported (Arriaga, 1985); therefore, the suggestion is to perform studies to evaluate the damages that it causes in citrus trees of the home gardens in Valladolid, Yucatan. However, this bird has traditionally been classified as an insectivore bark species, similar to the Yucatan

		Manilkara zapota 01	*Hibiscus rosa-sinensis* 02	*Malvaviscus arboreus* 03	*Cascabela thevetia* 04	*Carica papaya* 05	*Spondias purpurea* 06	*Citrus reticulata* 07	*Annona reticulata* 08	*Annona squamosa* 09	*Tamarindus indica* 10	*Citrus sinensis* 11	*Capsicum annum* 12	*Sabal yapa* 13	*Allophylus cominia* 14	*Spathodea campanulata* 15	*Bougainvillea glabra* 16	*Musa paradisiaca* 17	*Azadirachta indica* 18	*Cedrela odorata* 19	*Psidium guajava* 20	*Cecropia obtusifolia* 21	*Maclura tinctoria* 22
Ortalis vetula	1	F																					
Cynanthus canivetii	2		N																				
Amazilia rutila	3			N	N																		
Melanerpes pygmaeus	4	F					F	F															
Melanerpes aurifrons	5					F			F	F	F	F											
Myiozetetes similis	6												F										
Cyanocorax yucatanicus	7													F									
Turdus grayi	8	F																					
Dumetella carolinensis	9														F								
Amblycercus holosericeus	10										F												
Icterus spurius	11															N							
Icterus cucullatus	12				N		F																
Icterus auratus	13															N	N						
Icterus gularis	14					F	F																
Icterus galbula	15																	F					
Dives dives	16					F																	
Piranga rubra	17																		F	F			
Quiscalus mexicanus	18																	F					
Thraupis episcopus	19																				F		
Thraupis abbas	20																					F	
Saltator grandis	21											L									F		F
Abbreviations: F: Fruits, **N:** Nectar, **L:** Leaves																							

Figure 4. Matrix of trophic interactions: bird-plant in a home garden in Valladolid, Yucatan, Mexico.

Figure 5. Damage on sweet orange (*Citrus sinensis*) by the Golden-fronted Woodpecker (*M. aurifrons*) in a home garden in Valladolid, Yucatan, Mexico.

Woodpecker (*Melanerpes pygmaeus* Ridgway) which was seen in this study feeding off 3 species of cultivated plants: sapodilla, plum and mandarin (Figure 4). Therefore, the suggestion is to analyze the trophic guilds and to ratify them with direct observations and not solely based on the literature, since as López-Muñoz *et al.* (2022) mention, sometimes birds are grouped under a single criterion, such as their main source of food, leaving aside their other foraging techniques and other resources.

CONCLUSIONS

The number of birds (21) that feed off cultivated plants is considerable, since it represents 31% of the total birds observed (67), and is higher than the percentage (24%) of species recorded by Sánchez-Soto (2018) consuming cultivated plants in a home garden in Tabasco. These data indicate that home gardens in Valladolid, Yucatán, provide resources to an important number of wild birds. It should be highlighted that with the exception of Golden-fronted Woodpecker (*M. aurifrons*), the other species do not seem to represent a threat for cultivated plants, since they were not observed causing considerable damage.

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Colegio de Postgraduados

The effect of predictor variables on cherry coffee yield in two regions of the state of Veracruz, Mexico

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ABSTRACT
Objective: To analyze the effect of predictor variables (sown area, harvested area, and production) on cherry coffee yield in the Huatusco and Córdoba regions of the state of Veracruz.
Design/Methodology/Approach: A mixed and correlational method was employed with a representative trial sample of 144 cases. The existing bibliography on cultivation, processing techniques, good practices, commercialization, and yield was subject to a critical analysis.
Results: The results were statistically significant (Pearson correlation=0.983) for the "final production" and "harvested area" variables. The hypotheses were positive, confirmed, and determined to be 95% reliable. The production was 2.532 and the sown area was 0.639. The coffee yield was higher in the Huatusco region, with a value of 1.72.
Study Limitations/Implications: The study was not hindered by any limitations.
Findings/Conclusions: Innovation is of great consequence for the enhancement of coffee productivity. Technified agriculture has the potential to speed up the production process. However, further research is necessary to ensure the optimal care of these crops and to guarantee superior yields of higher quality.

Keywords: predictor variables, yield, coffee, and production process.

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INTRODUCTION

Coffee (*Coffea arabica* L.) is consumed by families around the world. It is enjoyed by many and consumed at any time of the day, even during working hours. Coffee keeps consumers active and relaxed. Many simply prefer it due to its taste and health benefits (Bonilla, 2017). The leading producers and exporters of coffee are Colombia, Brazil, Guatemala, Honduras, Peru, Nicaragua, Costa Rica, and Mexico. Additionally, Vietnam, Indonesia, India, and Uganda make a significant contribution to global coffee production. The United States, Germany, France, and Japan are the primary consumers of coffee (Argoti and Belalcazar, 2017).

The main plantations in Mexico are located in Chiapas, Oaxaca, and Veracruz. Approximately 511,679 producers grow coffee on 697,366.22 hectares (ha), representing 91% of the country's total coffee production (SAGARPA, 2018). As reported by the Mexican Agrifood and Fisheries Information Service (SIAP), the most productive crop varieties were identified and categorized according to their prevalence in large-scale plantations across 15 states (SIAP, 2021). In the state of Veracruz, several varieties of coffee (*Coffea arabica* L.), including *Typica*, *Bourbon*, and *Caturra*, are grown under shade. Most of these plants are grown in areas with varying altitudes to achieve an average production per plant (López-García *et al.*, 2016). The *Typica* variety from Ethiopia was one of the first to be introduced in Mexico in the XVIII century. *Typica* is distinguished by its grain size, although it is highly susceptible to rust (Escamilla Prado *et al.*, 2015). Mexico's abundant biodiversity —resulting from the climate, biogeography, and ecology that characterize some of its tropical regions— accounts for 60-70% of the domestic coffee production (INEGI, 2017).

Interestingly, a quality product requires adequate environmental care, complemented by innovative technical systems to safeguard the product (Flores Vichi, 2014). In some cases, such as in Mexico, coffee is cultivated on plots by small-scale producers who invest very little capital (Calo and Wise, 2005). A study conducted by Medina-Meléndez *et al.* (2016) corroborated that 41.20% of coffee farmers identified climate change as a factor contributing to crop losses. For some farmers, coffee production is a primary source of income (Argoti and Belalcazar, 2017). This is particularly noteworthy, given the experience these farmers have in the management of their production and the commercialization of coffee (Lopes *et al.*, 2020). Verifying the climate, ecological conditions, and productivity is essential for the selection of coffee beans (Monsalve-Vásquez, 2022). The climate in Córdoba, Veracruz, has a wide range of conditions, from tropical to temperate to dry. The latitude ranges from 20° to 36°, while the altitude varies from 600 to 1,500 meters above sea level (Rivera *et al.*, 2013). An assessment was conducted to determine the potential influence of air temperature on the onset of flowering and fruit growth in the Huatusco region (Villers *et al.*, 2009).

According to Thomas *et al.* (2015), innovative production processes are an effective technology for groups with implicit needs. For their part, Sampedro and Díaz (2016) consider that innovation occurs in two distinct spheres: innovation for development (CONCEIÇÃO *et al.*, 2000) and innovation for inclusion (Alzugaray *et al.*, 2012; George *et al.*, 2012; Hall *et al.*, 2012). The former may be defined as new improvement alternatives, while the latter is aimed at groups seeking support for their fields. In Mexico, the implementation of innovative techniques for agricultural products has not been a prevalent practice. For some producers, it entails an additional financial burden, whereas for others, it signifies a major advancement in the development of their crop (Amaro-Rosales and De Gortari-Rabiela, 2016).

There are two types of coffee production processes: artisanal and technified. Artisanal coffee growers have empirical knowledge about cultivation and pest and disease control (Staver *et al.*, 2001). The second process emphasizes the role of technified systems (Egea, 2016).

Medina-Meléndez *et al.* (2016) indicated that the coffee-growing region of Veracruz encompasses 82 municipalities (approximately 842 communities) that have implemented various production strategies, including small-scale and large-scale operations, in order to reduce costs and achieve positive results. These strategies have involved the use of diversified and innovative designs (Licona-Vargas *et al.*, 2006).

In the Huatusco region, the Costa Rica cherry coffee variety recorded an efficiency of 277.77 kg, resulting in the production of 55.9 kg of parchment. Meanwhile, the *Garnica* variety had a total yield of 288.94 kg, resulting in 56.5 kg of parchment (Sánchez-Hernández *et al.*, 2018). The expected average yield of coffee was 1.80 kg/plant for the technified system, as reported by Villavicencio-Enríquez (2012). In contrast, Córdoba (located in the central zone of Atoyac) is considered one of the ten best coffee-producing regions (Sánchez *et al.*, 2019). Since 2015, yields have fluctuated between 7.3 and 10.8, in the 18,832 hectares where coffee is grown (López, 2021).These crops are characterized by their aroma, body, and acidity (López-García *et al.*, 2016).

This research is divided into three sections. The first section provides an overview of coffee and its context in the state of Veracruz. The second section focuses on the mixed and correlational method, which involves the use of a representative sample to support the research. The third section presents the statistical analysis of the main results and variables. To support this research, the following hypotheses were proposed: H1: The sown area is directly related to the yield of cherry coffee. H2: The harvested area is positively correlated with the yield of cherry coffee. H3: The final production is closely related to the yield of cherry coffee.

MATERIAL AND METHODS

This research employed a quantitative, mixed, and correlational approach, with a sample selected through judgment method (Delphi) of two groups of coffee farmers in the Córdoba and Huatusco regions of Veracruz. The sample was obtained from a 2018-2023 database, sourced from the Agrifood and Fisheries Information Service (SIAP). In comparison to previous years, the minimal variation in yields was largely attributed to the seasonal cycles established by SIAP and the policies implemented for the benefit of producers. From January to December, a total of 144 cases were observed, with 72 in Córdoba and 72 in Huatusco. Information related to the cherry coffee crop was used, with the independent variables comprising sown area, harvested area, and production. The dependent variable was yield per hectare. The data were then imported into an Excel database and subsequently transferred to the SPSS Statistics 25 software. The Cronbach's alpha statistic was employed to assess the validity of the research instrument, with a confidence level of 95% and a margin of error of 5%. The stepwise regression model was used to correlate the multiple linear regression variables, in the SPSS Statistics 25 software.

RESULTS AND DISCUSSION

Table 1 shows the average data of cherry coffee crop, including sown area, harvested area, and production, as well as the regional yields for Huatusco and Cordoba. This information provided by SIAP covers the period from 2018 to 2023. Both descriptive and

Table 1. Average data of the cherry coffee crop.

State	Municipality	Year	Crop	Area sown (ha)	Area harvested (ha)	Production	Yield (udm ha^{-1})
Veracruz	Cordoba	2018	Café cereza	1900	1109.17	1160.71	1.15
		2019	Café cereza	1900	1055.00	1371.50	1.30
		2020	Café cereza	1990	539.88	631.16	1.17
		2021	Café cereza	1990	580.00	681.36	1.18
		2022	Café cereza	1826	531.67	696.48	1.20
		2023	Café cereza	1992	1341	1756.71	1.31
	Huatusco	2018	Café cereza	7495	5458.33	9997.75	1.83
		2019	Café cereza	7470	5637.50	10255.50	1.82
		2020	Café cereza	7465	6100.00	10405.53	1.71
		2021	Café cereza	1990	580.00	681.36	1.18
		2022	Café cereza	7467	6101	11530.89	1.89
		2023	Café cereza	7467	6826	12901.16	1.89

inferential statistics were calculated, supporting the information resulting from the study variables.

Descriptive statistics

As suggested by Hernández-Samipieri *et al.* (2014), the internal consistency of the instrument was validated using the Cronbach's Alpha statistic (Cronbach, 1951): a >0.7 value for this parameter is considered significant (Table 2).

Table 3 presents the descriptive statistics. The mean of each variable was calculated and the dependent variable (yield per hectare) showed an average value of 1.5151. The independent variables included the following values: sown area (4,716.08), harvested area (3,412), and production (5,889.63). These values were used to formulate the multiple linear regression model:

$$\textit{Average value}\left(X_1\right) = 4{,}716.08, X_2 = 3{,}412 = \left(X_3\right) = 5{,}889.63, \text{ and } Y = 1.5151$$

Table 2. Cronbach's Alpha statistic values

N of elements	Cronbach's Alpha based on the typed items
144	.972

Table 3. Descriptive statistics.

	Mean	Standard deviation	N
Yield (udm/ha)	1.5151	.33065	144
Area Sown(ha)	4716.08	2765.212	144
Harvested Area(ha)	3412.00	2646.411	144
Production	5889.63	5095.927	144

Source: Developed by the authors.

Inferential statistics

Inferential statistical data are calculated by determining significant associations according to Pearson's Correlation. Values lower than 1, with a 95% reliability and $p \leq 0.05$, indicate a significantly positive correlation between the data. The following results were obtained when the variables were crossed with yield: 0.848 (sown area), 0.840 (harvested area), and 0.899 (production). Production had a higher positive correlation. In summary, the four variables had positive correlations (>0.840). These findings are the closest to the results obtained by Reyna (2022) for the Amazonas region. The correlation of coffee production calculated for that region reached a score of 0.878, indicating a positive association with the variables shown (Table 4). Although there are some differences both obtained similar results.

The correlation was determined using the stepwise regression model and included the selected variables: production, harvested area, and sown area. The F-test results ranged from 2.70 to 3.84. Subsequently, the variables that had the greatest influence on the dependent variable (*i.e.*, yield per hectare) are presented. Model 1 in R resulted in the highest predictor variable (0.954), a corrected R-squared of 0.907 accounted for a data reliability of 90.7%, and 1.016 with Durbin-Watson, indicating that the residuals are dependent. These data corroborate a range of 0 to 4, which is predominantly associated with a positive autocorrelation. According to Belts (2011), the estimated model offers first-order evidence (Table 5).

In relation to the ANOVA of Model 1, the F-test yielded a result of 468.584, with a significance of 0.000, indicating a statistically positive result. To test the hypotheses, the typified coefficients with the following β values were provided as reference. The results of the analysis indicate that β_1 production has a value of 2.579, β_2 harvested area has a value of -2.237, and β_3 sown area has a value of 0.554. These values are significant at the 95% level, which supports the acceptance of the hypotheses. The t-value for production was 16.803, while the values for harvested and sown area were -12.459 and 5.777, respectively.

Tabla 4. Correlations

		Yield (udm/ha)	Area Sown (ha)	Harvested Area (ha)	Production
Pearson correlation	Yield (udm/ha)	1.000	.848	.840	.899
	Area Sown(ha)	.848	1.000	.964	.951
	Harvested Area(ha)	.840	.964	1.000	.986
	Production	.899	.951	.986	1.000

Source: Developed by the authors.

Table 5. Summary of model[b].

Model	R	R square	R square co-regulated	Standard error of estimation	Durbin-Watson
1	.954[a]	.909	.907	.10057	1.016

a. Predictor variables: (Constant), Production, Area Sown (ha), Area Co-harvested (ha).
b. Dependent variable: Yield (udm/ha).

Source: Developed by the authors.

These variables were found to be significant at the 0.000 level, with positive results. The collinearity statistics met the <1 tolerances (Table 6).

Table 6. Coefficients[a].

Model	Unstandardised coefficients	Typified coefficients	T	Sig.	Collinearity statistics	
	B	Beta			Tolerance	FIV
(Constant)	1.171		56.060	.000		
Area sown (ha)	.0000730	.554	5.777	.000	.070	14.210
Harvested area (ha)	.000	−2.237	−12.459	.000	.020	49.838
Production	.0000734	2.579	16.803	.000	.027	36.410

Source: Developed by the authors.

The mathematical equation of the multiple linear regression with the unstandardized coefficients and with the values of β is shown below. The constant is equivalent to $\beta_0 = 1.71$, production $\beta_1 = 0.0000734$, sown area $\beta_2 = 0.000$, and harvested area $\beta_3 = 0.000$. Therefore, the mathematical equation is proven according to the model that was proposed from the beginning (Table 5):

$$Y = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \beta_3(X_3) =$$

$$Y = 1.171 + .0000730(4{,}716.08) + .000(3{,}412) + .000(5{,}889.63) = 1.5151$$

Finally, both municipalities were compared using the Kolmogorov-Smirnov test for independent samples. This comparison proves the hypotheses, indicating that both are symmetrical, with a normal distribution and a positive significance (0.000). The test result for the Córdoba zone was 2.444, with an average yield of 1.21. In contrast, the test result for the Huatusco zone was 2.992, with an average yield of 1.72. According to Castillo (2013), in other states (such as Puebla), yields were higher and even reached 1.92, which is considered one of the highest national averages.

The results clearly demonstrate the crucial role and the high profitability of coffee production in these states. The Huatusco region has benefited from its central location, which has led to increased crop yields. This zone has been developed and conserved thanks to the effective management of its technified system. However, Cordoba, located in the south, still needs to improve its cultivation methods. Therefore, the farmers' experiences must be reorganized to enhance their local, regional, state, and international competitiveness.

