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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.

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Characterization of cocoa (*Theobroma cacao* L.) seeds and paste through physicochemical and functional analysis

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ABSTRACT

Objective: The objective of this study was to determine the functional properties and antioxidant capacity of the seeds and paste from five varieties of cocoa (*Theobroma cacao* L.).

Design/Methodology/Approach: Polyphenol content was quantified using spectrophotometry, measuring absorbance at wavelengths ranging from 500 to 760 nm over a defined period. Antioxidant capacity was assessed using the $DPPH^+$ (2,2-diphenyl-1-picrylhydrazyl) assay. A multivariate analysis was performed on the resulting data. Morphological characterization of the germplasm was conducted using 20 descriptors, which were subsequently analyzed through principal component analysis (PCA).

Results: The morphological descriptors most strongly associated with varietal differentiation, with a correlation coefficient $p>0.70$, were fruit length and fruit width. For physicochemical characterization, a one-way ANOVA revealed statistically significant differences among the varieties. This was further confirmed through a homogeneous group analysis at $\alpha=0.05$. Among the studied varieties, 'Rabo Lagarto' and 'Calabacillo' exhibited significant differences in polyphenol content compared to the others.

Limitations/Implications of the Study: This study is limited to cocoa varieties cultivated by smallholder producers in the localities of Miahuatlán and town C-11, within the municipalities of Cárdenas and Cunduacán, Tabasco.

Findings/Conclusions: The results demonstrate that the evaluated cocoa varieties can be distinctly characterized based on morphoagronomic traits, polyphenol content, and antioxidant capacity. These findings contribute valuable insights into the functional properties of cocoa and its derivatives.

Keywords: Cocoa, characterization, antioxidants.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a plant native to the humid tropics of South America (Lanaud *et al.*, 2024). It belongs to the family Sterculiaceae and is naturally distributed in tropical regions characterized by warm and humid climates (Rangel-Fajardo *et al.*, 2012). The Amazon rainforest of Ecuador exhibits the greatest genotypic variability of

the species, where at least three cocoa phenotypes Landrace, Forastero, and Trinitario are found (Zarrillo *et al.*, 2018). According to SIAP (2022), cocoa production in Mexico is concentrated in three southern states: Tabasco (64%), Chiapas (35%), and Guerrero (1%). The economic significance of cocoa cultivation in this region lies in the distinctive and highly prized flavor of the beans, which sets them apart from those produced elsewhere in the world (Jiménez *et al.*, 2018). The chemical composition of cocoa beans and paste confers notable nutritional properties, particularly due to the presence of various antioxidant and anti-inflammatory compounds known as polyphenols, which provide multiple health benefits when consumed (Chacón, Mori, & Chavez, 2021; Rodríguez-Sánchez *et al.*, 2015). In recent years, the complexity of cocoa compounds has drawn significant attention due to the dynamic interactions between environmental conditions and product development. These factors are key drivers of variations in both the quality and nutritional value of cocoa (Jurado-Teixeira *et al.*, 2016). Polyphenols are classified into phenols, flavonoids, and procyanidins groups of secondary metabolites with a broad range of chemical structures (Rojo-Poveda *et al.*, 2020). Cocoa seeds and paste contain polyphenolic compounds such as flavanols (catechin and epicatechin), methylxanthines (theobromine and caffeine), and other bioactive molecules like phenylethylamine (PEA) (Morales *et al.*, 2012). Antioxidants are defined as substances that, at low concentrations, have the ability to reduce, delay, or prevent the oxidation of a substrate (López-Medina & Gil-Rivero, 2017). The antioxidant potential of cocoa polyphenols is attributed to their ability to inhibit oxidative cellular stress through modulation of redox reactions and their metal-chelating properties (Rossin *et al.*, 2021). These mechanisms offer potential health benefits, as they may help reduce the incidence of chronic degenerative diseases (Oliveira *et al.*, 2022). Cocoa contains both endogenous (enzymatic) and exogenous (non-enzymatic) antioxidants, which work by transforming free radicals into less reactive compounds (Chávez-Rivera & Ordoñez-Gómez, 2018). According to Ferreira de Oliveira *et al.* (2021), consumers are increasingly concerned with the quality of cocoa-derived products, especially regarding traceability and nutritional value including mineral content and antioxidants. Thus, understanding whether the type of cocoa consumed influences antioxidant content is of great importance. Such knowledge could enhance the valuation of certain varieties over others, depending on their intended use, and foster the development of niche markets. This presents opportunities for local agro-industries and contributes to preserving cocoa cultivation, especially in the face of displacement by more economically profitable crops such as sugarcane (Tadeo-Sánchez & Tolentino-Martínez, 2020). The aim of the present study was to characterize the seeds and paste of cocoa from Tabasco through physicochemical and functional analyses, in order to identify their phytochemical and functional properties.

MATERIALS AND METHODS

Study material

In the present study, five cocoa (*Theobroma cacao* L.) accessions were evaluated, corresponding to the varieties 'Amelonado', 'Calabacillo Type', 'Improved White Landrace Clone Carmelo', 'Landrace Type', and 'Rabo Lagarto'. The collection activities were carried out during March and April 2022. The variety 'Improved White Landrace Clone

Carmelo' was selected due to its recognized organoleptic profile and its distinction granted by the Mexican government under the Plant Varieties Law through a breeder's title. The remaining four varieties were chosen based on information provided by local producers, taking into account the general fruit morphology and the experiential knowledge of producers from the municipalities of Cárdenas and Cunduacán, Tabasco, as illustrated in Figure 1. It is important to note that the collected samples were subjected to a varietal validation process to ensure the accuracy of their classification. The sampling followed methodologies proposed by Restrepo-Quiroz *et al.* (2018) and López-Hernández *et al.* (2021). Each sampling site was georeferenced using a Global Positioning System (GPS), and mature fruits were randomly harvested in each plantation to ensure representativeness and minimize selection bias. Subsequently, the samples were recorded and labeled using a passport data system, which included information regarding the locality of origin, geographic coordinates, collection date, producer, and relevant morphoagronomic characteristics such as fruit and seed descriptors.

Physical (Morphoagronomic) Characterization)

Each cocoa sample was assigned an identification code that included the sample number, variety, and place of origin. The samples were processed at the Food Technology Laboratory of the Multidisciplinary Academic Unit Reynosa-Aztlán, part of the Autonomous University of Tamaulipas. For the physical characterization, morphoagronomic descriptors were used, consisting of physical measurements obtained using a HER-411 digital Vernier caliper (STEREN brand), an analytical balance, and a colorimeter (model NR110). Table 1 presents the twenty morphoagronomic descriptors employed in this study.

Chemical characterization (phytochemicals)

Phytochemicals present in cocoa seeds and paste were extracted following the methodology proposed by Ordoñez *et al.* (2020). From the collected cocoa seed and paste samples, 2.5 g of dry sample was mixed with 25 mL of a methanol-water solution

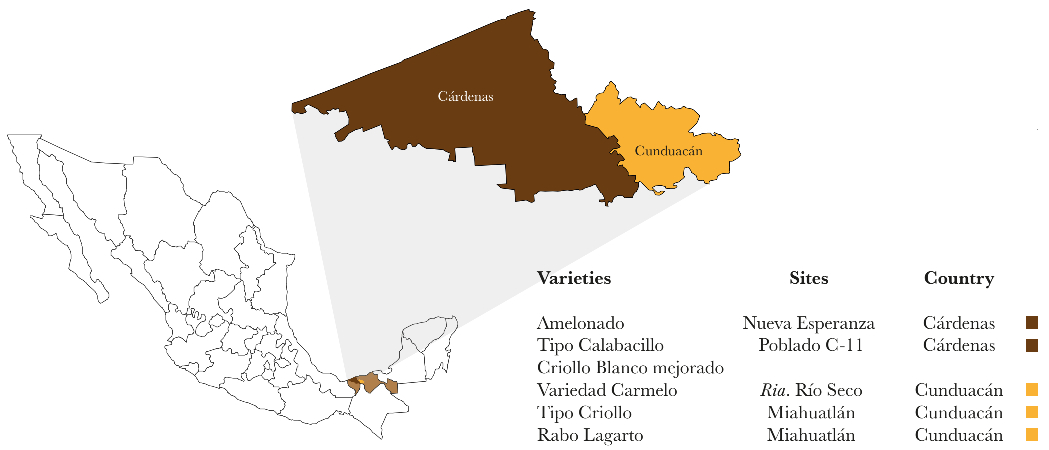

Figure 1. Geographical location of the collection sites for five cocoa varieties in the municipalities of Cárdenas and Cunduacán, Tabasco, Mexico. Source: Authors' own elaboration.

Table 1. Descriptors used for the morphoagronomic characterization of five cocoa varieties.

#	Descriptor	Code	#	Descriptor	Code
1	Color of ripe fruit.	CFM.	11	Total number of seeds per ear.	NSTM.
2	Shape of the apex.	FAF.	12	Number of rows.	NH.
3	Basal construction of the fruit.	CBF.	13	Wet seed mass.	MHS
4	Shape of the fruit.	FF.	14	Dry seed weight.	PSS
5	Roughness of the fruit.	RF.	15	Seed length.	LS.
6	Anthocyanin intensity in fruit spines.	IALF.	16	Seed diameter.	DS.
7	Separation between pairs of spines.	SPL.	17	Thickness of the seed.	GS.
8	Fruit length.	LF.	18	Longitudinal shape of the seed.	FLS.
9	Fruit width	AF.	19	Cross-sectional shape of the seed.	FSTS.
10	Thickness of the fruit.	GF.	20	Predominant color of the cotyledon.	CPC.

Source: Restrepo-Quiroz *et al.*, 2018.

(50:50 v/v). The suspension was subjected to constant agitation at 160 rpm for 24 hours at 20 °C using an Innova 4900 incubator. The mixture was then filtered using a Büchner funnel, filter paper, and a Buchi V-300 vacuum pump. The filtrate was centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatant was transferred to porcelain capsules and dried under controlled conditions at 40 °C±2 °C. The dried extract was stored in amber glass bottles with screw caps at 3 °C±1 °C until phytochemical characterization.

Determination of phenolic compounds

Total phenols (TP) were quantified using the Folin-Ciocalteu (FC) method. A 125 μL aliquot of the extract was mixed with 500 μL of deionized water and 125 μL of Folin reagent (Sigma-Aldrich, St. Louis, MO). The mixture was left to stand for six minutes in the absence of light. Then, to neutralize the reaction, 1,250 μL of 7% sodium carbonate (Na_2CO_3) solution was added. The final volume was adjusted with 1,000 μL of deionized water. After 90 minutes, absorbance was measured at 750 nm using a spectrophotometer. Quantification was performed using a gallic acid standard curve (Sigma Chemical Co.) within the range of 1,000-10 μg/mL, with an R^2=0.9926. Results were expressed as mg gallic acid equivalents per gram of dry sample (mg GAE g^{-1}).

Determination of Flavonoids

Flavonoid content (FL) was determined using the method of Adom and Liu (2002). A 250 μL aliquot of cocoa seed or paste extract was mixed with 75 μL of 5% (w/v) sodium nitrite ($NaNO_2$), 150 μL of 10% (w/v) aluminum chloride ($AlCl_2$), 500 μL of 1 M sodium hydroxide (NaOH), and 2,500 μL of distilled water. After five minutes of rest, absorbance was measured at 517 nm. Quantification was performed using a quercetin standard curve ranging from 10-500 mg/mL, with an R^2=0.9962. Values were expressed as mg quercetin equivalents per gram of dry sample (mg QE g^{-1}).

Determination of procyanidins (tannins)

Condensed tannins (procyanidins) were quantified following the methodology of Broadhurst and Jones (1978). A 50 μL aliquot of the extract was mixed with 3,000 μL of 4% (w/v) methanol-vanillin solution and 600 μL of concentrated hydrochloric acid (HCl). The mixture was allowed to react for 15 minutes at room temperature, and absorbance was measured at 500 nm using methanol as a blank. Quantification was based on a quercetin standard curve within a range of 10-500 mg/mL, with an $R^2=0.9707$. Results were expressed as mg quercetin equivalents per gram of dry sample (mg QE g^{-1}).

Evaluation of antioxidant capacity

The antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) method, as described by Brand-Williams *et al.* (1995). A 100 μL aliquot of the cocoa seed and/or paste extract was mixed with 3,900 μL of DPPH* reagent. Absorbance was measured at 517 nm using a spectrophotometer, with readings taken every 3 minutes for 30 minutes. Antiradical activity was expressed in μmol equivalents. All samples were analyzed in duplicate.

Statistical analysis

Statistical analysis was initially conducted using STATGRAPHICS Data Analysis Solution. A multivariate analysis was performed to identify descriptors with the highest degree of correlation ($p>0.70$). For the physicochemical characterization, ANOVA ($p<0.05$) was employed to verify model assumptions. Data normality was assessed using the Anderson-Darling test prior to ANOVA application. When necessary, homogeneity of variances was verified using Levene's test. Data were transformed and standardized (mean=0, standard deviation=1) to avoid scale-related bias. To perform the multivariate analysis Principal Component Analysis (PCA) Python 3.11 was used, utilizing the 'Scikit-learn' and 'Scipy' libraries for computation and 'matplotlib' for visualization. Each variable was measured in five independent samples with three biological replicates (n=15 observations per variable), applied to the 20 descriptors, ensuring the robustness of the experimental design.

RESULTS AND DISCUSSION

Physical analysis, morphological descriptors

The qualitative and quantitative morphological descriptors allowed for the differentiation and characterization of cocoa fruit and seed traits. This analysis was conducted using 20 morphological descriptors, through which the cocoa accessions were distinguished based on both qualitative and quantitative features of fruits and seeds. Tables 2 and 3 present the results of the morphoagronomic analysis applied to the studied varieties.