CONCLUSIONS

The agriculture sector is of great importance to producers who are fully devoted to farming activities. Specialty crops, such as cherry coffee, are an essential component of the food supply of Mexico and the rest of the world. The cultivation-to-harvest relationship is essential, as evidenced by the SIAP database, which shows that coffee yields in Córdoba and Huatusco, Veracruz, increased from 2018 to 2023. The variables under study have positive correlations, interrelated with the yield of the cherry coffee crop. In this regard, innovation is of great importance to enhance coffee productivity. Technified agriculture facilitates accelerated production processes. However, further studies are necessary to ensure optimal crop care and yield quality.

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Colegio de Postgraduados

Mexico's sage richness, traditional uses and chemical composition: a review

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ABSTRACT
Objective: To report the genus richness, the traditional uses and the main chemical constituents of *Salvia* species distributed in Mexico.
Design/Methodology/Approach: A bibliographic review was made in several databases such as Scopus, Web of Science, ScienceDirect and Google Scholar to know the diversity of the genus, compile the traditional uses and the main chemical constituents of *Salvia*. Books and theses available in the repositories of the National Autonomous University of Mexico (UNAM in Spanish) were also reviewed.
Results: In Mexico there are 318 species distributed in three subgenera with an endemism of about 84%; 63 native species of *Salvia* have traditional uses, mainly medicinal and only 17 species are edible, ornamental and ceremonial. Sages are used to treat 141 ailments, the most reported being stomach pain, diarrhea, insomnia, fever, susto, bile, cough and dysentery. Terpenes are the most diverse and abundant constituents in *Salvia* species, followed by phenolic acids and flavonoids.
Study Limitations/Implications: This review provided insight into the great diversity of Mexican salvias and their medicinal importance in treating various ailments. However, few species have been studied phytochemically and pharmacologically.
Findings/Conclusions: In the future, with prior implementation of their cultivation, Mexican sages could be a promising resource as a herbal remedy and/or as a source of bioactive compounds to provide medical care in the treatment of diseases, mainly of the digestive system.

Keywords: Biodiversity, ethnobotany, phytochemistry, flavonoids, alternative medicine, traditional medicine, terpenoids.

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INTRODUCTION

The species of the genus *Salvia* are known in Mexico as chias, myrtles or salvias (sages) and belong to the family Lamiaceae, which in Mexico and the world is one of the families with the largest number of species. The name *Salvia* comes from the Latin word *salvus*, meaning to save or intact, referring to the healing properties attributed to numerous species (Martínez-Gordillo *et al.*, 2017). The genus is well represented with

more than 1000 species around the world, with Mexico and South America recognised as sites of diversification as they contain a high number of species. In Mexico, the genus *Salvia* has a 318 species richness, and is distributed in all states of the country (Martínez-Gordillo *et al.*, 2017; Martínez-Gordillo *et al.*, 2023). The genus includes herbs and shrubs, often aromatic, with simple, opposite leaves and a 4-sided stems. The flowers are grouped in spikes, bilabiate, zygomorphic, coloured in white, yellow, purple, red, violet or blue. The diagnostic characteristic of the genus is the presence of two stamens with elongated connective, which function as a staminal lever that moves when pollinators visit and allows pollen to adhere to the pollinator's body (Martínez-Gordillo *et al.*, 2023). The wide variety of uses of *Salvia* dates back to ancient times. In Europe, medicinal use has been recorded since the first medical school was founded in Salerno, Italy (Inić & Gašparac, 2023). There is a proverb alluding to its medicinal importance "Why should a man die whilst sage grows in his garden?". Salvias are also appreciated for their great beauty, which is why they are commonly found in gardens as ornamental plants and are also used as a condiment in the cuisine of Eurasian countries (Ortiz-Mendoza *et al.*, 2022). There are species of *Salvia* that are used in traditional and alternative medicine in various regions of the world. Among the most common uses that have been attributed to it are useful to prevent infections, relieve pain, cure digestive problems and serve as painkillers. Dried extracts of the root of *Salvia miltiorrhiza* Bunge, also known commercially as Danshen or Tanshen in China, is one of the most popular natural products in Asia and has been used extensively as a treatment for heart and kidney diseases. In China, 700 companies produce the preparation (Hernández-Agero *et al.*, 2002). For Mexico, there are reports of the use of sages since the 16^{th} century, as food, medicine and for ceremonial use (Miranda & Valdés, 1991; Sahagun, 1975). Such is the case of *S. hispanica* L., which in Mesoamerica was a species known as chia, a nahuatl word meaning "oily", due to the large number of oils found in the seed. The Mayans used it as a medicine and the Aztecs used it as an important food source (Cahill, 1996). A common practice in pre-columbian Mexico was to make flour from the seeds, known as "chianpinolli", which was incorporated into tortillas, tamales and a drink called "chianatole". For the Aztecs, the relationship they had with this species was very important, because they used it in rituals, to worship the goddess Chicomecóatl, who was the goddess of subsistence, especially of maize and also of fertility. Another example is the pastora or ska pastora, *Salvia divinorum* Epling *et* Játiva, which is used in Mexico by the mazatec people to treat culture-bound syndrome or for divinatory medicine, and at the same time its hallucinogenic power has been proven (Cahill, 1996; Díaz, 2014). The different uses of *Salvia* species are justified by the great diversity of chemical compounds they produce (Hernández-Agero *et al.*, 2002; Ortiz-Mendoza *et al.*, 2022). The aim of the present work is to report the genus richness, traditional uses and main chemical constituents of native *Salvia* species in Mexico.

MATERIALS AND METHODS

The information presented is the result of an extensive literature review that included a systematic search in databases such as Scopus®, Web of Science®, ScienceDirect®, and Google Scholar®. For this search, the keyword "*Salvia*" was used in combination with

"Mexico" and without year restrictions. Results related to species that were not native or endemic to Mexico were excluded. Additionally, for traditional uses of *Salvia*, printed materials available in both Spanish and English were reviewed from the library of the Institute of Biology at the National Autonomous University of Mexico (UNAM). The computer search was conducted using the terms "*Salvia*" in conjunction with specific epithets and the words diversity, ethnobotany, phytochemistry, Mexico, and secondary metabolites. The information was organized and compiled into tables for analysis. To represent the chemical molecules in this manuscript, ChemDraw Professional 17.0.0.206 software was used.

RESULTS AND DISCUSSION

Diversity and endemism of the genus in Mexico

In Mexico, the genus *Salvia* presents a richness of 318 species, distributed in the subgenera *Calosphace*, *Heterosphace* and *Audibertia* (Rose *et al.*, 2021) with an endemism of 84% (Figure 1), being Mexico the main center of diversity of *Calosphace* with 301 species and 249 endemics. The subgenus *Audibertia* occurs mainly in Baja California, with 14 of the 19 species of this taxon, while *Heterosphace* is the least diverse with only three species (*Salvia henryi* A. Gray, *S. roemeriana* Scheele, and *S. summa* A. Nelson), which are found in the north of the country (Walker & Eiisens, 2001).

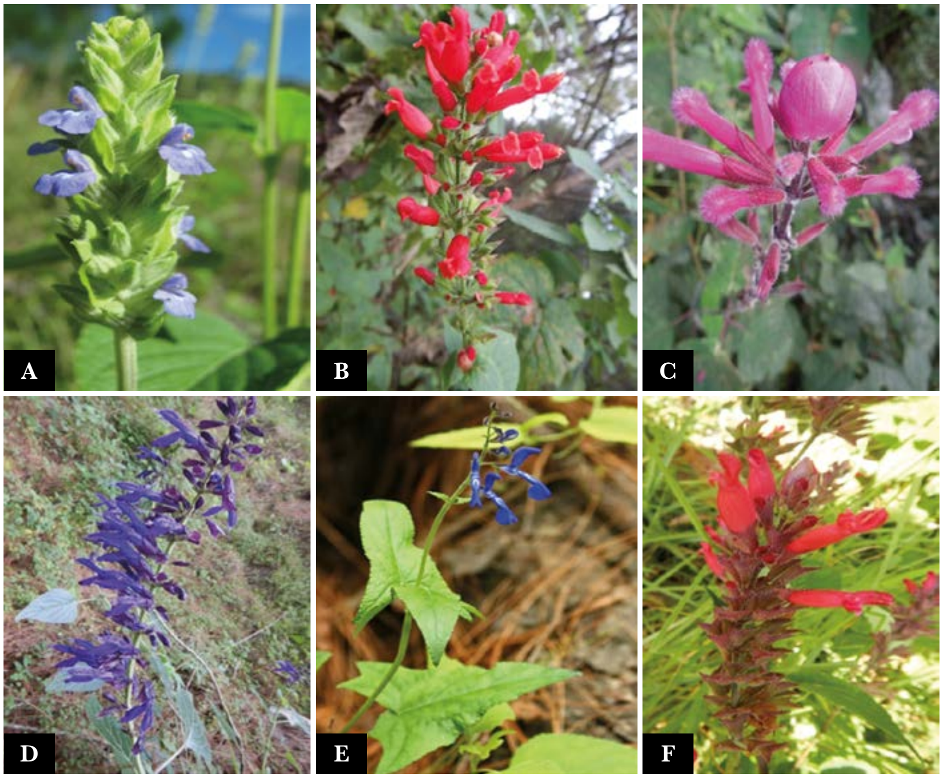

Figure 1. Salvias from Mexico: A) *Salvia hispanica*, B) *S. holwayi*, C) *S. involucrata*, D) *S. mexicana*, E) *S. vitifolia* and F) *S. wagneriana*.

Distribution of the genus *Salvia* in Mexico

The genus is distributed in all the federal states of the country, the states with the greatest presence of *Salvia* are Oaxaca, Guerrero, Puebla, Jalisco and Michoacán, and they are found in mountainous regions, especially in Sierra Madre Occidental (at west), Sierra Madre del Sur (at south) and Eje Neovolcánico Transversal (Trans-Mexican Volcanic Belt), where the vegetation types that harbor most species are temperate forests, particularly coniferous and oak forests, although they can also be found in tropical forests and arid zones, the latter being preferred by the subgenus *Audibertia* (Martínez-Gordillo *et al.*, 2017).

Uses of *Salvia* in Mexico

In Mexico, *Salvia* has a long history of interaction with humans. Many of the ancestral uses are still preserved; but others are falling into disuse and are under-represented today. An example of this is *S. hispanica*, which was a very important crop in ancient Mexico (Cahill, 1996) and is now a minor crop in localities such as Atzitzihuacan and Tochimilco in Puebla, although it is also cultivated in Jalisco, Michoacán, Puebla, Querétaro and Zacatecas, with yields up to 0.740 tons/hectare (Gobierno de la Ciudad de México, 2022). Something similar occurs with *S. apiana* Jeps. and *S. columbariae* Benth., whose seed is reported as edible among the Kumiai, but this use seems to be disappearing, as in interviews with several members of this culture, carried out in May 2023, all of them stated that they no longer eat them. The case of *S. divinorum* is the opposite of the previous one, after its use by the Mazatecs for divinatory medicine became known and it was proven that it has hallucinogenic effects, it is now used for 'recreational' purposes and is available in many parts of the world, being acquired via the internet. In Mexico, 63 native species are recorded as having traditional uses, mainly medicinal, and only 17 species are edible or ornamental. Six species are reported as edible by consumption of the seeds (*S. apiana*, *S. carduacea* Benth. *S. columbariae*, *S. hispanica* and *S. mexicana* L.) or the flowers (*S. apiana* and *S. fulgens* Cav.) (Alonso-Castro *et al.*, 2015; Bello-González *et al.*, 2015; Cornejo-Tenorio & Ibarra-Manríquez, 2008). Nine species are reported as ornamentals (*S. clinopodioides* Kunth *S. coccinea* Buc'hoz ex Etl., *S. elegans* Vahl *S. hispanica*, *S. leucantha* Cav. *S. mexicana*, *S. microphylla* Kunth, *S. polystachya* Cav. and *S. purpurea* Cav.) (Cornejo-Tenorio & Ibarra-Manríquez, 2008; Estrada *et al.*, 2007; Standley, 1920; Villavicencio & Pérez Escandón, 1995). The latter also include *S. splendens* Sellow ex J.A. Schultes, which is introduced (Martínez *et al.*, 1995). Ceremonial species are *S. gesneriiflora* Lindl. *et* Paxton, *S. mocinoi* Benth., *S. purpurea* and *S. thyrsiflora* Benth. (Bello-González & Salgado-Garciglia, 2007; Naranjo, 2012). Two species have domestic use: *S. cinnabarina* M. Martens & Galeotti, for brooms and face dye, as make-up and *S. mexicana* whose leaves are used as a scouring pad (Bello-González & Salgado-Garciglia, 2007; Naranjo, 2012). Considering its use in traditional medicine, *Salvia* is used to treat 141 ailments, grouped into 15 apparatuses and systems of the human body (Ortiz-Mendoza *et al.*, 2022). The most reported ailments are stomach pain, diarrhea, insomnia, fever, fright, bile, cough and dysentery. The most commonly reported diseases are digestive system, female reproductive system and culture-bound syndrome, which are also the categories with the highest number of species (Table 1). The most commonly used species are *Salvia microphylla*, *S. coccinea* and *S. lavanduloides* Kunth, and they are also the ones with the highest number of use categories (Ortiz-Mendoza *et al.*, 2022).

Table 1. Ailments grouped by human body apparatus and systems.

Apparatus and systems	Ailments	*Salvia* species	References
Digestive system	Digestive affections, bile, gallstones, stomach colic, baby colic, constipation, anger, diarrhea, dysentery, red dysentery, stomach pain, gas, gastritis, hemorrhoids, liver, indigestion, stomach and bowel cleansing, upset stomach, purging, torcijón (acute abdominal pain), vomiting.	*S. adenophora, S. amarissima, S. axillaris, S. ballotiflora, S. breviflora, S. carduacea, S. chamaedryoides, S. cinnabarina, S. coccinea, S. elegans, S. gesneriiflora, S. herbacea, S. hispanica, S. karwinskii, S. lavanduloides, S. leucantha, S. melissodora, S. mexicana, S. micrantha, S. microphylla, S. polystachia, S. protracta, S. purpurea, S. reflexa, S. reptans, S. semiatrata, S. shannonii, S. serotina, S. tiliifolia, S. thymoides, S. xalapensis*	Argueta, 1994; Bello-González & Salgado-Garciglia, 2007; Calzada & Bautista, 2020; Domínguez-Vázquez & Castro-Ramírez, 2002; González *et al.*, 2004; Lozano, 1996; Maldonado Almanza, 1997; Martínez *et al.*, 1995; Mercado, 2013; Ortiz-Mendoza *et al.*, 2022
Female reproductive system	Abortifacient, contraceptive, cramps, menstrual disorder, dysmenorrhoea, recent childbirth pain, pregnancy care, gynecological diseases, infertility, vaginal bleeding, childbirth inflammation, lactogen, cleansing women from childbirth, irregular menstruation, to conceive family, to facilitate childbirth, difficult childbirth, postpartum care, menstrual problems, recaída de señora (set of pains and discomforts that women usually suffer after childbirth)	*S. ballotiflora, S. cacaliifolia, S. cinnabarina, S. coccinea, S. fruticulosa, S. gesneriiflora, S. hispanica, S. holwayi, S. involucrata, S. karwinskii, S. laevis, S. lavanduloides, S. leucantha, S. melissodora, S. mexicana, S. microphylla, S. polystachia, S. purpurea, S. prunelloides, S. reptans*	Alonso-Castro *et al.*, 2015; Argueta, 1994; Bello-González & Salgado-Garciglia, 2007; Campos-Xolalpa *et al.*, 2021; Cruz-Pérez *et al.*, 2021; Domínguez-Vázquez & Castro-Ramírez, 2002; Espinosa, 1985; González *et al.*, 2004; Lozano, 1996; Navarro & Avendaño, 2002; Ortiz-Mendoza *et al.*, 2022
Culture-Bound Syndrome	Aire, aire en oídos, aljorra, calentar coyunturas, dolor de aire, empacho, espanto, vergüenza, fiebre, herido por rayo, llanto, mal aire, mal aire de muerto, mal de ojo, panzón de borrego, pérdida del alma o espíritu, purificar o limpiar ambiente, susto, susto de niños	*S. amarissima, S. apiana, S. chamaedryoides, S. cinnabarina, S. coccinea, S. fruticulosa, S. gesneriiflora, S. involucrata, S. lasiantha, S. lavanduloides, S. leptostachys, S. leucantha, S. melissodora, S. microphylla, S. patens, S. purpurea*	Argueta, 1994; Cruz-Pérez *et al.*, 2021; Domínguez-Vázquez & Castro-Ramírez, 2002; Solano-Picazo & Blancas, 2018
Respiratory system	Respiratory conditions, asthma, bronchitis, constipated catarrh, nasal congestion, sore throat, flu, colds, coughs, whooping cough (pertussis)	*S. apiana, S. elegans, S. lavanduloides, S. leucantha, S. mellifera, S. mexicana, S. microphylla*	Argueta, 1994; Martínez-Moreno *et al.*, 2016; Ortiz-Mendoza *et al.*, 2022; White-Olascoaga *et al.*, 2013
Skin and adnexa	Hair (hair loss, care, alopecia), erysipelas, scald, buried thorns, wounds, infected wounds, skin inflammation, "quemado" (dermatitis), hives	*S. adenophora, S. amarissima, S. iodantha, S. lavanduloides, S. mexicana, S. microphylla, S. misella, S. patens, S. polystachia, S. protracta, S. sessei, S. tiliifolia*	Aburto, 2013; Cruz-Pérez *et al.*, 2021; Esquivel-García *et al.*, 2018; Heras & Ariza, 2007; Lozano, 1996; Molina-Mendoza *et al.*, 2012
Musculoskeletal system	Arthritis, dislocation, muscle pain, oedema, rheumatic fever, bone strengthening, bumps, bruises, sprains, rheumatism	*S. apiana, S. coccinea, S. elegans, S. hispanica, S. keerlii, S. mellifera, S. misella, S. purpurea, S. reflexa, S. reptans, S. serotina*	Argueta, 1994; González *et al.*, 2004; Martínez *et al.*, 1995; Ortiz-Mendoza *et al.*, 2022

Table 1. Continues...

Apparatus and systems	Ailments	*Salvia* species	References
Signs and symptoms	Pain, headache, waist pain, chills, fever, lack of appetite, swelling, children's infections, swollen feet, primary stabbing headache	*S. cinnabarina, S. coccinea, S. elegans, S. holwayi, S. lavanduloides, S. leucantha, S. melissodora, S. mexicana, S. microphylla, S. misella, S. purpurea, S. reptans*	Argueta, 1994; Cuevas-Morales *et al.*, 2022; Domínguez-Vázquez & Castro-Ramírez, 2002; González *et al.*, 2004; Ortiz-Mendoza *et al.*, 2022
Sense organs	Eyes health (Conjunctivitis, eye pain, conjunctival irrigation, ocular discomfort, eye conditions, foreign object in the eyes, cleansing chincuapos, for the eyes), Ear health (earache, hearing impairment)	*S. coccinea, S. elegans, S. hispanica, S. mexicana, S. microphylla*	Argueta, 1994; Domínguez-Barradas *et al.*, 2015; Martínez *et al.*, 1995; Ortiz-Mendoza *et al.*, 2022; Soto, 1987; Zamora-Martinez *et al.*, 1992
Other	Cancer, caustic, tonic, weakness, viper bite, paralysis, parasites	*S. gesneriiflora, S. herbacea, S. lavanduloides, S. leucantha, S. microphylla, S. misella, S. purpurea, S. shannonii, S. tiliifolia*	Argueta, 1994; Calzada & Bautista, 2020; González *et al.*, 2004; Ortiz-Mendoza *et al.*, 2022
Therapeutic practices	Postpartum bath, bathing a newborn, steam therapy, wound cleansing, limpias or barridas (the therapist literally sweeps the body of the patient), massage	*S. elegans, S. involucrata, S. iodantha, S. leucantha, S. microphylla, S. patens, S. protracta, S. rubiginosa*	Aburto, 2013; Argueta, 1994; Breedlove & Laughlin, 1993; Lozano, 1996; Ortiz-Mendoza *et al.*, 2022
Circulatory system	Anaemia, heart, hemorrhage, nose bleed, blood pressure	*S. coccinea, S. hispanica, S. lavanduloides, S. microphylla, S. polystachia, S. regla*	Argueta, 1994; Ortiz-Mendoza *et al.*, 2022
Nervous system	Epilepsy, insomnia, children's insomnia, nervousness, calming children	*S. elegans, S. fulgens, S. leucantha, S. melissodora, S. microphylla*	Argueta, 1994; Ortiz-Mendoza *et al.*, 2022
Infectious diseases	Abscesos, fogazos, malaria, paperas, sarampión Abscesses, cold sore, malaria, mumps, measles	*S. coccinea, S. elegans, S. fulgens, S. reflexa, S. tiliifolia*	González *et al.*, 2004; Ortiz-Mendoza *et al.*, 2022
Urinary system	Urinary tract stones, kidney problems, kidney	*S. laevis, S. lavanduloides, S. leucantha, S. melissodora, S. mexicana, S. microphylla*	Cruz-Pérez *et al.*, 2021; Lozano, 1996
Metabolic diseases	Diabetes	*S. amarissima, S. fruticulosa, S. lavanduloides, S. leucantha, S. mexicana, S. oaxacana, S. tiliifolia*	Argueta, 1994; Ortiz-Mendoza *et al.*, 2022

Chemical constituents of the genus *Salvia*

Sages synthesize a large number of secondary metabolites, so called because they originate from chemical compounds formed from primary metabolism. The main groups are terpenoids and phenolic compounds, which have been isolated and identified from around 90 salvias distributed in Mexico (Ortiz-Mendoza *et al.*, 2022).

Terpenes constitute a very broad group of metabolites isolated in *Salvia* species, classified according to the number of carbons in their chemical structure, with compounds with 10, 15, 20, 25, 30 and 40 carbon atoms, many of which are the main constituents of essential oils, some examples are α-pinene, β-pinene, camphene, camphor, borneol, menthol, phellandrene, limonene, thymol, carvacrol, p-cymene, myrcene, linalool, farnesol and geraniol, among others (Figure 2) (Imanshahidi & Hosseinzadeh, 2006; Ortiz-Mendoza *et*

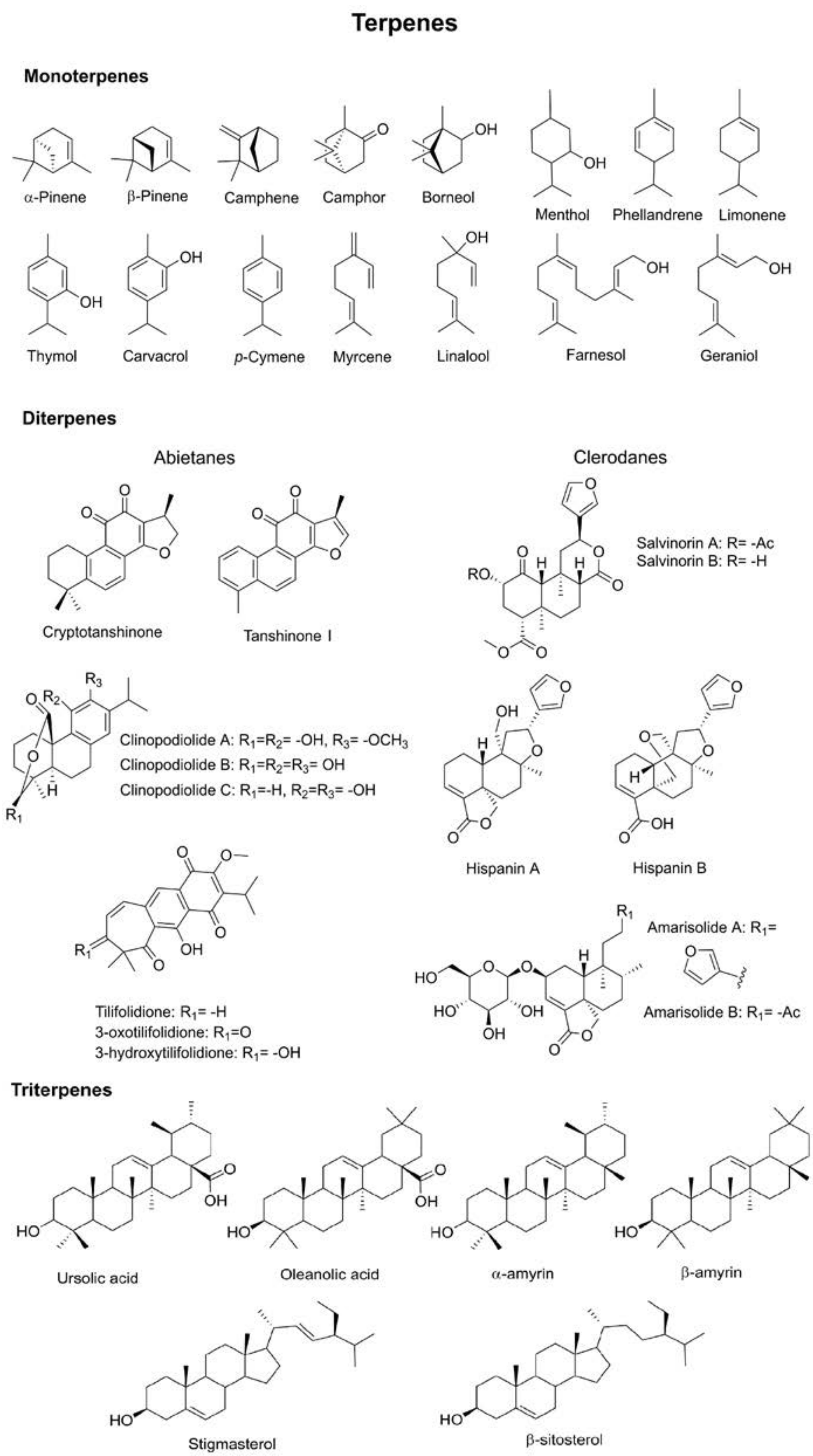

Figure 2. Structures of terpenes isolated from *Salvia* species.

al., 2022; Wu *et al.*, 2012). The major terpenoids in *Salvia* are compounds with 20 and 30 carbons, better known as diterpenes and triterpenes, respectively. Diterpenes stand out in sages, mainly abietanes (*i.e.* tanshinones, clinopodiolides, tilifolidiones) and clerodanes (*i.e.* salvinorins, hispanines, amarisolides, among others) with the highest number of structures (Figure 2) (Adams *et al.*, 2005; Bigham *et al.*, 2003; Bustos-Brito *et al.*, 2019; Esquivel & Sánchez, 2005; Fan *et al.*, 2019; Salinas-Arellano *et al.*, 2020). The psychoactive compound, Salvinorin A, a clerodane isolated from *S. divinorum*, is considered to be a naturally occurring hallucinogen, and unlike other psychoactives such as alkaloids (morphine), it lacks nitrogen. The mechanism of action of Salvinorin A is via the opioid receptors, which also exerts an analgesic effect, as does morphine; however, what has attracted the attention of the pharmaceutical industry is that it is a powerful antidepressant. It is banned for both consumption and research purposes in some countries, such as Germany, Japan, Poland, Russia and Belgium. In Norway, Finland, Estonia and Iceland, the plant is legal to use for medicinal purposes and can only be obtained by prescription (Cahill, 1996; Ortiz-Mendoza *et al.*, 2022). Within the triterpenes, it is common for sages to synthesise ursolic acid, oleanolic acid, α-amyrin, β-amyrin, stigmasterol and β-sitosterol (Figure 2). Ursolic acid and oleanolic acid are generally isolated from *Salvia* species with high yields (Ortiz-Mendoza *et al.*, 2022; Wu *et al.*, 2012). These compounds have such varied biological activities, even a single compound has several health benefits, as in the case of ursolic acid, which has been attributed anti-inflammatory, analgesic, gastroprotective, antimicrobial, antiviral, antitumour and hepatoprotective properties (Hussain *et al.*, 2017).

Other important compounds in salvias are characterized by having one or more hydroxyl (-OH) groups attached to an aromatic ring, commonly called phenols (Figure 3). One of the main characteristics of these compounds is that they are powerful antioxidants, and in general protect the body from damage caused by oxidizing agents, such as ultraviolet rays, environmental pollution and toxic substances present in some foods. Within this large group of compounds are phenolic acids, the most common of which in *Salvia* are caffeic acid, ferulic acid and rosmarinic acid. Flavonoids are widely distributed in sages, the main ones being apigenin, luteolin, quercetin, kaempferol and rutin. These compounds are characterized by their antioxidant, antitumour, anti-inflammatory, antimicrobial, chemopreventive and neuroprotective properties. Quercetin promotes health by lowering blood pressure and cholesterol, as well as reducing inflammation, by preventing the development of chronic diseases such as diabetes, hypertension and cancer, among others. The fatty acids, especially omega-3 fatty acids, present in chia seeds, as well as the flavonoids kaempferol, quercetin and rutin have been found to have an effect on patients with type 2 diabetes (these patients are characterized by insulin resistance and high blood glucose levels), whose blood glucose levels are kept under control with a chia diet. In addition to reducing the risk of cardiovascular and brain diseases (Hernández-Pérez *et al.*, 2020; Ortiz-Mendoza *et al.*, 2022; Ullah *et al.*, 2016) (Figure 3).

Cultivation, potential and perspectives

Salvia is the most diverse genus in Mexico, and is an integral part of Mexican biodiversity and culture. Its cultivation, whether for ornamental, medicinal or economic use, reflects

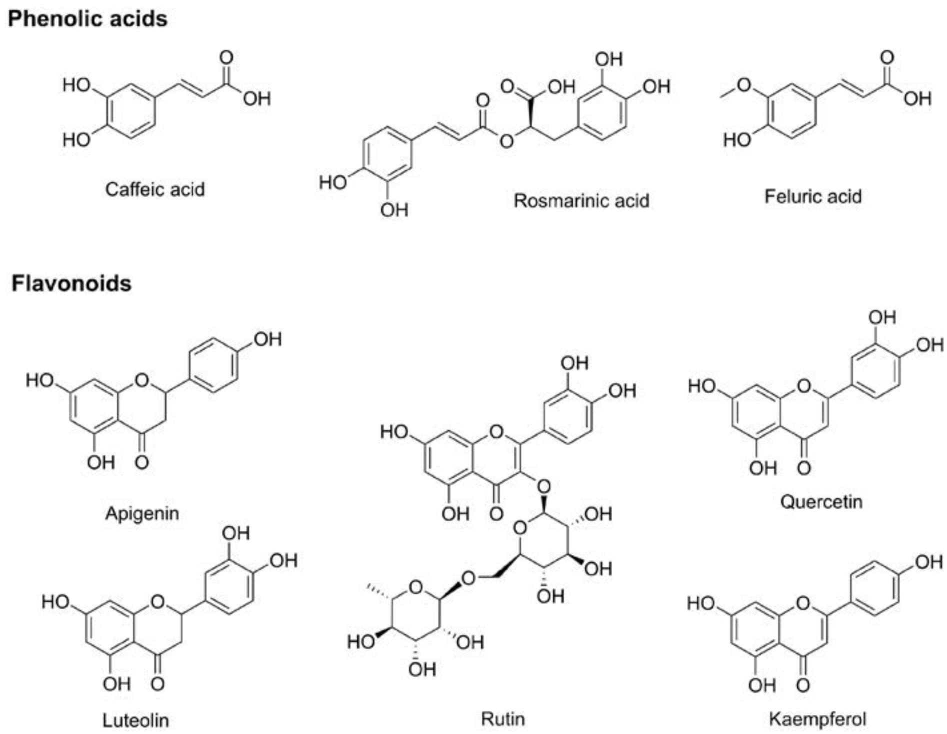

Figure 3. Structures of phenols isolated from *Salvia* species. This figure was created using Chem Draw Professional 17.0.0.206.

the richness and diversity of the phylogenetic resources of this genus. It is essential that its cultivation and conservation be promoted sustainably. Conservation strategies should be established, according to localities with ideal characteristics for its growth. There are *Salvia* species considered rare and at risk of germplasm loss due to habitat loss (Flores-Tolentino *et al*., 2020). Native species, in particular, play an important role in ecosystems and traditional culture. It is essential to highlight the salvias that have traditionally been cultivated in Mexico, either for their medicinal properties, ornamental value or cultural importance; however, despite their high use value, few species are cultivated. Chia stands out with a planted area in Mexico of 6,238 hectares, with a production of 4,771 tonnes and a production value of around 273 million pesos (Gobierno de la Ciudad de México, 2022). Other species that are cultivated, although exact figures are not available, are *Salvia divinorum*, *Salvia coccinea* and *Salvia leucantha* Cav. The cultivation of sages not only safeguards an essential part of Mexico's biological and cultural heritage, but also represents an invaluable economic opportunity for local communities (Ramírez *et al*., 2016). Encouraging plant-based agriculture not only helps to preserve the genetic diversity of the species, but can also generate sustainable income and promote environmentally friendly farming practices. As the world moves towards an environmentally conscious approach and seeks sustainable resources, salvias offer a unique combination of cultural, medicinal, economic and ecological benefits. It is imperative that society recognises and capitalizes on these advantages by encouraging research, education and investment in salvias cultivation and conservation.

CONCLUSION

Medicinal plants have played an important role in the history and culture of the people of Mexico, and the use and application of remedies is knowledge that has been passed down orally from generation to generation. The genus *Salvia* is not only the most diverse in Mexico but has also been a key element in traditional Mexican medicine, particularly those species used to relieve physical ailments as well as those that address spiritual or emotional issues. Out of the 318 species distributed in Mexico, it is known that 20% have some use, totaling approximately 63 species, many of which are used in traditional medicine, primarily to alleviate digestive problems.

Moreover, Mexican *Salvia* species have significant potential as ornamental plants, making it crucial to investigate this aspect. This is particularly important to avoid the fate of other native Mexican species, such as *Dahlia* spp., *Euphorbia pulcherrima* Willd. ex Klotzch, and *Tagetes* spp., which have become prominent in the global ornamental plant trade —a sector in which Mexico currently has very low participation.