Table 4 presents a summary of the statistical analysis of the morphological descriptors evaluated in the cocoa samples, which allow for a statistical assessment of the intrinsic characteristics of the studied variety. Although the analysis framework encompasses the morphology of the cacao tree including stems, flowers, leaves, fruits, and sedes this study

Table 2. Qualitative descriptors of seeds and fruits from five cocoa (*Theobroma cacao* L.) varieties.

Variety	Location	CFM.[A]	FAF.[B]	CBF.[C]	FF.[D]	RF.[E]	IALFM.[F]	SEPL.[G]	FLS.[H]	FSTS.[I]	CPC.[J]
Amelonado	Nueva Esperanza Cárdenas	3	5	1	2	0	1	0	3	1	2
Type Calabacillo	Poblado C-11, Cárdenas	2	6	0	5	0	0	1	3	1	3
Landrace Var. Carmelo	Ria. Río Seco. Cunduacán	3	4	2	2	2	1	3	4	1	1
Type Landrace	Miahuatlán, Cunduacán	3	4	1	1	2	1	3	2	3	1
Rabo Lagarto	Miahuatlán, Cunduacán	2	2	1	4	3	0	4	4	1	3

Where: CFM, color of the ripe fruit; FAF, shape of the fruit apex; CBF, basal construction of the fruit; FF, fruit shape; RF, fruit roughness; IALFM, intensity of anthocyanins on the fruit ridges; SEPL, separation between pairs of ridges; FLS, longitudinal shape of the seed; FSTS, cross-sectional shape of the seed; CPC, predominant color of the cotyledon.

A) 1 intense yellow; 2 intermediate yellow; 3 light yellow; 4 orange-yellow; 5 light orange-yellow; 6 intense red; 7 intermediate red; 8 reddish-orange. B) 1 attenuated; 2 serrated; 3 sharp; 4 nipple-shaped; 5 obtuse; 6 rounded. C) 0 absent; 1 slight; 2 intermediate; 3 strong. D) 1 elliptical; 2 oblong; 3 obovate; 4 ovate; 5 orbicular; 6 oblate. E) 0 absent; 1 slight; 2 intermediate; 3 strong. F) 0 absent; 1 slight; 2 intermediate; 3 intense. G) 1 fused; 2 slight; 3 intermediate; 4 wide or equidistant. H) 1 oblong; 2 elliptical; 3 ovate; 4 irregular. I) 1 flattened; 2 intermediate; 3 rounded. J) 1 white; 2 violet; 3 purple.

Table 3. Quantitative descriptors of seeds and fruits from five cocoa (*Theobroma cacao* L.) varieties.

Variety	Location	n	PF[A]	LF[B]	AF[C]	GF[D]	NSTM	NH	MHS[E]	PSS[F]	DS[G]	LS[H]	GS[I]
Amelonado	Nueva Esperanza Cárdenas	3	428	135.33	83.33	11.40	48	6	2	1.3	13	22.7	4.5
Type Calabacillo	Poblado C-11, Cárdenas	3	554	136	81.33	9.93	40	5	3	1.2	12	20.07	6.5
Landrace Var. Carmelo	Ria. Río Seco. Cunduacán	3	572	169.67	85.33	12.97	44	5	3.54	1.1	12.5	20.47	6.2
Type Landrace	Miahuatlán, Cunduacán	3	650	186.33	86.67	14.83	45	5	3.20	1.6	11.5	19.97	7.2
Rabo Lagarto	Miahuatlán, Cunduacán	3	406	162	71.67	8.97	35	5	3.0	1.2	12.2	20.73	7.3

Where: n, number of fruits; PF, fruit weight; LF, fruit length; AF, fruit width; GF, fruit thickness; NSTM, total number of seeds per pod; NH, number of rows; MHS, fresh seed mass; PSS, dry seed weight; DS, seed diameter; LS, seed length; GS, seed thickness. A) fruit weight in grams (g); B) fruit length in millimeters (mm); C) fruit width (g); D) fruit thickness (mm); E) fresh seed mass (g); F) dry seed weight (g); G) seed diameter (mm); H) seed length (mm); I) seed thickness (mm).

focused on values obtained from fruits and seeds. These data enabled differentiation among cocoa types based on fruit length and width (Restrepo-Quiroz *et al.*, 2018).

Table 5 shows that the variance associated with each principal component differs and decreases in order. The Kaiser criterion (eigenvalue ≥1) was used to select significant eigenvalues, as it is appropriate for the model employed. The first component accounted for 38.41% of the total variance. The distribution of coefficients in the first vector and correlation values (≥0.63) indicated that fruit length (FL) contributed most positively to this component. In contrast, the variables fruit apex shape (FAS), fruit shape (FS), number of rows (NR), seed length (SL), seed diameter (SD), and seed longitudinal shape (SLS) contributed negatively. The second principal component explained 28.74% of the variance, with fruit width (FWi) showing a negative correlation (≥0.63) and being the main contributing variable.

Table 4. Characteristic vectors of 20 morphological descriptors measured in cocoa (*Theobroma cacao* L.).

Descriptor	PC_1	PC_2	PC_3
CFM.	0.009	−0.057	−0.073
FAF.	−0.037	−0.123	0.027
CBF.	0.017	−0.016	−0.110
FF.	−0.045	0.0138	0.065
RF.	0.035	0.070	−0.214
IALF.	0.210	−0.030	0.036
SPL.	0.460	0.098	−0.104
LF.	0.989*	0.064	−0.014
AF.	0.064	−0.710*	0.051
GF.	0.072	−0.0217	0.031
NSTM.	0.120	−0.0607	−0.054
NH.	−0.011	−0.027	−0.109
MHS	0.290	0.021	0.113
PSS	0.105	0.012	0.012
LS.	−0.028	−0.041	−0.036
DS.	−0.022	−0.004	0.026
GS.	0.033	0.088	0.065
FLS.	−0.007	0.060	−0.051
FSTS.	0.029	−0.037	0.028
CPC.	0.030	0.088	0.038
$\sum^2$=Eigenvalue	2.865	1.6422	1.257

Source: own elaboration

The third principal component accounted for 14.73% of the variance; however, some variables in this component did not meet the Kaiser criterion. The total accumulated variance was 81.96%. The eigenvalues of each component revealed two highly explanatory variables, primarily found in the first and second principal components, which presented the highest coefficients. Table 6 summarizes the variance values for the three principal components.

In this context, several studies have demonstrated the impact of morphoagronomic characterization on cocoa seeds. For example, Montaleza-Armijos *et al.* (2020) evaluated

Table 5. Variance values corresponding to the three principal components obtained.

Principal Component (PC)	Explained Variance (%)	Cumulative Variance (%)
1	38.49	38.49
2	28.74	67.23
3	14.73	81.96

Source: own elaboration.

Table 6. Quantification of polyphenols in five varieties of cocoa beans.

Cacao seeds sample	Phenols[1]	Flavonoides[2]	Procianidinas[2]
Amelonado	6.55±0.10	28.90±0.10	3.37±0.64
Calabacillo	8.66±0.05	31.23±0.09	3.72±0.66
Criollo	6.27±0.10	29.40±0.07	2.68±0.31
Clon Carmelo	7.08±0.08	16.52±0.005	1.62±0.59
Rabo Lagarto	8.95±0.009	38.62±0.05	3.96±0.36

[1] Milligrams of gallic acid equivalents per gram of dry sample.
[2] Milligrams of quercetin equivalents per gram of dry sample.
Source: Authors' own elaboration.

37 accessions of nacional cocoa in southern Ecuador using 22 morphoagronomic descriptors derived from leaves, flowers, fruits, and seeds. They identified eight principal components explaining 77.62% of the total variance and found that components 3 and 4 were correlated with the descriptors corresponding to fruit length (FL) and fruit width (FWi), which are consistent with the findings of the present study. Similarly, Ballesteros-Possu (2011) characterized 102 cocoa genotypes and explained the observed variation using five principal components. The descriptors contributing to these components included fruit length (FL), fruit width (FWi), shell thickness (STh), fruit width-to-length ratio (FWLR), and seed weight (SW), yielding a cumulative variance of 70.17%. The first principal component was found to contain the variables with the highest positive (FL) and negative (FWi) loadings, results that align with those of the current research. Additionally, López-Hernández *et al.* (2021) performed morphoagronomic characterization of ten cocoa accessions and identified nine key descriptors explaining the variability within their collections: fruit weight (FW), fruit length and diameter (FL, SD), seed index using dry seed weight (SID), seed weight with mucilage (SWWM), seed length and diameter (SLSD), ratio of seed weight without mucilage to seed weight with mucilage (SWWM/SWM), shell weight (ShW), percentage of seed with mucilage (SWM), and seed index (SI). This analysis shares only one descriptor with those found in the present study.

Chemical analysis: polyphenol characterization in cocoa seeds and paste

The polyphenolic content of cocoa seeds and paste was evaluated in this study. For the quantification of polyphenols, calibration curves were prepared for total polyphenols and flavonoids. Standard solutions were prepared using gallic acid and quercetin for the quantification of total phenols, flavonoids, and procyanidins, respectively. Each concentration corresponded to an absorbance value according to Heimler *et al.* (2005) for total phenols, Ghafar *et al.* (2017) for flavonoids, and Oña and Novillo (2017) for procyanidins. The quantification of polyphenol content was based on the linearity of the method; for this purpose, standard solutions of gallic acid and quercetin were prepared, and calibration curves were generated at concentrations ranging from 10 to 1000 μL for total phenols, flavonoids, and procyanidins.

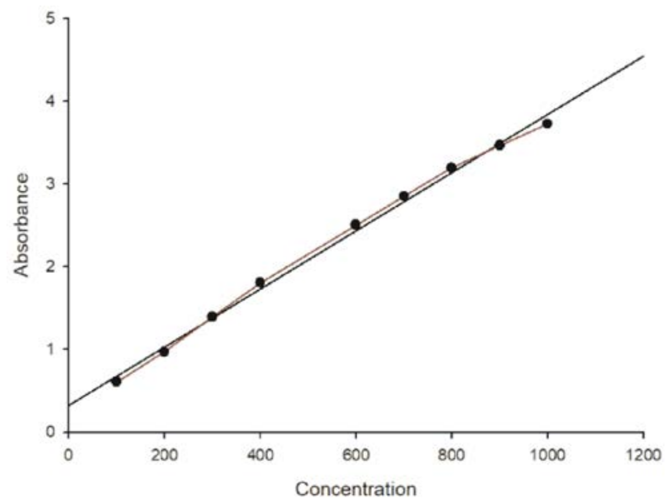

Figure 2. Gallic acid curve.

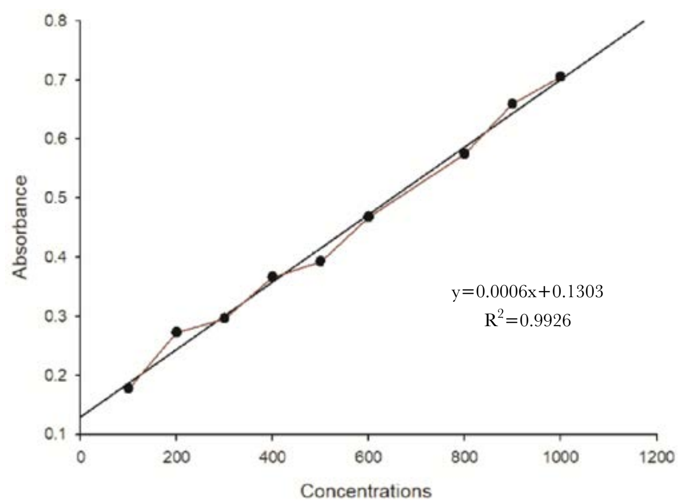

Figure 3. Quercetin curves.

In a study conducted by Urbanska and Kowalska (2019), it was reported that the polyphenolic content present in cocoa beans depends on the regions where the crop is grown, and that the concentration of these compounds is primarily influenced by temperature. Polyphenols are the most abundant phytochemicals in plants. They are secondary metabolites that play a protective role in plants and influence flavor due to their astringent properties (Wollgast & Anklam, 2000). The presence of polyphenols in cocoa has been documented in numerous studies. Urbanska and Kowalska (2019) noted that although polyphenols naturally occur in cocoa beans, their concentrations are influenced by external factors such as geographic region and climate specifically temperatura as a determinant of plant response and compound accumulation in the beans. Additionally, Benítez-Correa *et al.* (2023) highlighted the importance of the solvent type on the kinetics of polyphenol extraction from cocoa seeds, a factor that must be considered in such analyses. Polyphenols in cocoa are mainly stored in cotyledon cells, whose coloration ranges from white to deep purple (Wollgast & Anklam, 2000). In the present study, the flavonoid content in cocoa seeds of the 'Rabo Lagarto' variety was 38.62±0.05 mg EQ/g, compared to 16.52±0.005 mg EQ/g for the 'Clon Carmelo' variety, as shown in Figure 4. For total phenols, the values were 8.95±0.009 and 6.27±0.10, with the highest concentration observed in 'Rabo Lagarto' and the lowest in the 'Amelonado' variety (Figure 5). In the case of procyanidins, the quantified ranges were 3.96±0.36 and 1.62±0.59, corresponding to the 'Rabo Lagarto' and 'Clon Carmelo' varieties, respectively (Figure 6). Table 6 presents a summary of the analyses performed. The variation in compound concentrations is attributed to environmental

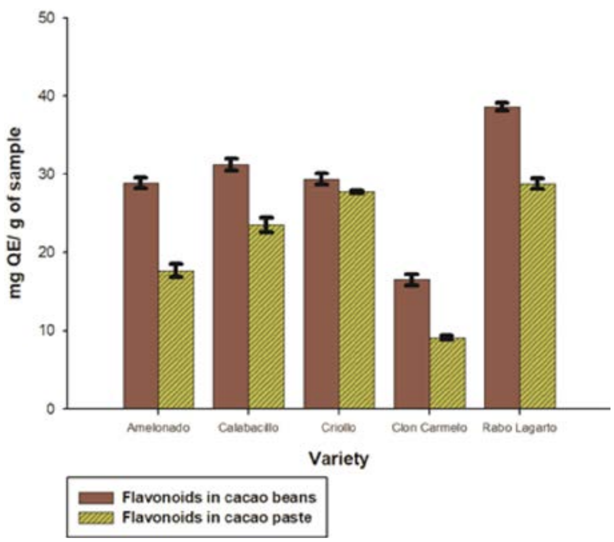

Figure 4. Average total flavonoid content by variety in cocoa seed and paste.