It is essential to emphasize the medicinal importance of *Salvia* and its active constituents to promote pharmacological research and the implementation of its cultivation for future use as phytopharmaceuticals. Additionally, exploring novel studies of the *Salvia* genus can uncover potential applications in cosmetics and personal care products due to its antioxidant and anti-inflammatory properties. Investigating its role in the food industry could also be promising, given its potential benefits for digestive and metabolic health. Metabolomics could further reveal new profiles of bioactive compounds, leading to the development of innovative products across various fields.

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Colegio de Postgraduados

Use of native corn (*Zea mays* L.) from two edaphoclimatic regions of Veracruz with potential as hydroponic green forage

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ABSTRACT
Objective: To evaluate native corn from two different edaphoclimatic regions, with potential as hydroponic green forage (HGF) without nutrient solution.
Design/Methodology/Approach: A completely randomized experimental design was used with 5 treatments and 3 repetitions each: T1 hybrid (HR), T2 Almolonga (ALF), T3 Coyutla (CPC), T4 Coyutla (CFJ), and T5 Coyutla (CMJ).
Results: The ALF treatment was superior in the height (28 cm) and root length (14.7 cm) variables. For its part, CMJ recorded the highest leaf width (2.8 cm) and length (20.5 cm) values. However, ALF had higher yield (36.80 kg m^2), dry matter (4.89 kg m^2), protein content (29.88%), crude fiber (39.28%), and mineral content (4.95% N, 4.95% P, 5.95% K, and 1.97 kg of dry matter).
Study Limitations/Implications: Native corn from two different regions are proposed as an alternative HGF.
Findings/Conclusions: Native ALF corn with irrigation and without nutrient solution recorded better results as an alternative HGF in the following agronomic variables: yield, protein content, crude fiber, and mineral content in biomass.

Keywords: native, protein, yield, crude fiber, and minerals.

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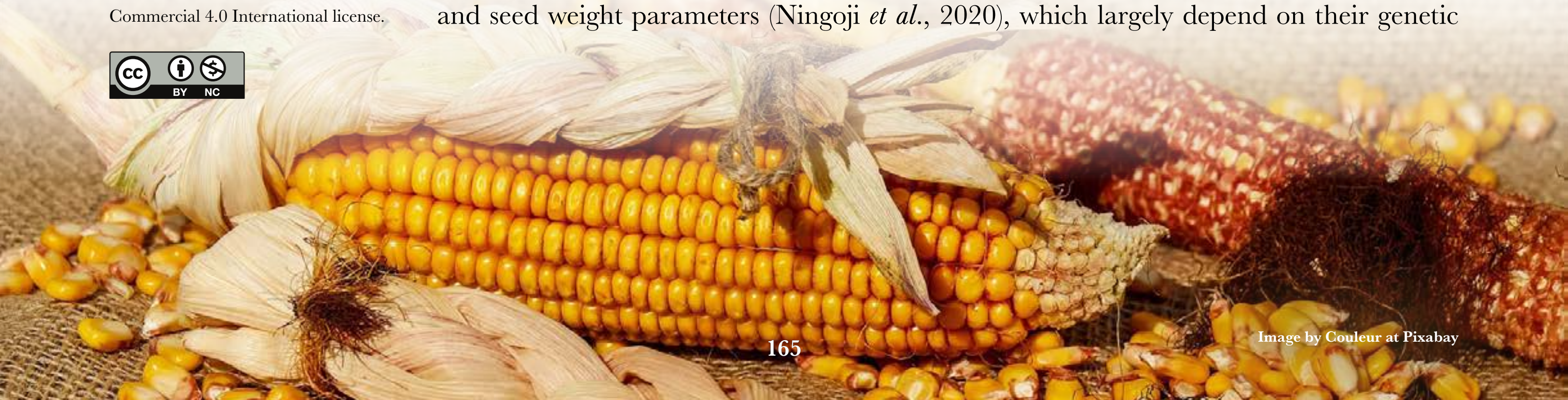
INTRODUCTION

Growing time, fertilizer requirement, rainy season, water scarcity, climate change-related natural disasters, and high input costs are the main limitations on green forage production, which in turn has a negative impact on the production and reproduction of livestock (Ramírez de la Ribera *et al.*, 2017).

In this sense, hydroponic systems used to produce forage have more efficient germination, water use, and yield (Bamikole *et al.*, 2020). In the case of corn, there are differences between yellow and white varieties, which record 6.92 and 6.74 kg·m^2 yields, respectively (Lamnganbi and Surve, 2017). These differences are attributed to shoot, root, and seed weight parameters (Ningoji *et al.*, 2020), which largely depend on their genetic



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characteristics and edaphoclimatic conditions. Yellow corn has been selected to produce Hydroponic Green Forage (HGF), given its 66.67% and 33.33% total yield in roots and shoots, respectively (Jemimah *et al.*, 2020); however, white corn is not far behind, considering that some varieties, such as "Morocho Blanco", record yields of 10.34 kg·m^2 (González *et al.*, 2015). In addition, these forages can contain on average 20.01% protein, 18.95% crude fiber, 4.5% ash, 7.44% ethereal extract, and 88.6% dry matter digestibility (Soto-Bravo and Ramírez-Víquez, 2018), which are parameters that indicate high nutritional quality in animal feeding. Therefore, the objective of this research was to evaluate native corn from two different soil-climatic regions, with the potential to produce hydroponic green forage without a nutrient solution and to study the hypothesis that some of the native corns from different edaphoclimatic conditions have high yield and quality values under a HGF system without nutrient solution.

MATERIALS AND METHODS

Plant material from the study areas

Table 1 describes the characteristics of the materials used. Additionally, an open-pollination hybrid material from the Universidad Autónoma de Chapingo was used as a control. This hybrid has high potential and optimal performance under moderate drought conditions, which allows it to adapt to different environments (Bonilla, 2018).

Study area

The experiment was established in a greenhouse at the Facultad de Ciencias Agrícolas-Xalapa of the Universidad Veracruzana. Average temperatures from 23 to 30 °C and relative humidity from 60 to 80% were recorded. Five-level racks with the following characteristics were used for the production of HGF: 1.06 m width, 1.30 m length, and 1.70 m height, and capacity for fifty trays with a 25 cm width, 53 cm length, and 2.5 cm height. Irrigation was carried out through micro-sprinklers.

Experimental design

A completely randomized experimental design with five treatments was used: T1 hybrid (HR), T2 Almolonga (ALF), T3 Coyutla (CPC), T4 Coyutla (CFJ), and T5 Coyutla (CMJ). One kg of seeds was placed per tray for each treatment, with three repetitions.

Seed selection and counting

The ears of each treatment were threshed and the damaged seeds were subsequently removed by hand. One kg of seeds from each sample was weighed and the number of seeds

Table 1. Edaphoclimatic characteristics of Coyutla and the town of Almolonga, municipality of Naolinco, Veracruz.

Provenances	Altitude (msnm)	Maximum temperature (°C)	Minimum Temperature (°C)	Rainfall (mm)	Soil type	Slope (%)
Coyutla, Coyutla	160	45	5	2, 985.3	Luvisoles	25-40
Almolonga, Naolinco	724	23	10	768	Arcillosos y pedregosos	12-18

in each repetition was counted. The seeds were washed and disinfected with a 1% NaClO solution for 30 minutes, followed by 2 to 3 rinses with clean water to remove residues. For the pre-germination procedure, a CaO solution was prepared at a concentration of 50 g·L^{-1} of water. The solution was added to the seeds in plastic bags for 24 h, before they were rinsed again with water and aired on trays for 24 h. Finally, the seeds were placed in 780 cm^2 trays, forming a uniform 1.5 cm thick layer, and put into the HGF rack inside the greenhouse. To promote germination, the trays were covered with a 60% black shade cloth. The quality analysis of the water used for irrigation purposes had the following characteristics: 0.63 mg·L^{-1} of nitrates, 1.60 mg·L^{-1} of phosphates, 0 mg·L^{-1} of SO_4, 0.1025 mL·L^{-1} of K, 0.1 mL· L^{-1} of Ca, and 0.3 mL·L^{-1} of Mg. A Steren timer was programmed to activate the micro sprinklers for 60s every 5 h, starting at 8:00 h and ending at 20:00 h, to avoid water oversaturation and seed removal. The shade cloth was removed after germination and the specimens were irrigated for 60 s every 3 h, during the 12 days of the experiment, with a daily water consumption per tray of 1,564 mL.

Variables evaluated

Total forage height, leaf length and width, root length, fresh weight yield (kg·m^2), and dry matter content were evaluated at 12 days after sowing (DAS). The dehydration method was used to evaluate the dry matter, applying the following formula:

$$dry\ matter = 100 - \left(pi - pf\ /\ pi \times 100\right)$$

where: *pi* is the initial weight and *pf* is the final weight.

Likewise, the N, P, and K content was determined according to the official methods of the AOAC (1990). The crude protein percentage was obtained with the micro-Kjeldahl method, multiplying the total N percentage by a factor of 5.83. The AOAC (2000) methodology was used to determine crude fiber. The P, K, and Ca content was determined with a Perkin Palmer® 2380 atomic absorption spectrophotometer (AAS) (Perkin-Elmer, 1996).

Statistical analysis

Statistical analysis was performed using Statistics Software version 12.0, verifying the assumptions of normality and homoscedasticity. Likewise, the ANOVA and Tukey's mean comparison tests at 5% ($\alpha = 0.05$) were developed.

RESULTS AND DISCUSSION

Agronomic variables

Livestock production currently requires higher quality food in less time and consequently nutritional solutions are used to increase the production of HGF systems (Girma and Gebremariam, 2018). However, the production of forage without a nutrient solution, chemical fertilizers, pesticides, or fungicides, using only water, results in inputs savings for producers (Abdula, 2022). In this sense, the statistical analysis carried out in this work showed significant differences between the agronomic variables ($P \leq 0.05$). The ALF treatment reported better results (28 cm total height and 13 cm root length) than Zagal-Tranquilino *et al.*, (2016), who recorded a 22.2 cm height after 13 days. In the case of leaf length (20.5 cm) and width (2.8 cm), CMJ was a better treatment than HR, which reached a leaf length of 19 cm and a leaf width of 2.7 cm (Table 2). Height is important for producers, because greater height results in greater availability of green matter to meet the nutritional requirements of the animals. Unlike other studies no nutrient solution was applied during the 12 day harvest time, clearly demonstrating that the use of a solution is not always necessary and consequently reduces costs to the producer (Suma *et al.*, 2020).

Yield and dry matter

HGF systems increase the yield of forage production. The best treatment was ALF with 36.80 kg·m^2, followed by CPC (32.06 kg·m^2) and HR (28.33 kg·m^2) (Figure 1a). All these treatments showed higher yields than those reported by Zeferino-Hernández *et al.* (2021): an average of 21.5 kg m^2 in native corn from Southern Veracruz, irrigated with nutrient solution and harvested after 10 days. Additionally, the yield and quality of the forages are affected by the management of the system considering several elements, including labor, weight and quality of seeds, water quality, irrigation frequency, temperature, humidity, light intensity, and harvest time (Dogrusoz, 2022). Regarding total dry matter, the ALF treatment stood out with a weight of 4.89 kg m^2 (Figure 1b). Better yield results are also obtained with this treatment, perhaps due to the larger seeds used and even more so to the climatic conditions of the ALF origin, which were similar in the greenhouse (23-30 °C).

Protein content in HGF

In the case of protein, the statistical analysis detected significant differences ($P \leq 0.05$) (Figure 1c). ALF (29.88%) had a higher percentage than HR (15.96%). These values are higher than the 19% and 26.19% percentages in native corn reported by Bedolla-Torres *et al.* (2015) and by Zeferino-Hernández *et al.* (2021), respectively. No specific protein concentration exists, since it varies depending on the HGF production conditions and the genetic material used.

Table 2. Agronomic variables of native corn.

Treatments	Total height of HGF (cm)	Leaf length (cm)	Leaf width (cm)	Root length (cm)
HR	1.49±20 c	1.35±19 b	0.48±2.7 b	5.10±12.3 c
ALF	3.8±28 a	2.12±18 c	0.17±2 d	4.16±14.7 a
CPC	1.68±20 c	1.26±19 b	0.45±2.7 b	0.76±10 d
CFJ	2.55±18 d	1.81±12 d	0.26±2.5 c	1±10 d
CMJ	1.63±22 b	2.17±20.5 a	1.28±2.8 a	1±13 b
$P \leq 0.05$				

Values with equal letters within columns are statistically equal (Tukey, $P \leq 0.05$) and ± Standard deviation.

Crude Fiber Content

ALF was the best treatment for crude fiber with 39.28%, followed by CPC with 11.26%, HR with 10.48%, CMJ with 10.22%, and CFJ with 9.58% (Figure 1d). Zeferino-Hernández *et al.* (2021) reported less crude fiber (38.68%) in the aerial matter of native corn from southern Veracruz than the values reported in this work. Furthermore, previous reports state that the use of organo-mineral nutrient solutions can reduce crude protein content (Adeyemi *et al.*, 2020). In contrast, this study recorded positive results without the addition of minerals.

Mineral content

The ALF treatment recorded highest mineral content in dry matter (DM) for N (4.95%), P, K, and Ca (4.95, 5.95, and 1.97 kg DM). This content may be related to root development, which allowed better absorption of nutrients intended for tissue formation (Table 3). Noteworthily, the mineral content in DM did not obtain minimum parameters compared to the HGF systems produced with nutrient solution, which recorded values of up to 0.6230 mg kg^{-1} Ca (Zainab *et al.*, 2019). These nutritional requirements vary according to the metabolic rate and the tissue formation and reconversion, since each plant is different, even if they belong to the same genus, variety, or species (Mejía and Orellana,

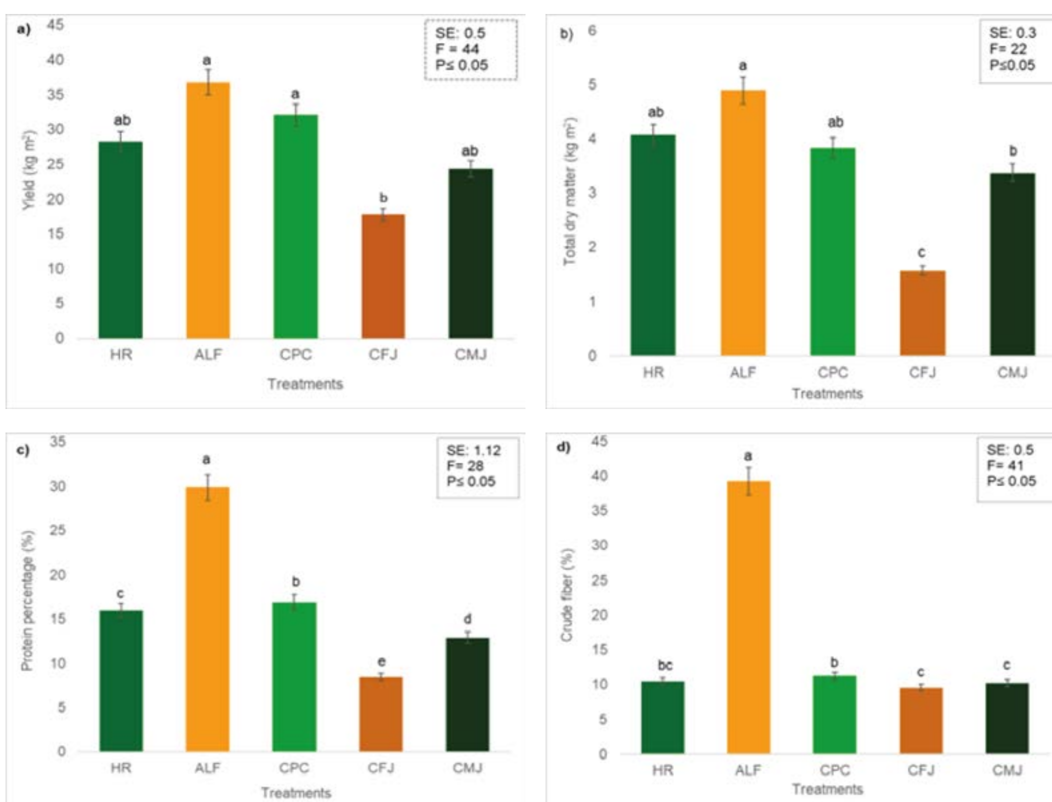

Figure 1. a) Yield of corns in HGF (kg·m^2), b) dry matter (kg·m^2), c) protein content (%), and d) crude fiber (%). Columns with the same letter are statistically equal (Tukey, P≤0.05). The vertical lines on the bars represent the standard error (±).

Table 3. Mineral content of green forage made from native corn.

Treatments	N (%)	P (Kg DM)	K (kg DM)	Ca (kg DM)
HR	0.01±4.09b	0.03±4.03b	0.03±4.66b	0.02±0.57b
ALF	0.05±4.95a	0.05±4.95a	0.05±5.95a	0.015±1.97a
CPC	0.11±3.84c	0.02±3.46c	0.22±3.80c	0.005±0.58b
CFJ	0.01±2.02e	0.01±1.56e	0.015±1.56e	0.025±0.23d
CMJ	0.015±3.02d	0.015±2.97d	0.19±2.77d	0.03±0.36c
P≤	0.05			

Note: DM (dry matter) ± Standard deviation. Same letters in the same column represent Tukey's statistical equality ($P \leq 0.05$).