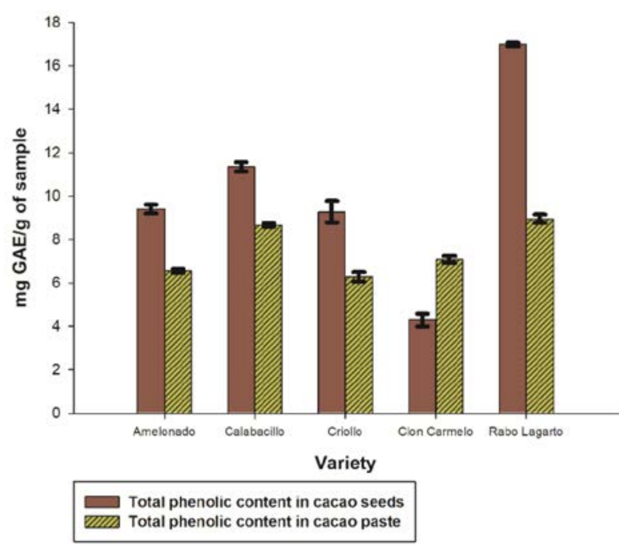

Figure 5. Average content of total phenols by variety in cocoa seed and paste.

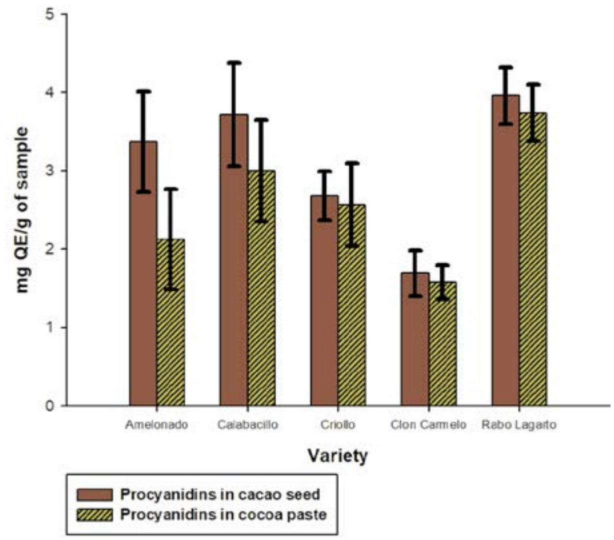

Figure 6. Average total flavonoid content by variety in cocoa seed and paste.

and physicochemical factors, as well as the type of solvent used (Benítez-Correa *et al.*, 2023; Niemenak *et al.*, 2006).

As is well known, epicatechins are a type of polyphenol with antioxidant properties. In this regard, Serafini *et al.* (2003) reported that these compounds can promote cardiovascular health. Similarly, Oracz and Zyzelewicz (2020) noted that cocoa-derived compounds are not only important in food applications but are also of significant interest to the cosmetic and pharmaceutical industries. Multiple studies have documented the positive effects of cocoa consumption on human health. The results of the present study confirm that flavonoids are the most abundant polyphenols in cocoa beans, specifically those of the procyanidin type, which consist of oligomers and polymers of (+)-catechin and (−)-epicatechin (Gu *et al.*, 2006). The polyphenols epicatechin and catechin present in cocoa beans undergo fermentation processes, during which they are oxidized into quinone structures. These oxidation products promote protein condensation, which affects the polyphenol profile and reduces the astringency and bitterness of the beans (Niemenak *et al.*, 2006). In this study, Figure 7 shows the degree of association between variables represented by vectors derived from the phytochemical characterization (polyphenol quantification) of cocoa seeds and paste, grouped into total phenols (TP), flavonoids (FL), and procyanidins (Pr). The distance between components, indicated by red lines, reflects differences among analytical groups, corresponding to the quantity of polyphenolic compounds present in each studied variety highlighting their relative importance. The biplot graphically illustrates the analysis of the five cocoa seed and paste varieties ('Amelonado', 'Calabacillo' type, 'Clon Carmelo', 'Landrace' type, and 'Rabo Lagarto'), each showing distinct associative behavior. The axes represent the principal components (PC1, PC2, and PC3), which capture the data variability in relation to polyphenolic content (see Figure 7).

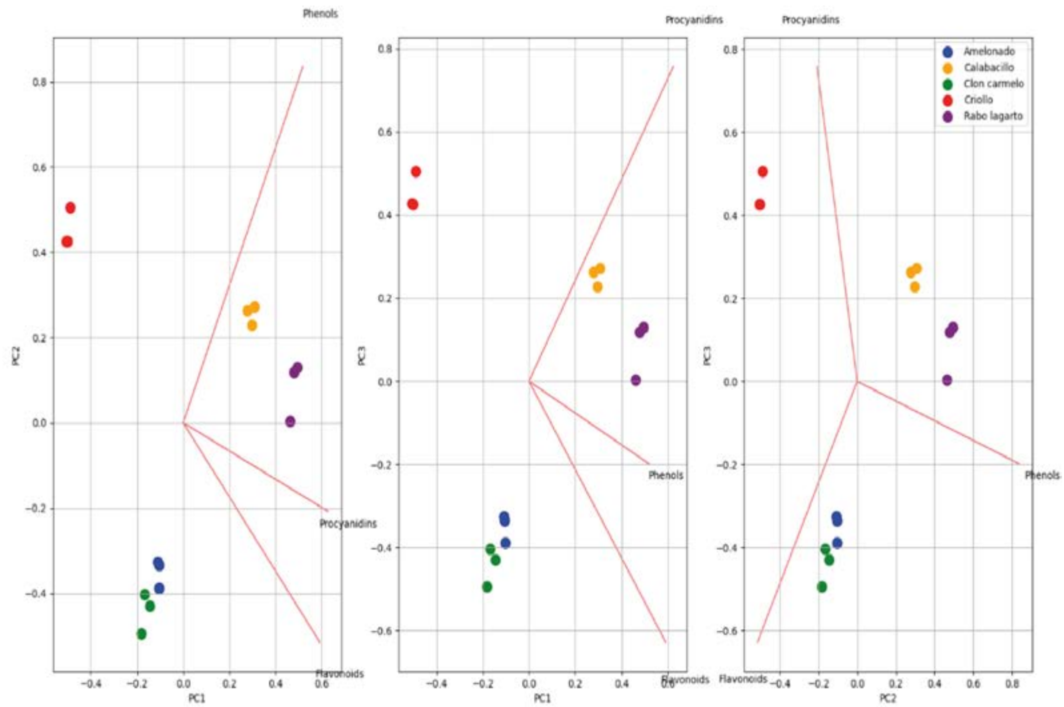

Figure 7. Distribution of accession variables on the first, second, and third principal components in the phytochemical characterization of cocoa seeds and cocoa paste.

CONCLUSIONS

The morphological and phytochemical analyses conducted on five cocoa varieties allowed for clear differentiation of both fruits and seeds based on qualitative and quantitative descriptors. Variables such as fruit length (FL) and fruit width (FWi) exhibited the greatest explanatory weight in the principal components, indicating that these parameters are key in characterizing the evaluated materials. The cumulative variance (81.96%) demonstrates that these variables effectively distinguish the diversity present among the accessions. Regarding the phytochemical analysis, significant variability was observed in the content of polyphenolic compounds (total phenols, flavonoids, and procyanidins). The phytochemical analysis enabled statistical differentiation ($p<0.05$) between cocoa seeds and paste. The 'Rabo Lagarto' variety exhibited the highest concentrations of these metabolites, positioning it as a material with high potential due to its antioxidant properties, whereas 'Clon Carmelo' showed the lowest values. Meanwhile, the 'Amelonado' and 'Calabacillo' varieties displayed similar profiles, while 'Landrace' and 'Clon Carmelo' shared some morphological characteristics but differed notably in their chemical profiles. These findings may inform preferences among both producers and consumers when health-promoting properties are of interest. The results suggest that both morphological traits and phytochemical profiles are complementary tools for differentiating and classifying cocoa germplasm. Furthermore, the variability observed is primarily associated with genetic factors, as 'Rabo Lagarto' corresponds to a Forastero-type cocoa, while 'Clon Carmelo' represents an improved Landrace variety. These findings not only contribute to the advancement of morphoagronomic characterization of cocoa in the region, but also highlight the importance of conserving and valuing genetic diversity for its impact on bean quality and potential applications.

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Colegio de Postgraduados

Financial evaluation of small-scale pig farming in central Hidalgo

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ABSTRACT

Objective: To evaluate the financial performance of small-scale pig farming in central Hidalgo.

Design/methodology/approach: Economic data from 32 pig farms were analyzed through the Cost-Benefit Analysis (CBA) methodology. This method includes a set of criteria to evaluate economically and financially an agricultural production system; among the most relevant there are Net Present Value (NPV), Internal Rate of Return (IRR), and Benefit-Cost Ratio (B/C R).

Results: The project is profitable in a horizon of 5 years, since the NPV was higher than 0 ($54,127.03), even when the initial capital investment is low ($141,008), the project financial yield (IRR=25.84%) indicates that pig fattening evaluated as an investment option exceeds 2.3 times the potential yield that can be obtained with the annual CETES rate (11.25%).

Limitations on study/implications: The size of the sample used suggests a challenge to generate the results, given the heterogeneity of the economic variables in the small-scale pig production systems.

Findings/conclusions: The financial yield evaluated through the IRR shows the highest return provided by pig fattening in comparison to the traditional investment instruments. In conclusion, this study supports the notion that small-scale pig farming constitutes a profitable and sustainable agribusiness, with the potential of significantly contributing to the economic progress of Mexican rural areas.

Keywords: Family livestock production, cost-benefit analysis, net present value, pig farming.

INTRODUCTION

Pig meat is the one with highest consumption globally and it is expected for its demand to continue increasing to 129 million tons in 2029 (OCDE/FAO, 2022; Bartlett *et al.*, 2023). In Mexico it represents 30% of the total meat consumption and given its versatility to

prepare dishes and the existence of a wide network of sale points, it has been positioned as one of the favorite meats to satisfy the growing demand of the Mexican middle class (COMECARNE, 2023).

In this scenario, small-scale pig farming represents a relevant activity since it contributes significantly with food security and income generation in rural communities of the entire world (Das *et al.*, 2021). In Mexico, it plays a crucial role in rural economy through the generation of sources of employment (Martínez-Castañeda and Perea-Peña, 2012) and through its contribution (22%) to national production (Amo, 2018).

However, despite the economic and social importance that small-scale pig farmers have in Mexico, they face considerable challenges in terms of access to credit, technical training and scarcity of economic resources for investment in new technologies (SENASICA, 2021). With these conditions, it is important to develop scientific research that analyzes both the financial and the structural aspects, given their critical impact on the growth and sustainability of small-scale pig farming in Mexico.

While some studies in Mexico have addressed the analysis of profitability and production costs in pig production systems (Benítez-Meza *et al.*, 2015; Venegas *et al.*, 2017), there is a clear research opportunity in the agricultural economics literature to conduct financial analyses of these production systems, both at the national level and in the study region. This deficiency limits a comprehensive understanding of the true economic potential and sustainability of small-scale pig farming under local production conditions.

While many small-scale pig farmers may lack familiarity with formal economic tools, such analyses can provide valuable insights for improving operational efficiency and competitiveness, it is certain that when it is implemented they can get useful information to execute their operations in a more efficient, profitable way and can be more competitive in the demanding environment of the present market (Tsiouni *et al.*, 2023). Based on the foregoing, the objective of this study was to evaluate the financial viability of small-scale pig fattening systems in central Hidalgo, Mexico, using Cost-Benefit Analysis to assess Net Present Value (NPV), Internal Rate of Return (IRR), and Benefit-Cost Ratio (B/C R).

MATERIALS AND METHODS

Study area

The study was conducted in 32 small-scale pig farms located in the municipality of Ixmiquilpan Hidalgo, Mexico, between coordinates 20° 29’ 03” of latitude North and 99° 13’ 08” of longitude West; the municipality has an altitude of 1,680 masl, semi-warm climate with rainfall of 300 to 500 mm and rains from June to September (INAFED, 2023).

Sampling

A non-probabilistic sampling design was used to obtain the information, following the guidelines proposed by Otzen and Manterola (2017). This approach eased the selection of the 32 production units that cooperated, which adequately represented the diversity of the pig production sector in the region of interest. To select the farms, criteria of inclusion were established, considering aspects such as the size of the production unit, the technological level, and the geographic location; meanwhile, the criterion of exclusion used was the lack of interest of producers to participate in the study. This approach guaranteed the representativeness of the sample obtained.

Although selection bias is an inherent limitation of non-probability sampling, this approach was adopted due to the lack of a clearly defined target population, as there was no formal producer association in the region. The selection process was based on prior field observations and a pilot survey of 20 production units, which verified key characteristics such as farm size and technology. While lack of interest was used as an exclusion criterion, the survey results demonstrated that the selected farms were not biased toward more successful producers, as they exhibited homogeneous characteristics in terms of size and technology. This ensured the representativeness of the sample within the regional context. The sample size (n=32) was defined considering the specific operational context of the study, particularly the limited accessibility to dispersed rural areas and the availability of producers to participate. Although a formal statistical power analysis was not conducted, previous research in the agricultural sector has employed similar or even smaller sample sizes (Albarrán-Portillo *et al.*, 2015; García-Martínez *et al.*, 2017; Hernández *et al.*, 2025), demonstrating their adequacy for obtaining valid results in descriptive, social, and economic studies. Given the descriptive and exploratory nature of this study, the sample size was deemed adequate for the objectives set. However, it is acknowledged that future research could expand the sample size to enhance the robustness and generalizability of the findings.