2019). Finally, based on the results, the hypothesis was accepted. Regarding the use of native corn, the use of these materials from other regions with different edaphoclimatic conditions should be expanded and their performance without nutrient solution in HGF systems should be verified. This measure would simultaneously generate a genotypic conservation and reduce the input costs of producers in the state of Veracruz.

CONCLUSIONS

In the case of HGF, AL was the best native corn from two different edaphoclimatic regions, due to its higher forage yield (dry matter), protein percentage, and crude fiber. Additionally, it recorded high N, P, K, and Ca content, without using a nutrient solution. In conclusion, it is an alternative for the producers of Almolonga, municipality of Naolinco, Veracruz.

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Colegio de Postgraduados

In-silico production of bioactive enrichment soil fertilizer from agricultural by-products towards bioeconomics perspectives

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ABSTRACT

Objective: To evaluate production facilities biorefinery design of bioactive enrichment soil fertilizer using food waste generated by farms in Baja California Sur (BCS), México, of five agricultural commodities towards bioeconomic perspectives.

Design/methodology/approach: BCS state was the study area. A mathematical model biorefinery design was made using a Mixed Integer Linear Program approach. Four layers of decision were used, Biomass, Production facilities, Storage and Consumer location. SuperPro Designer was used to biorefinery design and model was formulated in Phyton.

Results: Model shows two optimal production facilities: Valle de Vizcaino (VV) and Valle de Santo Domingo (VSD). VV processes strawberry and tomato with net profit of 50.28. While VSD processes orange and asparagus with net profit of 35.48. Production line goes from May to April. Ascorbic acid and quercetin were considered to enrichment the soil fertilizer.

Limitations on study/implications: This study did not produce bioactive compounds in industrial way only use mathematical modeling program and equations to predict the optimal scenario to production facilities location in an agricultural region.

Findings/conclusions: Model showed the importance of consider four interconnected decision layers. The framework was developed for capturing food waste generation dynamics. These dynamics display a very important role in all other dimensions' decisions and that an efficient biorefinery design must account for these dynamics.

Keywords: Agri-food, Biorefinery design, Food waste, bioeconomics, bioactive compounds.

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INTRODUCTION

Environmental, economic, and societal pressures necessitate a transition from linear "take-make-dispose" models of consumption and production to value networks and business models aligned with circular economy principles [1]. The so-called circular economy framework is the system in which the consumption of materials and waste generation are minimized while also recycling and reusing the residues to produce new materials [2].

In this way, the concept of turning waste into a resource is especially interesting in terms of sustainability.

Agri-food industries (AFI) generate high amounts of processing waste [3]. Some well-established applications of these wastes include their use as animal feed or soil fertilizers [4-5]. However, these AFI by-products are remarkable rich in wide range of high added-value bioactive components, such as polyphenolic compounds [6]. Polyphenols are a vast group of secondary plant metabolites that comprise thousands of compounds [7]. Polyphenols are scavengers of free radicals, which are harmful products of aerobic metabolism leading to oxidative stress in the organism [7]. Multiple *in-vitro*, *in-vivo*, and epidemiological studies have shown that polyphenols are of great interest in the prophylaxis and treatment of cardiovascular and neurological diseases, cancer, and aging-related disorders, mainly due to their remarkable antioxidant activity [8-9].

Bioactive phenolic acids and flavonoids are on the top of the well-known value pyramid of biomass, which makes AFI waste very attractive from circular bioeconomy approach, which great prospects in the pharmaceutical, cosmetics, and food industries [10]. In this context, recovering natural polyphenols from AFI waste is an excellent option, in line with the United Nations Sustainable Development Goals [11]. The overall strategy is even more remarkable when green technologies are applied, with the design of processes aiming to eliminate, or at least reduce significantly, the use and generation of hazardous substances and prevent environmental and health impacts [12]. In this scenario, the extraction, characterization, and purification of phenolic acids and polyphenols stir up an increasing scientific and commercial interest [10].

Biorefining consists of biomass exploitation to produce chemicals and energy and emerges as a potential alternative for more sustainable industrial models [13]. Nonetheless, the abundance of biomass sources, conversion technologies, and potential target-products makes the efficient design of biorefinerías a very complex task [14].

Mathematical programming has been extensively employed to facilitate this task [15, 16]. Frameworks to choose optimal technological pathway for converting a set of feed-stocks into a set of desired chemical represent important tools for deciding technological aspects of the biorefinery design [17]. However, they do not incorporate location-specific information into the decision-making process [18]. As biomass physic-chemical properties and availability are highly dependent on geography [19], this location-specific information should also be considered in the decision making-process, which should account not only for conversion technologies but for the whole biorefinery supply chain.

Biomass supply chain aspects were incorporated into a mathematical programming framework [20]. They proposed a framework for designing biomass-based energy systems that explicitly decides on cultivation, harvesting, and the centralization/decentralization of processing facilities. However, the framework applies only to a local level as it does not consider the possibility of integrating supply and demand from different regions.

From the above mentioned, the present study aims to assess the *in-silico* biorefinery design for bioactive and biofertilizer production from agricultural residues of AFI along five perishable vegetable species (commodities) supply chains in Baja California Sur, México. By using data collected at the harvest level, we examine the process towards bioeconomic perspectives.

MATERIALS AND METHODS

This study was conducted with data collected in Baja California Sur State (BCS), in five municipalities (Figure 1). FLW data of every commodity was assessed in harvest stage of supply.

Framework and mathematical model

The framework consists of two steps: (i) Model formulation and solution, and (ii) Results and evaluation. Model formulation and solution aims at instantiation the mathematical model with the data of [21] and at retrieving the instance's optimal solution. Optimal solution was drawn, as it shows in Figure 2.

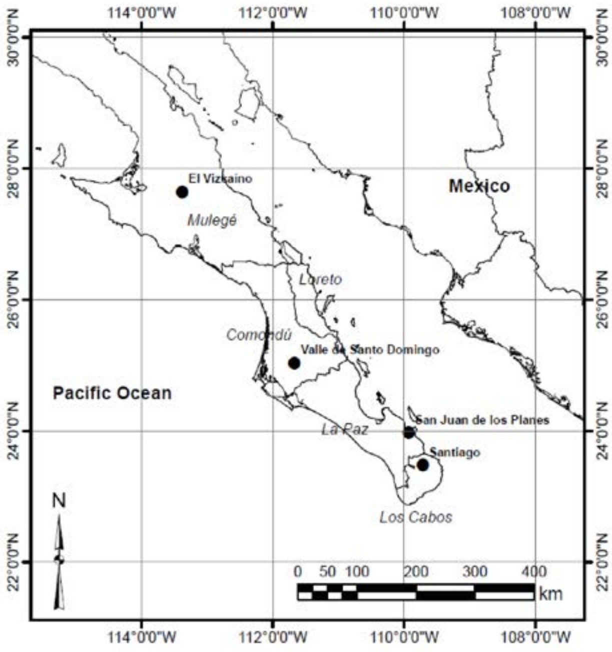

Figure 1. Map of Baja California Sur indicating the municipalities and localities included in the study. (Source Martinez-Camacho *et al.*, 2024).

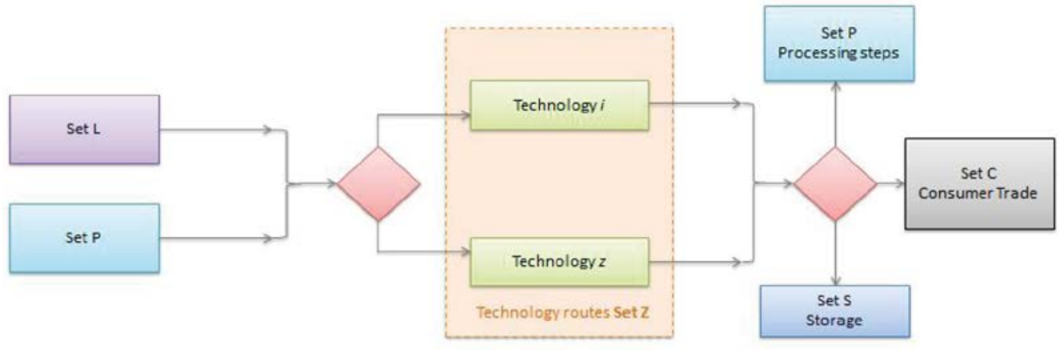

Figure 2. Mass Flow within Production Layer P. Demand is translated to a chemical species flow according to a biomass composition parameter and summed over all units. The species flows coming from sums up to those transferred from other production facilities and are split into the conversion technologies z.

The mathematical model proposed for the framework consists of a Mixed Integer Linear Program that interconnects four layers of decision (Figure 2). Each layer is represented by a set of n possible instances: Set L (Biomass cultivation locations); Set P (Possible Production facilities location); Set S (storage locations); and Set C (Possible Consumer location). The interconnections flows are referenced with a subscript q (or b) indicating a chemical species q from the Set Q (or a biomass b from Set B) and a subscript t is indicating a period from the Set T. All variable data is shown in Table 1.

The flow of biomass b transferred to the production facilities is converted to a flow of a chemical species q of the Set Q $\left(FFP_{p,q,t}\right)$ according to a composition parameter defined for each biomass b at each harvest location $f\left(X_{f,b,q}\right)$ (Eq. 1). The mass balances for the production, storage and consumer layers are developed in function of the chemical species flow.

$$FFP_{p,q,t} = \sum_{f \text{€} F} \sum_{b \text{€} B} X_{f,b,q} F^{demand} F_{f,p,b,t} \forall \left(p \text{€} P, q \text{€} Q, t \text{€} T\right) \qquad \text{(Eq. 1)}$$

The demand of biomass from all the production facilities is summed into the demand of harvested biomass at each harvest location $\left(FF^{harvested}{}_{f,b,t}\right)$ (Eq. 2).

$$FF^{harvested}{}_{f,q,t} = \sum_{p \text{€} P} F^{demand} F_{f,p,b,t} \forall \left(f \text{€} F, b \text{€} B, t \text{€} T\right) \qquad \text{(Eq. 2)}$$

The harvested biomass is transferred to the production layer for conversion. The amount of chemical q that is fed to the production facility p may be split into the z technologies available within the Set Z. The chemical species qprod produced by a technology z can have several destinations. These flows are subjected to a mass balance for each species q at each production facility p for each period t.

Table 1. Mathemathical model Data. Supplemented data to *in-silico* model for biorefinery design.

Set	Location	Set	Species		Time
			Chemical	**Biomass**	
Set L	Valle de Vizcaíno, Valle de Santo Domingo, Los Planes, Santiago, Todos Santos.	Set Q	Ascorbic acid, quercetin		
Set P	Valle de Vizcaíno, Zona Ejidal, Loreto, Ciudad Constitución, Ciudad Insurgentes, La Paz, Los Planes, Santiago, Todos Santos.	Set B		Asparagus, Mango, Orange, Strawberry, Tomato.	
Set S	Valle de Vizcaíno, Loreto, Ciudad Constitución, La Paz, Todos Santos	Set T			May to April, monthly assessment
Set C	Valle de Vizcaíno, Loreto, Valle de Santo Domingo, La Paz, Cabo San Lucas, San José del Cabo, Todos Santos				

This model does not need to have predefined sequential processing steps. Instead, it assumes a parallel processing network where the output of any technology may serve as input for any other technology. Each technology has its own conversion parameters defined for each pair of chemical on Set Q. When converting chemical q to qprod it is also possible to produce utilities as a co-product. Some utilities are also needed for enabling a given conversion. The utilities that are produced by a technology z can be used by another technology z' within the same production facility. The surplus (or lack) of any utility produced (or demanded) can be sold within the same production facility.

Production operational and capital costs are estimated as a function of the chemical species flow fed to each technology. The capacity of each technology on each production facility is taken as the maximum chemical species flow within this facility on all operational periods.

Storage facilities are considered for implementing minimal storage policies and transport modal shifts. For instance, it may be defined a port location on Set S that receives some material by road transport (from production to storage facilities).

The objective function is defined as the Net Present Values (NPV) of the cash flow generated by the operation. Production and Storage Investments are incidents in the first period of operation. Profit is incident in the middle of each period and is discounted to present value by an interest rate.

Case of study

The framework presented in section above is illustrated through a case study on the in silico production of biofertilizer and enrichment bioactive compound serum produced via fermentation of five commodities waste. The potential locations for the operation implementation are all in BCS state (Harvest, production and storage), but trade steps could be worldwide.

The biomass cultivation layer is segmented according to agricultural municipalities inside BCS state (Figure 1). The strawberry, mango, tomato, asparagus and orange planted land are taken according to official data from SIAP database [22]. The commodities species selected for the model are the most productive in BCS state. The productivity data and planting, maintenance, and harvesting costs were taken from [21] and they were also used for estimation.

The Set P with all possible production facilities location was defined as the union of agricultural region point and distributor market location sets. This enables production facilities to be close to the biomass supply. The production facilities design allows producing biofertilizer and bioactive compound serum, with the possibility to recirculate used water. The only technology considered for producing serum in the model is the fermentation/extraction process. The process consists on several operations grouped in four: (i) commodity preparation, responsible for the transport and processing of raw waste, washing, cutting and selection; (ii) inoculum preparation, responsible for the processing of microorganism for fermentation; (iii) fermentation/extraction, responsible for mixing and fermentation parameters; (iv) separation, responsible of centrifugation, drying stage and packing operations. A water recovery stage was design after primary washing. A water evaporator was considered to burn residual water. A visual summary was made with SuperPro Designer of all technologies considered as is shown in Figure 3.

RESULTS AND DISCUSSION

The mathematical model shows two optimal production facilities in two different locations: Valle de Vizcaino (VV) and Valle de Santo Domingo (VSD). Figure 4 shows the NPV breakdown for the two optimal solutions for the operation and return rate smaller than 2 years in which chemical logistics and production/operation costs are the major negative contributors. The total investment on the operation is paid back during the 3rd year of operation, already considering the 1.5 years construction period.

The revenues reported in Figure 4 are already net of taxes. Thus, the overall effect of taxes on the operation's feasibility is not shown in the waterfall. The operation revenue comes exclusively from chemical sales. The only chemicals produced were ascorbic acid, quercetin and biofertilizer. Other organic acids are not considered because the purification process increases the operation cost that affects net profit in significant way [23]. Energy had presented a diminished relevance for the operations NPV. However, it is important to notice that only a single type of recirculation process is considered. Water recirculation is crucial to allow the continuity of the overall process because de production facilities are located in desert and extreme weather region [24]. Figure 4 also shows how important raw material costs are to the operations NPV. In this case of study, chemicals are completely dependent of raw material available state, so the raw material transportation was taken as opened dump tank [25].

Valle de Santo Domingo is a very competitive and well-positioned spot for production [22]. It presents high productive harvest lands availability [22]. Also, its routes to reach production facilities are near to [18]. VSD facilities process orange and asparagus waste

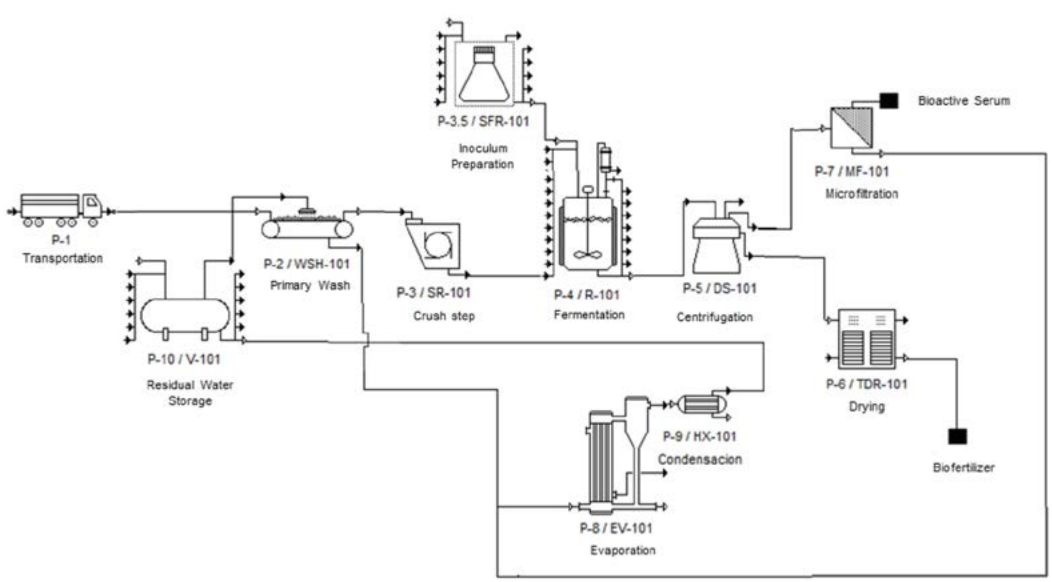

Figure 3. Visual representation of technologies and steps considered. Equipment was represented as visual symbol.

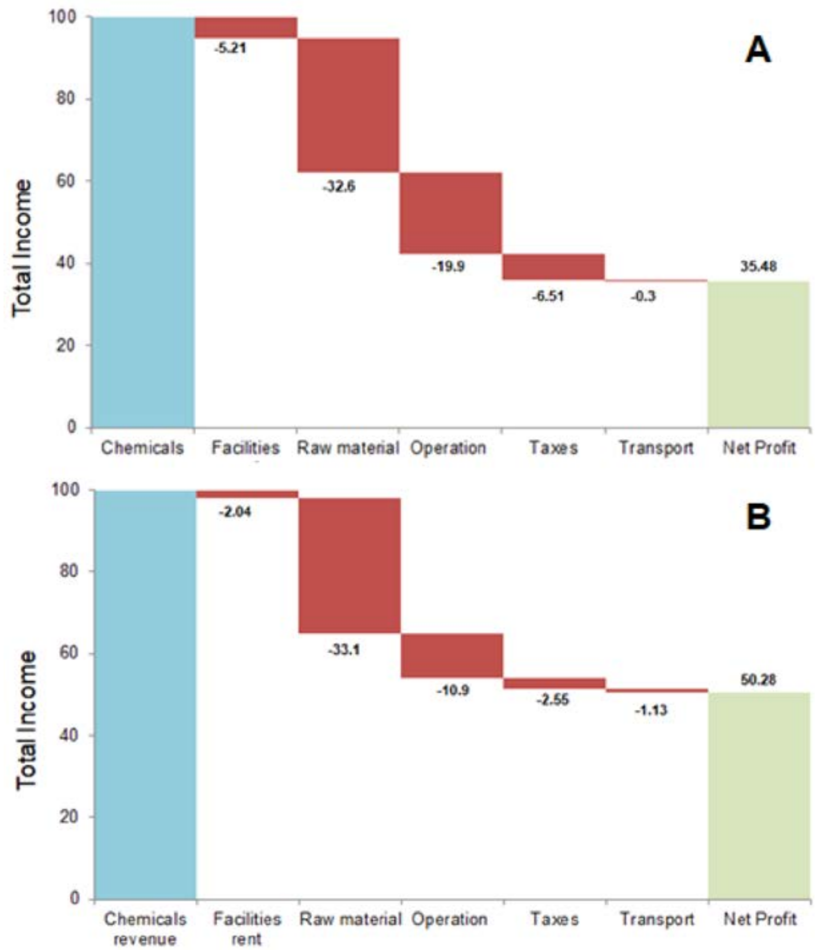

Figure 4: Breakdown of the operations Net Present Value. Revenues are already net of sales taxes. A) Valle de Vizcaino scene; B) Valle de Santo Domingo scene.

in a timeline going from May to April ensuring a complete production year. Harvest scheduling is intimately related to food waste generation, which is one of the most critical parameters for biorefinery modeling [26]. This scheme is only feasible with the assumption that the simple transference of raw material between locations of the same owner (no sale transaction involved) is free of taxes [27].

Productivity affects several aspects of operations dynamics, especially the amount of harvested land needed to buy raw material. Also, the extra land needed may push the operations to collect biomass on further distances, as the extra land required may not be available on the limits of production facilities. This extra distance might under hinder the operations performance and are not fully captured in the present model.

CONCLUSIONS

A mathematical programming framework was presented for assessing the optimal design of biorefineries considering four interconnected decision layers: Biomass, Technology, Operation, and Consumers. The framework was illustrated through a case study on bioactive compound enrichment that may use water recirculation. The framework was developed for capturing food waste generation dynamics. It was shown that theses dynamics display a very important role in all other dimensions' decisions and that an efficient biorefinery design must account for these dynamics. Finally, the technological aspects of the biorefinery were able to be captured within the parallel processing representation proposed in the present work. This representation has the advantage of supporting the addition of new process technologies to the model with little effort even if it has several interconnections to other existing technologies.

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Colegio de Postgraduados

Scientific research on crustacean farming in Mexico: a scientometric scenario

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ABSTRACT

Objective: The objective of this article is to evaluate the research effort developed by Mexican scientists in relation to the study of native and exotic crustacean species, based on articles hosted in Scopus.

Design/methodology/approach: Species were selected based on documentary research and personal communication with researchers related to the subject. All scientific articles published between 1993 and 2023 related to culture, which included the species name in the title, abstract and keywords, were selected from the Scopus® database. The number of publications, publication timelines, topics addressed, institutions, sponsors and type of access were obtained.

Results: The search yielded a total of 1,240 articles published by Mexican institutions, penaeid shrimps representing 85% of the total, and *P. vannamei* represents almost 75%. In general, UNAM and CIBNOR lead scientific production followed by the IPN and the CIAD. The most common topic areas include nutrition, aquaculture, morphophysiology and genetics. The main source of financing is CONAHCYT and 46% of the publications are available in open access.

Limitations on study/implications: The study is restricted to the Scopus database, recognized for including the largest number of journals worldwide, although the omission of other data-bases could cause bias in the results. However, we consider that this limitation will not significantly affect the identified trends.

Findings/conclusions: Research has predominantly focused on the marine shrimp *P. vannamei*, reflecting significant technological development in its culture, while other species such as *C. quadricarinatus* and the genus *Macrobrachium* have received limited attention. It is crucial to encourage research and technological development in native and alternative species, secure funding and institutional support, promote equitable access policies, and improve regulation for sustainable practices.

Keywords: Aquaculture, *Macrobrachium*, marine species, *Penaeus vannamei*, social impact.

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INTRODUCTION

Mexico is a megadiverse country in terms of crustacean diversity. Alvarez *et al.* (2014), report a total of 1,775 species classified in 537 genera and 115 families of decapod crustaceans,

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which represents 11.9% of the total number of species in the world and 57.5% of the families. Of this total, only a few species have been studied as feasible for culture. According to Chong-Carrillo *et al.* (2023), research directed to these purposes by the Centros Públicos de Investigación (Public Research Centers; CIP) of the Consejo Nacional de Humanidades, Ciencias y Tecnologías (National Council of Science, Humanities and Technology; CONAHCYT) has focused, for the most part, on marine species with high added value, while freshwater species have been poorly attended to. In relation to cultured crustaceans, although Mexico has an enormous diversity, only a few species have been studied with the purpose of developing culture technologies. Penaeid shrimps have received the most scientific and technological attention, followed by far by river shrimps of the genus *Macrobrachium* (Chong-Carrillo *et al.*, 2023). Research on cultivable crustaceans in Mexico has been less than on fish and mollusks because native crustacean species that can be taken to aquaculture have been little studied. However, the commercial value of shrimp production in culture is enormous and this has led to practically only one species being of priority interest. According to the Secretaría de Agricultura y Desarrollo Rural (SADER), 192,600 tons of farmed marine shrimp were produced in 2023, with an economic value of more than 19.8 billion pesos.

Research on native crustacean species in Mexico with aquaculture potential has been relevant and large governmental funds have been allocated for this purpose. However, most of these funds have been directed to one native species, the Pacific white shrimp, *Penaeus vannamei* (Chong-Carrillo *et al.* 2023) with a high economic value, especially in international markets. The study also mentions the Australian lobster, *Cherax quadricarinatus*, which is a proven exotic species with a great invasive capacity (Rodríguez-Cruz *et al.*, 2023), which has not been an impediment to public funding for its research for aquaculture purposes.

The objective of this article is to evaluate the research effort developed by Mexican scientists in relation to the study of native and exotic crustacean species, based on articles published in international and regional journals hosted in Scopus. This is done to identify the strengths, weaknesses and areas of opportunity in the study of these aquatic organisms, whose ecological, social and economic importance is relevant for the country.

The above considering the hypothesis that the scientific research on crustacean aquaculture in Mexico is predominantly focused on the shrimp *P. vannamei*, leading to significant technological development in its cultivation, while native and alternative species have been underexplored, limiting the potential for aquaculture diversification in the country.

MATERIALS AND METODS

Crustacean species were selected based on documentary research and personal communication with researchers related to the subject. All scientific articles or reviews, published between 1993 and 2023 related to culture, which included the species name (*Penaeus*, *Litopenaeus*, *Farfantepenaeus*, *Macrobrachium*, *Cherax* and *Procambarus*) in the title, abstract and keywords, were collected from the Scopus® database (Elsevier, The Netherlands). The choice of Scopus over other databases can be justified for several key reasons, making it particularly suitable for bibliometric studies in scientific research. It is the largest abstract and citation database of peer-reviewed literature, covering over 24,000 titles; is updated more frequently compared to other databases, ensuring that the bibliometric analysis reflects the most up-to-date information available; it tends to include more literature from developing countries and regional journals, which is critical for this study focused on the scientific output of Mexican institutions.

The database obtained was subjected to a filtering process that focused on studies carried out by Mexican institutions. Subsequently, those articles that did not comply with the parameters established for the genus and species of the crustacean under study were eliminated. The complete records were transferred to Excel® (Microsoft, USA) data sheets for subsequent analysis. The filtered database was organized to obtain, by species and in general, the number of publications, publication timelines, topics addressed, institutions, sponsors and type of access (open or paid). The results were graphed using Excel®.

RESULTS ANS DISCUSSION

The SCOPUS search yielded a total of 1,240 articles published by Mexican institutions on native and exotic crustaceans that are being studied in Mexico for culture purposes. The study focused on 11 species, which are mentioned individually below.

Cherax quadricarinatus (Australian red claw crayfish)

Commonly known as Australian freshwater lobster or red claw crayfish (*Cherax quadricarinatus*, von Martens 1868), it is a decapod crustacean of the family Parastacidae, native to Australia and Papua New Guinea. It inhabits freshwater bodies such as rivers, streams and lakes (Ghanawi *et al.*, 2012). It prefers warm, well-oxygenated waters and refugia such as rocks, logs and aquatic vegetation. It is one of the most cultivated freshwater species worldwide (Ghanawi *et al.*, 2012) so it can be found in the Americas, including Mexico (Bortolini *et al.*, 2007). It is in high demand in the international market for its flavor and nutritional value, which has led to a significant increase in aquaculture production in recent decades and also as an ornamental species in aquariums (Jones *et al.*, 1994; FAO, 2022). In Mexico, it is cultured in intensive and semi-intensive systems. The states of Baja California, Veracruz and Tamaulipas stand out as the main producers of this species (IMIPAS, 2018). Research, in Mexico, directed to this crustacean has focused on aspects of aquaculture and nutrition, mainly. The Centro de Investigaciones Biológicas del Noroeste (Northwest Biological Research Center; CIBNOR) is the CPI of CONAHCYT that has dedicated more studies to this exotic species (Figure 1) and CONAHCYT has granted most of the resources for its financing.

Macrobrachium acanthurus (Cinnamon river shrimp)

The cinnamon river shrimp (*Macrobrachium acanthurus*) is a freshwater prawn endemic to the Americas that is distributed in rivers, lagoons and estuarine areas along the Atlantic coast, from North Carolina in the United States to southern Brazil (Torati *et al.*, 2011). It has a high economic value due to its flavor, high protein content and visual attractiveness (Kent, 1995; García-Guerrero *et al.*, 2013). In Mexico, *M. acanthurus* is consumed regionally and represents a source of income for fishers in the states of Veracruz and Tabasco (Hernández-

Abad *et al.*, 2018). Due to its demand in the regional market, fishing volumes of this species have been reduced, and in some parts of Mexico have evidenced the disappearance of natural populations such as in the rivers of the Papaloapan and Coatzacoalcos Basins (García-Guerrero *et al.*, 2013). More studies aimed at the development of its culture are required, as there are still gaps in knowledge that prevent the successful achievement of its complete culture technology (García-Guerrero *et al.*, 2013; Villafuerte Mojica *et al.*, 2016). Research, in Mexico, directed to this crustacean has focused mainly on aquaculture and nutrition aspects. The Universidad Nacional Autónoma de México (UNAM) is the university that has dedicated the most studies to this native species and also has provided most of the resources for its financing.

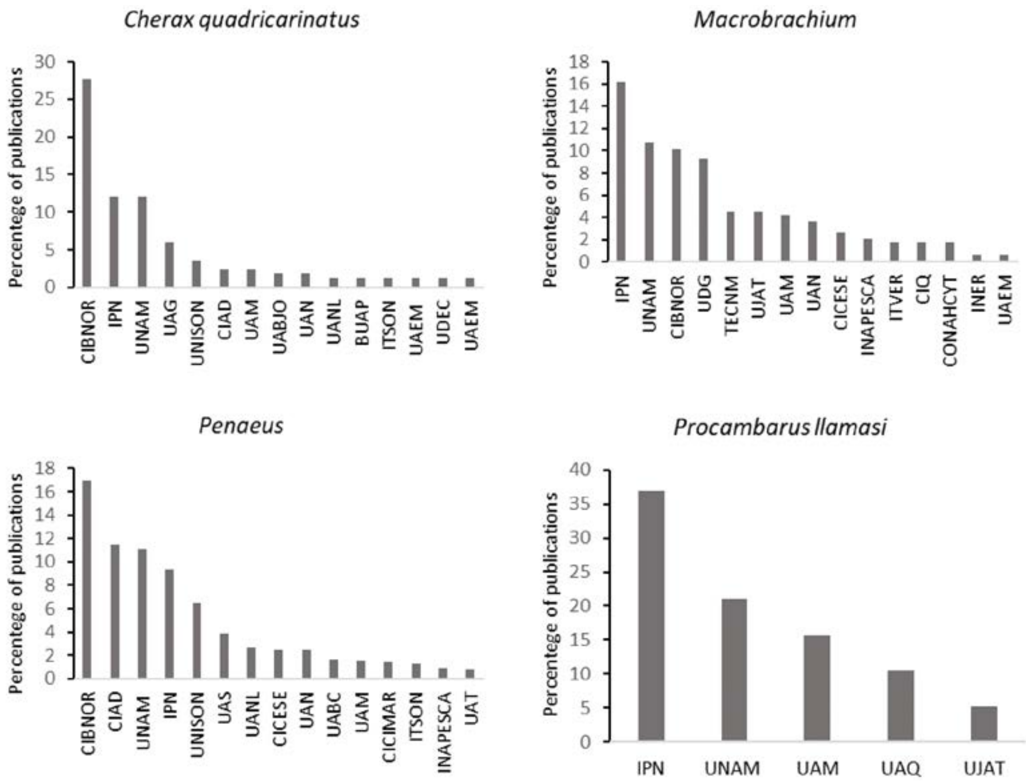


Figure 1. Mexican institutions that conducted studies of crustaceans with culture technology developed, under development or with culture potential in Mexico according to Scopus. The graphs show the 15 institutions with the most studies for each genus. Nomenclature of institutions in alphabetical order: BUAP=Benemérita Universidad Autónoma de Puebla, CIAD=Centro de Investigación en Alimentación y Desarrollo, CIBNOR=Centro de Investigaciones Biológicas Del Noroeste, CICESE=Centro de Investigación Científica y de Educación Superior de Ensenada, CICIMAR=Centro Interdisciplinario de Ciencias Marinas, CIQ=Centro de Investigaciones Químicas, CONAHCYT=Consejo Nacional de Humanidades, Ciencias y Tecnologías, INAPESCA=Instituto Nacional de Pesca y Acuacultura, INER=Instituto Nacional de Enfermedades Respiratorias, IPN=Instituto Politécnico Nacional, ITSON=Instituto Tecnológico de Sonora, ITVER=Instituto Tecnológico de Veracruz, TECNM=Tecnológico Nacional de México, UABC=Universidad Autónoma de Baja California, UABJO=Universidad Autónoma Benito Juárez de Oaxaca, UAEM=Universidad Autónoma del Estado de México, UAG=Universidad Autónoma de Guadalajara, UAM=Universidad Autónoma Metropolitana, UAN=Universidad Autónoma de Nayarit, UANL=Universidad Autónoma de Nuevo León, UAQ=Universidad Autónoma de Querétaro, UAS=Universidad Autónoma de Sinaloa, UAT=Universidad Autónoma de Tamaulipas, UDEC=Universidad de Colima, UDG=Universidad de Guadalajara, UJAT=Universidad Juárez Autónoma de Tabasco, Universidad Nacional Autónoma de México, UNISON=Universidad de Sonora.

Macrobrachium americanum **(Cauque river prawn)**

Macrobrachium americanum, colloquially known as cauque, has good potential for culture because it reaches a large size and currently has a well-established local market in Mexico as an edible product (García-Guerrero and Apun-Molina, 2008). This river shrimp is distributed along the Pacific slope of the Americas from Baja California in Mexico to Peru, as well as in the Cocos and Galapagos Islands (Holthius, 1980). Pressure from local fisheries has severely depleted natural populations, but there is a lack of studies that can provide a better scenario of the current status of their populations. The Instituto Politécnico Nacional (IPN) is the institution most mentioned in published studies on the species, followed by CIBNOR. Funding sources have come mainly from CONAHCYT and the IPN.

Macrobrachium carcinus **(Bigclaw river shrimp)**

The bigclaw shrimp is one of the largest river shrimp in the world, measuring up to 300 mm in length. It is distributed from Florida in the United States to southern Brazil (Lima *et al.*, 2014). This species is an important local source of protein for human consumption in the Atlantic Ocean slope in Latin America (Benítez-Mandujano and Ponce-Palafox, 2014), in addition to having high commercial value (Valenti, 2007). Although adult specimens of *M. carcinus* live and breed in freshwater, larval development occurs strictly in brackish water (Benítez-Mandujano and Ponce-Palafox, 2014). However, due to intensive overexploitation by artisanal fisheries, pollution and destruction of natural environments, it is listed as a vulnerable species in several countries (Coelho-Filho *et al.*, 2018). Domestication attempts have been made to keep it in culture, with partial success, generating management handbooks as results (García-Guerrero *et al.*, 2013). The Universidad Juárez Autónoma de Tabasco (UJAT) is the institution that demonstrates the greatest production in publications and the financing has been provided by CONAHCYT and UNAM.