Obtaining the information

The data analyzed were obtained using the procedure proposed by Herrera *et al.* (2019), which involves collecting information through face to face surveys with producers. The survey included open ended, closed ended, and categorical questions, addressing technical, economic, and social aspects of the production process. Following the initial survey, the data were processed in an Excel spreadsheet, and the results were validated through a second visit to the producers. This feedback process allowed the questions to be refined and ensured that they accurately reflected the realities of the production system under study.

Economic and financial analysis

The methodology used to evaluate the financial behavior of pig fattening was the CBA. This technique is easy to use and explain, and it adjusts to new data and to possible extrapolation of results to other agricultural systems (Márquez and Castro, 2015). CBA includes a set of criteria to economically and financially evaluate a production system; among the most relevant there are the NPV, IRR and B/C R.

The NPV is an indicator that represents the sum of the net effects on the economic life of a project, reduced or discounted at present time (Cetkovic *et al.*, 2022). The equation to calculate it is the following:

$$NPV = -Io + \left(\frac{FNE_1}{(1+TREMA)^1}\right) + \left(\frac{FNE_2}{1+TREMA^2}\right) + \ldots + \left(\frac{FNE_n + VS}{(1+TREMA)^n}\right)$$

where: $-Io$ is the initial capital investment; *FNE* is the net flow in cash in the year *n*; *TREMA* is the discount rate, and *VS* is the salvage value of fixed assets. If the *NPV* is 0 or positive, it indicates that a project must be accepted, given that the financial return generated pays for the opportunity cost of the capital invested.

The IFF is the updating rate that seeks to equalize the NPV of a project to 0. This rate reflects the efficiency of the project and the eligibility criterion, and under this indicator the IRR should be higher than the discount rate (Kim *et al.*, 2021). The B/C R shows how much net benefit can be achieved by unit of cost and it is calculated as the updated value of the benefit divided by the updated cost value (Márquez and Castro, 2015).

The financial analysis for this study was built according to the following assumptions: 1) the evaluation was carried out in Mexican pesos, 2) the financial analysis used real prices (market prices in the purchase of inputs and the product), 3) the initial year of analysis was 2023, 4) the planning period of the project was five years, 5) the production cost and sale price per pig for the five years of project planning were adjusted with the foreseen inflation data for the 2023-2027 period extracted from Statista (2023), 6) The discount rate (TREMA) used to update future cash flows was 13.65%. This rate was derived by adding a 10% risk premium, , to the average inflation rate observed over the 5-year project planning period (2023-2027) (Statista, 2023), in accordance with the methodology proposed by Dorantes-Coronado *et al.* (2025), and 7) the benefits derived from the project were analyzed in relation with the poverty lines defined by the National Council for Evaluation of the Social Development Policy (Consejo Nacional de Evaluación de la Política de Desarrollo Social, CONEVAL), as a measure to evaluate its financial capacity in terms of family upkeep.

RESULTS AND DISCUSSION

The results from the study show that in a horizon of 5 years, the project is profitable (Table 1), and this is because the NPV was higher than 0 ($54,127.03), which indicates that the benefits exceed the costs of investment, positioning the small-scale pig fattening project as a financially attractive option (Santiago-Santiago *et al.*, 2020). The Benefit-Cost Ratio (B/C R=1.31) confirms the profitability of the evaluated system, indicating a net return of 0.31 monetary units for every unit invested. This value is higher than that reported by Martínez-Castañeda *et al.* (2024) for pig fattening systems in central Mexico (B/C R=0.98), suggesting greater economic efficiency under the conditions assessed in this study. This behavior highlights that the project is economically sustainable and offers a solid financial yield during its useful life (Bonazzi *et al.*, 2021; Ivanović *et al.*, 2015), since the initial investment is recovered and benefits are obtained in each year of the project (Ivanović *et al.*, 2015).

On the other hand, the IRR value of 25.84% exceeds a TREMA of 13.65%, indicating a robust margin for the investment made by the evaluated farms. Previous studies on pig finishing systems in Mexico and Brazil reported lower IRRs of 11.06 and 10.08%, respectively (Martínez-Castañeda *et al.*, 2024; Krüger *et al.*, 2024). These findings position the evaluated model as a more economically attractive alternative within the context of pig production in the study region.

However, it is important to highlight that under the conditions observed on the farms analyzed, although producers have between 2 and 5 hectares available for food cultivation and an average of four family members (spouse, children, and elderly adults) providing labor, expanding production capacity presents significant challenges. These include technological limitations, lack of technical training, inadequate infrastructure, limited access to credit (SENASICA, 2021), and difficulties in product marketing. Despite these limitations, the farms evaluated represent a source of monetary income and constitute an effective model for leveraging human capital and generating economic value in the family business.

Considering the initial capital investment ($141,008), the results from the study show that the financial yield (IRR=25.84%) in the pig farm evaluated as an option of investment exceeds 2.3 times the potential yield that can be obtained with the annual CETES rate (11.25%; CONDUSEF, 2023), which indicates that small-scale pig farming is a good option since it can be a profitable business that can be promoted and undertaken (Obayelu *et al.*, 2017) with low investment.

It has been proven that small-scale pig farming in Mexico is an efficient business option that allows peasant families to have an alternative to complement their income (Martínez-Castañeda and Perea-Peña, 2012). This result can be confirmed with the values obtained in the project's annual net cash flows, which has the capacity to cover the annual food basket expenses of approximately 1.10 people, according to the rural poverty line defined by CONEVAL (2023). This organization established a monthly cost of $3,102.31 per person for that year, which when multiplied by 12 months is equivalent to an annual expense of $37,227.72. By dividing the project's average annual net cash flow ($41,127.72) by this value, it is estimated that the income generated can cover the basic food needs of 1.10 people per year. These results support the economic viability of the project and suggest that if small-scale pig production is reproduced in the Mexican rural communities, this business could be an efficient instrument to help eradicate poverty among peasant families; thus contributing to the fulfillment of two of the 17 sustainable development goals of the 2030 United Nations agenda (FAO, 2019).

Although the research results allow for an analysis of the financial performance of the studied farms, factors such as feed costs and pork prices are subject to variability, influenced by fluctuating input prices and the dynamics of the local market and regional demand. A sensitivity analysis of these factors would allow for an assessment of their impact on profitability, particularly in volatile economic contexts. However, despite this being a limitation in the present study, the cost and revenue projections are based on the macroeconomic trends expected for Mexico during the period 2023-2027. Therefore, the projected results, although dependent on external factors, are in line with the trends observed in recent years and can be considered realistic within the Mexican market.