Macrobrachium tenellum **(Longarm river prawn)**

The longarm river prawn inhabits fresh and brackish waters, and can be found in estuaries, rivers, and coastal lagoons throughout its range from the Baja California Peninsula to the Chira River in northern Peru and Galapagos Island (Holthuis, 1952). *Macrobrachium tenellum* is an amphidromous species with great commercial value and is the subject of artisanal fisheries by rural communities in Mexico for direct consumption and for sale. It plays an important role in food chains, contributing to the flow of energy and nutrients in limnetic environments (García-Guerrero *et al.*, 2013). It has been considered a good candidate for aquaculture, mainly because of its tolerance to environmental factors and its low aggressiveness compared to its congeners *M. americanum* and *M. carcinus* (López-Uriarte *et al.*, 2020; De los Santos-Romero *et al.*, 2021). Cultures of this species have been developed at experimental and pilot levels in rustic, semi-rustic ponds, circular pools, earthen ponds and in pond cage systems (Ponce-Palafox *et al.*, 2006; Vega-Villasante *et al.*, 2011; Ponce-Palafox *et al.*, 2013; López-Uriostegui *et al.*, 2014) with yields ranging from 560 to 2013 kg h^{-1} (Vargas-Ceballos *et al.*, 2021). However, the scarcity of studies on the larval stages of this species has not allowed it to be cultivated for commercial purposes

(Vargas-Ceballos *et al.*, 2020). The Universidad de Guadalajara (UDG) appears as the most productive institution in articles, followed by IPN and CIBNOR. Most of the funding for the study of this species has been provided by CONAHCYT.

Macrobrachium rosenbergii (Giant River Prawn)

The Malayan prawn is native to the tropical Indo-Pacific region, but its distribution has spread significantly through introduction and culture in different parts of the world (New and Valenti, 2009). The natural habitat of *M. rosenbergii* is characterized by warm, tropical rivers and streams. In its natural distribution, it is found in areas with moderate currents and depths ranging from 0.5 to 5 meters. The species can adapt to different water conditions, including temperatures between 20 and 30 °C and pH between 6.5 and 8.5 (New, 2002). Modern cultivation of this species originated in the 1960s in Malaysia, where it was found that larvae required brackish conditions to survive (FAO, 2009). The institution with the highest number of publications is UNAM and funding has been granted by CONAHCYT.

Penaeus californiensis (Yellowleg shrimp)

The yellowleg shrimp *P. californiensis* (Holmes 1900, subgenus *Farfantepenaeus*) is a native species of the Gulf of California in Mexico, with populations from San Francisco Bay, California, USA, to the coast of Paita in Peru (Peña-Rodríguez *et al.*, 2017); with marine habits, but with larval development in estuaries (Villareal *et al.*, 2003). It is able to reproduce in different salinity gradients and develop at winter temperatures (around 20 °C), with high fishery value and aquaculture potential (Martínez-Córdoba *et al.*, 1998). It has been fished since 1930 in Mexico and its catch represents around 40% of the total shrimp fishery in the Mexican Pacific, with a catch similar to *P. stylirostris* and higher than *P. vannamei* (Barbosa-Saldaña *et al.*, 2012).

Aquaculture of *P. californiensis* has been under development in Mexico since the end of the last century, although it has been overshadowed by the adaptability and technological development of *P. vannamei* (Villareal *et al.*, 2003). Its monoculture has been carried out in ponds with aeration (Martínez-Córdova *et al.*, 1998), although since 2011 its culture efforts in the Pacific have been focused more on co-culture with the alga *Ulva clathrata* (Peña-Rodríguez *et al.*, 2017), the same alga that has been reported to have a positive effect on the growth of penaeid shrimp (Portillo-Clark *et al.*, 2013). CIBNOR is the CPI with the highest production of scientific articles, while funding has been obtained from CONAHCYT, for the most part.

Penaeus setiferus (Northern white shrimp)

The northern white shrimp *P. setiferus* (Linnaeus 1767), is a crustacean native to the northern Atlantic distributed mainly from the coasts of New Jersey in the USA to Campeche in Mexico, caught mainly in the Gulf of Mexico and the coasts of North Carolina and Florida (Valenzuela-Jiménez *et al.*, 2020). It is a euryhaline organism, with marine life, but develops to adulthood in estuarine waters (Baker *et al.*, 2014). In the last century, the fishing potential of the species was extensively studied (Gracia *et al.*, 1999), while at the same time, its nutrition was developed by the National Autonomous University of Mexico (UNAM) (Guzmán *et al*., 2001; Gallardo *et al*., 2002; Arena *et al*., 2007).

Research on its overfishing, reproduction, nutrition and behavior developed interest in its cultivation in the 1990s, and due to its high commercial value (Valenzuela-Jiménez *et al*., 2020). However, there are still no intensive productions of the species. (Valenzuela-Jiménez *et al*., 2020). UNAM has developed the greatest research efforts regarding its culture. It is also UNAM that has provided funding for studies on this species, followed by CONAHCYT.

***Penaeus stylirostris* (blue shrimp)**

The blue shrimp *P. stylirostris* (Stimpson 1874) is a crustacean native to Mexico, distributed from the upper Gulf of California in the USA to the coasts of Peru, with the greatest presence (and importance) in the Gulf of California, being caught mainly in the states of Sonora, Sinaloa, Baja California and Nayarit in Mexico (Ramírez-Rojo and Aragón-Noriega, 2006). Together with *P. californienses*, it is the native shrimp with the largest catch in Mexican fisheries, representing together with *P. vannamei* 90% of the total catch in the Pacific (Barbosa-Saldaña *et al*., 2012); in addition to having a high commercial value and being more in demand because it is larger than other penaeids in capture zones (Leyva-Vázquez *et al*., 2021). It has a life cycle similar to other penaeids, with larval stages that develop in estuarine systems throughout the Mexican Pacific (Aragón-Noriega *et al*., 2016).

Its culture has been developed since 1975 in the Gulf of California, and the main research efforts have been carried out by CIBNOR, addressing since 1985 topics such as: nutrition, physiology, genetics, environmental impact, health and management (Pérez-Enríquez *et al*., 2016). Its technology is highly developed thanks to research on its reproduction in captivity (Alfaro-Montoya, 2010), and the genetics of captive populations (Aubert & Lightner, 2000). The Universidad de Sonora (UNISON) and CIBNOR are the institutions most represented in the articles published. Most of the funding has been provided by CONAHCYT.

***Penaeus vannamei* (Whiteleg shrimp)**

The white shrimp (*Penaeus vannamei*) is a marine crustacean that is widely distributed in warm and subtropical waters of the Pacific. Native to the eastern coast of the Pacific Ocean from Sonora, Mexico to Peru and introduced to the coasts of the Gulf of Mexico by aquaculture activity (IMIPAS, 2018). It is distributed in coastal areas and estuaries, where it feeds on small invertebrates and plants. It is a popular edible species and is cultured on a large scale in many countries, including Ecuador, Mexico and other Latin American countries (FAO, 2009). It is grown in all systems: extensive, semi-intensive, intensive and hyper-intensive. Its biotechnology is fully developed and standardized (IMIPAS, 2018). The topics addressed by the articles identified in Scopus, show a balance in the different lines of research with a preponderance of genetics, immunology and nutrition, followed by cultivation, biochemical aspects and morphophysiology. This indicates a research process that aimed to fill the gaps in knowledge in order to achieve the integral biological management of the species and, with this, improve the capacity for efficient culture. CIBNOR is emerging as the most relevant institution in the study of this crustacean and CONAHCYT as the sponsoring organization that has granted the most funding.

Procambarus llamasi (Yucatecan crayfish)

It is a species distributed in the Yucatan Peninsula and Tabasco. It inhabits rivers, lakes, seasonally flooded areas, jagüeyes, coastal marshes, streams, water holes, canals in agricultural fields (Rodríguez-Serna,1999; Grajeda-Zabaleta *et al.*, 2024). This species is part of the biocultural heritage of communities in its area of distribution and has aquaculture potential. It has been studied in various aspects such as feeding in captivity (Rodríguez-Serna *et al.*, 2010), in recirculation systems and also biofloc (Grajeda-Zabaleta *et al.*, 2024). This species has received attention from the IPN and UNAM, as they are the institutions that appear most often in published articles. CONAHCYT has provided most of the funding for its study.

General scenario

Table 1 lists the crustacean species that have been identified as those that have been studied for culture and have generated scientific publications. Only two species are exotic species (*C. quadricarinatus* and *M. rosenbergii*), while most are native or endemic to Mexico.

The number of publications that were selected was 1,240 in total. Of these, penaeid shrimps account for 1057 publications, representing 85% of the total, and the species *P. vannamei* alone represents almost 75%. *Macrobrachium* shrimps are the second most productive genus with 107 articles (8.6% of the total), distributed among five species, with *M. tenellum* being the most studied with 43. However, individually, *C. quadricarinatus* is the species with the second highest number of publications, 68 (5.4%), even though it is considered an exotic and invasive species. Of the crayfish of the genus *Procambarus*, only *P. llamasi* (an endemic species) recorded publications related to culture, but only marginally (eight articles).

Table 1. Crustacean species with culture technology developed, under development or with culture potential in Mexico. The scientific name, common name, status (exotic/native) and number of publications found in Scopus are shown.

Cientific name	Common name (Spanish)	Common name (English)	Status	Number of publications
Cherax quadricarinatus	Langosta australiana	Australian red claw crayfish	Exotic	68
Macrobrachium acanthurus	Langostino prieto	Cinnamon river shrimp	Native	10
Macrobrachium americanum	Langostino cauque	Cauque river prawn	Native	31
Macrobrachium carcinus	Acamaya	Bigclaw river shrimp	Native	7
Macrobrachium rosenbergii	Langostino Malayo	Giant river prawn	Exotic	16
Macrobrachium tenellum	Langostino chacal	Longarm river prawn	Native	43
Penaeus californiensis	Camarón amarillo	Yellowleg shrimp	Native	45
Penaeus setiferus	Camarón blanco del Golfo	Northern white shrimp	Native	38
Penaeus stylirostris	Camarón azul	Blue shrimp	Native	53
Penaeus vannamei	Camarón blanco del Pacífico	Whiteleg shrimp	Native	921
Procambarus llamasi	Acocil yucateco	Yucatecan crayfish	Native	8

The above results should not come as a surprise, since the development of research on crustacean culture in Mexico has been directed, almost entirely, to marine shrimp. Chong-Carrillo *et al.* (2023) analyze the number of projects directed to scientific research on aquatic culture and their financing in the CPI of CONAHCYT, based on information provided by the CPI themselves. The CIBNOR is the one that has directed more projects (44) to research on crustaceans, with a clear dedication to marine shrimp and *P. vannamei*, although projects have also been carried out with shrimp of the genus *Macrobrachium*, specifically *M. americanum* (but in very low numbers). The funding that CIBNOR has received for the development of these projects (from CONAHCYT) was 53,484 million pesos in approximately 25 years. With fewer scientific projects on crustacean culture (mainly *P. vannamei*), Centro de Investigación en Alimentación y Desarrollo (Research Center in Food and Development; CIAD) was detected with eleven projects, also financed by CONAHCYT, with 15.705 million pesos. Finally, the Centro de Investigación Científica y de Educación Superior de Ensenada (Ensenada Scientific Research and Higher Education Center; CICESE) reported six projects, with funding of 2.090 million pesos. This clearly demonstrates that CONAHCYT's CPIs have directed their greatest research effort in crustacean culture toward one species. The possibility of increasing the production of Pacific white shrimp, which is a high value-added species with high demand in international markets, has been, almost without a doubt, the origin of the scientific policies that have directed research toward this species. Marginally, research has been carried out with other native species that would require more studies to complete the biotechnology that would make them competitive.

The only species that has shown an almost constant upward trend since 1996 is *Penaeus vannamei*, which on average has produced 30 articles per year in three decades (Figure 2C). The rest of the penaeid species have remained with a low number of publications in the same period. *Cherax quadricarinatus* records publications since 2002, with a maximum of ten in 2003 to subsequently show few, if any, annually (Figure 2A). Of the prawns of the genus *Macrobrachium*, two species stand out from the end of the first decade of the 21st century, *M. americanum* and *M. tenellum*, and it is evident that interest in the study of both has restarted after a long unproductive period, which has continued to date, although with decreases (Figure 2B). Two peaks of article production, very similar, are observed in 2012 and 2018 with these two species. The exotic species, *M. rosenbergii*, has also been of scientific interest with some publications in the period studied, but with totally unproductive years. The same is observed for the other native species of *Macrobrachium*, which have barely maintained a discrete production of articles over the course of three decades. In the case of *P. llamasi* there was a period of only ten years in which it was of scientific interest, related to its culture (Figure 2D). From 2010, the year in which the largest number of articles was produced (3), to date, there is no record of publications.

Analysis of the timelines also suggests that there is some decline in the number of articles published from the time the COVID-19 pandemic occurred in late 2019 and early 2020. Although, for some species there is an increase and decrease in publications over the years, the decrease from 2020 onwards is notable especially in the publication of the genera *Penaeus* and *Macrobrachium*, which suffered declines after notable increases in

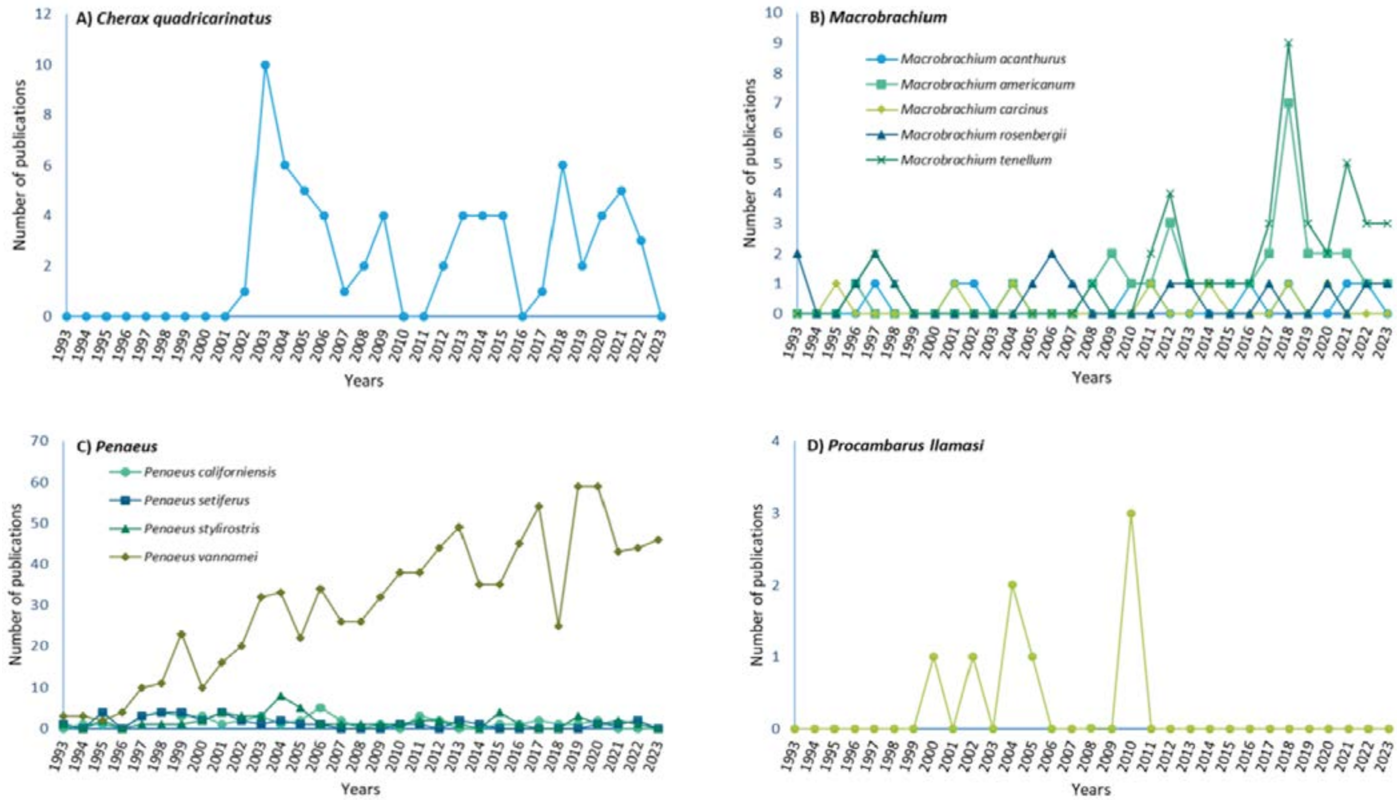


Figure 2. Timeline of publications of crustacean species with culture technology developed, under development or with culture potential in Mexico according to Scopus. A) *Cherax quadricarinatus*, B) Species of the *Macrobrachium* genus, C) Species of the *Penaeus* genus, D) *Procambarus llamasi*.

previous years and the recovery has been slow. This phenomenon is not the product of a light interpretation but has been studied by other authors. Gao *et al.* (2021) mention that the pandemic had important, long-term effects on the creation of new projects and on the performance of scientists, especially women and men with young children. Riccaboni and Verginer (2022) study the number of publications generated in the first years of the pandemic and mention that there was a general decrease, except for those related to health issues and the pandemic itself. Jamali *et al.* (2023), comment that the closure of laboratories and the impossibility of carrying out field work had a severe impact on the development of young scientists in training and on their productivity, an important sector in population science since it is the main workforce of research groups. The consequences of the COVID pandemic on scientific productivity in Mexico should be further studied and is part of our future studies in aquaculture research.

The 20 institutions that have conducted the most research on crustacean culture are presented in Table 2, considering the total number of articles generated by each one and the respective percentage per institution. CIBNOR leads the percentage of publications, together with UNAM (20% both). The participation of CIBNOR is remarkable in studies directed to aquatic cultures in Mexico. This has already been mentioned in previous reports (Chong-Carrillo *et al.*, 2023; Nieves-Rodríguez *et al.*, 2024; Chong-Carrillo *et al.*, 2024).

The participation of the other two CPIs with a vocation in aquaculture research (CIAD and CICESE) is also relevant in this case. However, the contribution of public universities is also preponderant.

UNAM, IPN, UNISON and Universidad Autónoma de Sinaloa (UAS), among others, show that these institutions have consolidated research groups in crustacean culture. As part of a study on crustacean research for farming purposes, it was found that the lines of research directed to *Penaeus vannamei* are diversified. The results show that the greatest effort for this species is directed to aquaculture and nutrition, representing 18% of the publications for each of these topics. However, areas such as biochemistry, genetics and morphophysiology also stand out. For the blue lobster (*Cherax quadricarinatus*), a similar situation occurs, where the dominant topics belong to aquaculture (30%) and

Table 2. Institutions most mentioned in articles on crustacean published by Mexican institutions and hosted in SCOPUS (as a percentage of total articles).

Institutions	% of published articles
CIBNOR	20
UNAM	20
IPN	15
CIAD	11.2
UNISON	6.2
UAS	3.8
CICESE	3
UAN	2.8
UANL	2.7
UAM	2.5
CICIMAR	2
UABC	2
INAPESCA	1.9
UAT	1.5
UDG	1.4
ITSON	1.2
UABCS	0.7
UAEM	0.4
ECOSUR	0.4
BUAP	0.2

Nomenclature of institutions in alphabetical order: BUAP=Benemérita Universidad Autónoma de Puebla, CIAD=Centro de Investigación en Alimentación y Desarrollo, CIBNOR=Centro de Investigaciones Biológicas Del Noroeste, CICESE =Centro de Investigación Científica y de Educación Superior de Ensenada, CICIMAR=Centro Interdisciplinario de Ciencias Marinas, ECOSUR=El Colegio de la Frontera Sur, INAPESCA=Instituto Nacional de Pesca y Acuacultura, IPN=Instituto Politécnico Nacional, ITSON=Instituto Tecnológico de Sonora, UABC=Universidad Autónoma de Baja California, UABCS=Universidad Autónoma de Baja California Sur, UAEM=Universidad Autónoma del Estado de Morelos, UAM=Universidad Autónoma Metropolitana, UAN=Universidad Autónoma de Nayarit, UANL=Universidad Autónoma de Nuevo León, UAS=Universidad Autónoma de Sinaloa, UAT=Universidad Autónoma de Tamaulipas, UDG=Universidad de Guadalajara, UNAM =Universidad Nacional Autónoma de México, UNISON=Universidad de Sonora.

nutrition (19%), while other areas such as morphophysiology and parasitology appear in a lower percentage. For species of the genus *Macrobrachium*, interest in technological development is also demonstrated, since aquaculture is among the main research topics (Figure 3). The aquaculture interest in research on crustacean species with culture potential is evident, since the studies directed at these species are mainly focused on that direction. This situation differs from research in Mexico oriented to native fish species, where although there is an interest in laying the foundations for aquaculture technological development, a large proportion of research indirectly affects aquaculture topics, with high percentages of studies focused on Ecology or Parasitology (Nieves-Rodriguez *et al.*, 2024).

Figure 4 shows the publications by species considering the access to them, either open access or paid access. Of the total number of articles published, 46% were open access through the paid access system of the respective journals, while only 54% were restricted with paid access. These percentages are similar to those reported in the analysis of fish studies carried out by Nieves-Rodríguez *et al.* (2024), which also shows that Mexican researchers use the paid access method in high-impact journals so that their research can be placed with greater visibility and "greater prestige".

According to the president of the aquaculture group of the Consejo Nacional de Fabricantes de Alimentos Balanceados y de la Nutrición Animal (CONAFAB) Jaime Almazán, aquaculture generates social benefits, such as: greater food security (access to fish and seafood at affordable prices), job creation (economic growth in aquaculture zones reduces the expulsion of labor to urban areas), among others (El Economista, 2024). Undoubtedly, the beneficial effects of aquaculture in terms of economic growth derived from job creation are indisputable. According to the Comité de Sanidad Acuícola del Estado Sonora, A.C. (COSAES), in Sonora alone, marine shrimp aquaculture is considered to generate 50,000 direct and indirect jobs, in 140 farms with 26,000 hectares of culture (personal communication with Miguel Ángel Castro Cosío). This confirms the job-generating vocation of the cultivation of this crustacean and, consequently, its economic benefits for a certain sector of the population. However, its participation in achieving food security is not so clear, at least in terms of products obtained through crustacean aquaculture. The Programa Nacional de Pesca y Acuicultura (PNPA) 2020-2024 (SADER, 2019), mentions that "the aquaculture sector will be promoted, with the purpose of contributing to food security, by virtue of the fact that, through this activity, a greater production of food of high nutritional value can be obtained". The above shows the spirit in which development policies in the fisheries and aquaculture sector should be carried out in the established period. However, in the same PNPA it is mentioned that there is low productivity in these sectors, which leads to low-income levels. The causes of this are identified as follows: 1) Low contribution of fisheries and aquaculture to food security, 2) Low levels of income and poverty in fishing and aquaculture communities, 3) Inadequate management of fishery and aquaculture resources, 4) Weak legal and institutional framework for fisheries and aquaculture. These causes, which are directly related to aquaculture, include "insufficient supply of fishery and aquaculture products at accessible and quality prices in rural communities," "deficient quality, adequate and

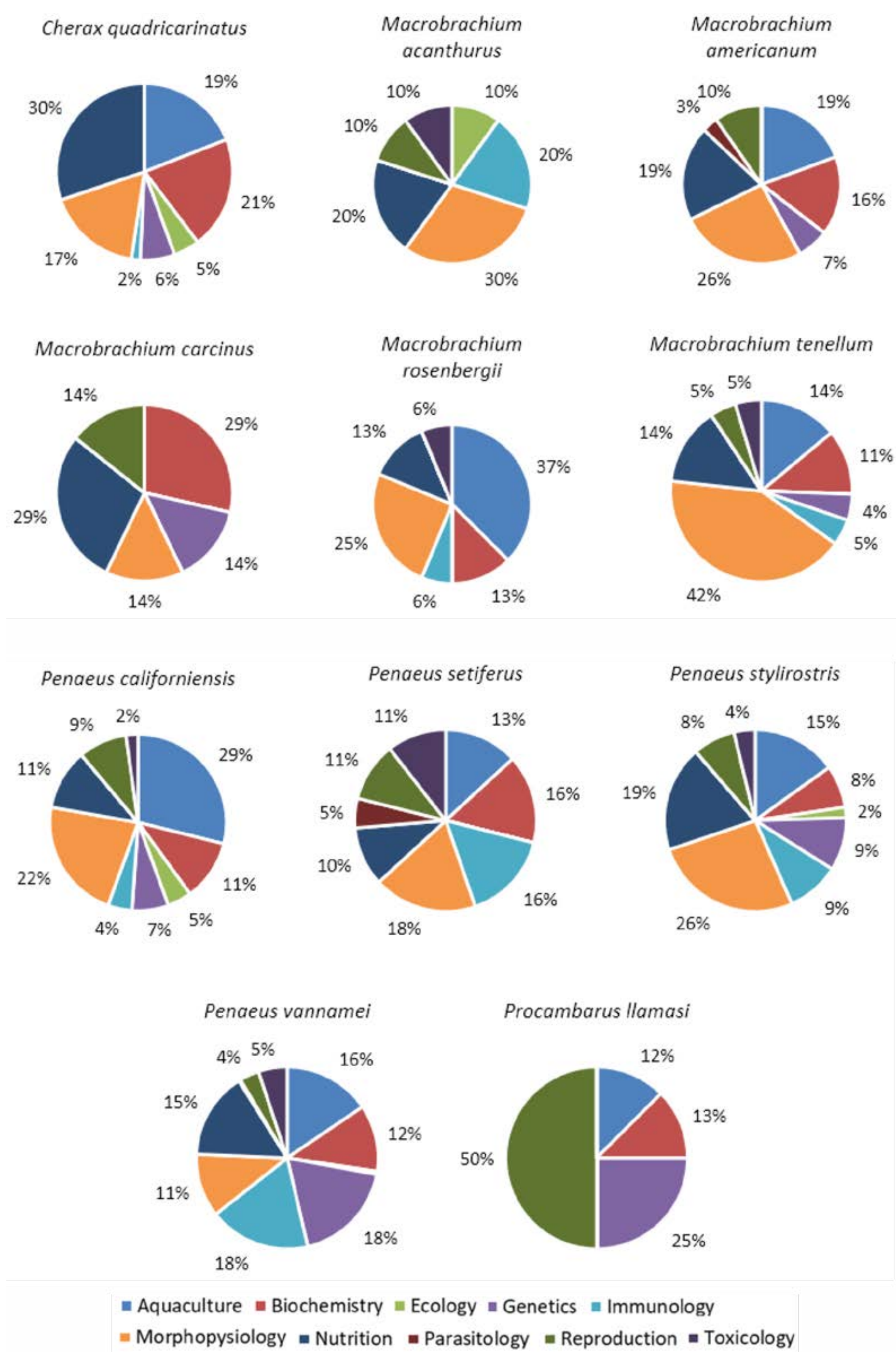
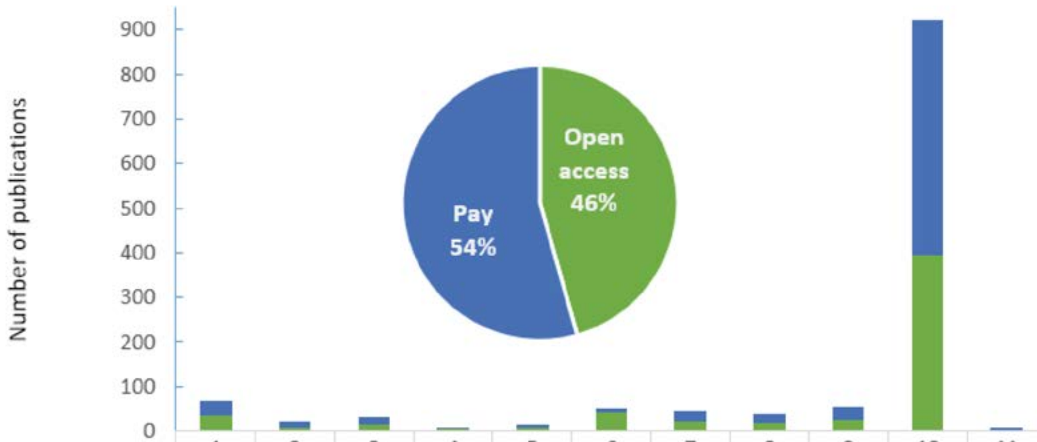


Figure 3. Percentage of publications by thematic areas of crustacean species with culture technology developed, under development or with culture potential in Mexico according to Scopus.



	1	2	3	4	5	6	7	8	9	10	11
Pay	34	10	17	2	8	11	23	19	28	526	6
Open access	34	10	14	5	8	40	22	19	25	395	2

Figure 4. Number of publications of crustacean species with culture technology developed, under development or with culture potential in Mexico according to Scopus and classified by access (open or paid access). 1. *Cherax quadricarinatus*, 2. *Macrobrachium acanthurus*, 3. *Macrobrachium americanum*, 4. *Macrobrachium carcinus*, 5. *Macrobrachium rosenbergii*, 6. *Macrobrachium tenellum*, 7. *Penaeus californiensis*, 8. *Penaeus setiferus*, 9. *Penaeus stylirostris*, 10. *Penaeus vannamei*, 11. *Procambarus llamasi*.

pertinent technical-scientific information," and "limited growth and diversification of aquaculture.

The social impact of the research carried out to develop the cultivation of the crustacean species included in this study is low. Most of the species studied and for which the complete technology is already available are of high added value. However, these crustaceans could be a valuable source of protein for human consumption, especially for sectors of the population with limited access to other sources of animal protein. The three species that are currently being cultured in Mexico (*P. vannamei*, *C. quadricarinatus* and *M. rosenbergii*) and that have been subjected to research in CPI and academic institutions, have sales value that is not accessible to a large sector of the population. According to information provided by the producers themselves, the retail price of Australian lobster (*C. quadricarinatus*) is around $250 pesos per/kg; and that of the Malaysian prawn (*M. rosenbergii*) at the farm is around $600 pesos/kg, both data obtained in Veracruz. In Mérida, Yucatán, in the Chedraui supermarket, the price of Malaysian prawn is $599 pesos/kg, while in San Pedro Pochutla, Oaxaca, it is $700 pesos in farm. In the case of head-on shrimp, prices at Walmart supermarkets (online) range from 199 pesos/kg to 299 pesos/kg, depending on size. According to Smattcom (agricultural, aquaculture and fishery products trader), the price of fresh shrimp per kg, wholesale, in May 2024, can have a minimum price of 82 to 146 pesos and a maximum of between 195 to 899 pesos (no information on size or origin). In a study currently carried out in Veracruz, Puebla and the La Viga market in Mexico City, it has been recorded that the price of *M. rosenbergii* can vary from $380 pesos per box with two pounds (wholesale), up to $450 pesos (retail) and even up to $700 pesos (for individuals and restaurants). For Australian lobster, prices have been recorded ranging from $80 pesos to $200 per kg (depending on size and freshness) (personal communication with Gonzalo Ammiel Gómez Salgado).For the Pacific coast, in the Cajón de Peñas dam (Jalisco), the price per kg of Australian lobster varies from $40 to $50 pesos and the price of river shrimp, *M. americanum*, is $500 pesos/kg (personal communication with fishermen of the Cajón de Peñas dam). It is worth mentioning that both species are the product of fisheries (in the same dam). The Australian lobster is already fished regularly in that area and represents a profitable activity for the inhabitants, despite being an exotic species that has been distributed both in the dam and in its canals (Vega-Villasante *et al.*, 2015). It is clear that a low-income family would not be able to acquire, on a daily basis, such products that are, in an obvious way, aimed at a population sector with greater purchasing power. Therefore, at least in the case of crustaceans, the insufficient supply at accessible and quality prices for rural populations is confirmed.

The deficient scientific-technical information and the scarce diversification of aquaculture, problems identified in the PNPA 2020-2024, are consequences of the same phenomenon. According to the results of the analysis of the publications in this study, scientific-technical information has been profuse and relevant for *P. vannamei*, and to a much lesser extent for shrimp of the genus *Macrobrachium* and *C. quadricarinatus*. Decades ago, there was interest in the scientific and technological study of native and alternative species for marine shrimp culture. The production of articles over time, shown in Figure 1, demonstrates this. However, the lines of research that would allow the aforementioned diversification were not maintained and only those directed to the study of Pacific white shrimp were (apparently) preserved. At least, for the study of crustaceans with a vocation for cultivation, there do not seem to be new lines that address new or forgotten species, except for the genus *Macrobrachium* and its native species. For *M. rosenbergii*, there is also no evidence to show that studies are being carried out to improve or make culture, reproduction or nutrition techniques more efficient, even though its cultivation is growing in several regions of the country.

CONCLUSIONS

The dominance of the marine shrimp *P. vannamei* is clear, since most of the research has focused on this crustacean, reflecting a clear preference and technological development in its culture. Despite initial mentions of studies on other species such as *C. quadricarinatus* and some of the *Macrobrachium* genus, the research and technology developed are limited, suggesting a lack of exploration towards culture diversification. Shrimp aquaculture generates significant employment and economic growth in specific areas, but the products obtained are of high value, limiting their accessibility to the low-income population. The sector faces challenges such as low productivity, insufficient supply at affordable prices, and limited research on native species and alternatives to *P. vannamei*.

Therefore, it would be advisable to encourage research and technological development in native species and alternatives to *P. vannamei*, such as shrimp of the *Macrobrachium* genus, in order to reduce dependence on a single species and explore new markets. Ensure sustained funding and institutional support for aquaculture research projects, especially those directed toward less studied species and innovative technologies. Promote policies that allow equitable access to the economic benefits of aquaculture, considering food production at affordable prices for rural and low-income communities. Improve the regulation and institutional framework to promote sustainable, efficient and ethical practices in aquaculture, ensuring responsible development of the sector. Encourage collaboration between research institutions, production centers, and international cooperation to share knowledge, technologies and best practices in aquaculture.

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