Table 1. Determination of the net cash flow and financial indicators in small-scale pig farming in Ixmiquilpan, Hidalgo.

Concept	**Years of project planning**					
	2022†	**2023**	**2024**	**2025**	**2026**	**2027**
Production of heads of pigs		45.00	45.00	45.00	45.00	45.00
Average weight per pig at sale (kg)		110.00	110.00	110.00	110.00	110.00
Sale price per pig ($/kg)		41.07	42.67	44.07	45.43	46.81
Income from pig sale ($/kg)		203,296.50	211,204.73	218,132.25	224,872.54	231,686.17
Total income per year ($)		203,296.50	211,204.73	218,132.25	224,872.54	231,686.17
Average cost per pig ($/kg)		3,776.85	3,923.77	4,052.47	4,177.69	4,304.27
Total costs per year ($)		169,958.16	176,569.54	182,361.02	187,995.97	193,692.25
Depreciation of fixed assets ($)		6,737.32	6,737.32	6,737.32	6,737.32	6,737.32
Amortization of differed assets ($)		1,052.07	1,052.07	1,052.07	1,052.07	1,052.07
Gross return ($)		33,338.34	34,635.20	35,771.23	36,876.56	37,993.92
Financial costs ($)		0.00	0.00	0.00	0.00	0.00
Return before taxes ($)		33,338.34	34,635.20	35,771.23	36,876.56	37,993.92
Taxes (0%)		0.00	0.00	0.00	0.00	0.00
Net return ($)		33,338.34	34,635.20	35,771.23	36,876.56	37,993.92
¶Depreciation and amortizations ($)		7,789.38	7,789.38	7,789.38	7,789.38	7,789.38
Net cash flow ($)		41,127.72	42,424.58	43,560.61	44,665.95	45,783.30
Fixed asset investment	96,008					
Working capital	45,000					
Initial investment	141,008					
Rescue value (residuals)						86,282.59
TREMA						13.65%
NPV						54,127.03
IRR						25.84%
B/C R						1.31

† Year zero or base.
¶ The method used in this study to calculate fixed asset depreciation was the straight-line method, as it is one of the methods recognized in Mexico for tax purposes.

CONCLUSIONS

The results of the financial indicators such as NVP and the B/C R confirm the viability of the project by showing that the benefits exceed the costs in each year of planning. The financial yield of the project (IRR=25.84%) indicates that pig fattening evaluated as an investment option exceeds 2.3 times the potential yield that can be obtained with the annual CETES rate (11.25%). These results support the perspective that pig production constitutes a promising alternative for small-scale producers, offering an additional source of income as a means to strengthen food security in rural communities.

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Colegio de Postgraduados

Economic Effects of Drought in Irrigation District 023 San Juan del Río, Querétaro

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ABSTRACT

Objective: To analyze the impact of various levels of drought and increase in groundwater use on agricultural income in Irrigation District 023 of San Juan del Río, Queretaro, and to determine water productivity as drought intensifies.

Design/methodology/approach: Linear programming was used; Four scenarios were built: a typical year with surface and groundwater, two with moderate drought levels and one, only with groundwater for irrigation. Additionally, four other scenarios with the same characteristics were considered, incorporating the cultivation of 60% of alfalfa to serve the dairy sector due to its importance

Results: Reductions in agricultural income range from 8.6% to 19.1% in the first set of models and from 9.7% to 23.1% when alfalfa planting is enforced. The most water-demanding crops cause a significant reduction in agricultural income. Shadow water prices range from \$800 per thousand m^3 to \$5,819 per thousand m^3 for the W-I cycle as drought intensifies.

Limitations on study/implications: The research included the most representative crops in the district and limited the area of crops with high profitability due to market aspects.

Findings/conclusions: It is concluded that droughts significantly affect agricultural income and that the shadow price of water increases significantly as the drought increases, so groundwater should be allocated to the most productive and less water-demanding agricultural activities.

Keywords: Water scarcity, shadow price, water productivity

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INTRODUCTION

Productive activities in agricultural production units are diverse. Various crops can be grown using a range of technologies and production techniques. The seasonality of agriculture leads to certain resources having a well-defined temporary use. These units have a specific amount of resources available to implement their production plans, such as land, labor, and, in particular, water.

In the literature, irrigation water has been recognized as the input that most significantly influences crop yields (FAO, 2004). Climate change has made rainfall patterns more erratic, and droughts are becoming increasingly frequent. This, combined with the development of well-drilling and pumping technologies, has led to the excessive use of groundwater worldwide, a phenomenon referred to as "the silent revolution of intensive groundwater use" (Llamas M. & Martínez-Santos, 2006).

It is important to make the best use of water resources. A useful tool in this task is linear programming (LP), which aids decision-making in agricultural production, incorporating theoretical advances that allow for solving systems of equations with inequality constraints based on the application of the Kuhn-Tucker conditions, duality theory, and, as a result, the demonstration of the symmetry between primal and dual problems. This approach highlights the usefulness of shadow prices for the resources used in the primal problem, which are derived through the dual problem without formulating and solving the dual problem itself, marking a significant advance (Hazell and Norton, 1986).

Linear programming (LP) is used to solve a wide range of optimization problems (Kaiser and Messer, 2011). Estimating water productivity is important to understand the contribution of non-priced productive inputs to total output. Young and Loomis (2014) note that LP models are an appropriate tool for assessing the productivity of inputs such as water. This method has been used to evaluate water productivity in an irrigation district in Mexico (Florencio *et al.*, 2002; Botello *et al.*, 2022). The Technical Committee on Groundwater (COTAS, 2024), in relation to the San Juan del Río Valley aquifer, reports a deficit in groundwater availability of nearly 57 million m^3.

The objective of the research was to analyze the impact of various levels of drought and groundwater use on agricultural income in ID 023 in San Juan del Río, Querétaro, and to determine water productivity. The hypothesis is that during drought conditions, when surface water is unavailable and only the concessioned groundwater is used, the crops that require the most water will experience a reduction in the area cultivated, leading to significant decreases in agricultural income.

MATERIALS AND METHODS

This research was conducted in the San Juan del Río Valley, Querétaro, with coordinates: Latitude 20° 29'ꝛ12.2" and Longitude 99° 44' 49.7", specifically in ID 023 San Juan del Río. The main user of groundwater in the San Juan del Río aquifer is the agricultural sector, which includes the central region of ID 023 San Juan del Río, as shown in Figure 1 (CONAGUA, 2023). The static water level evolution for the period 2014-2023 recorded declining values across most of the exploited area, ranging from 1 to 10 meters, with some areas showing declines of up to 15 meters, representing annual reductions of 0.1 to 1.7 meters. The greatest declines, ranging from 1.1 to 1.7 meters annually, occur in the northern and northwestern areas of the agricultural zone.

Models were constructed using LP to estimate the effects of a range of droughts, from moderate to severe, on economic income in the region, cropping patterns, and water productivity. An LP model consists of an objective function and a set of constraints. The objective function (1) is made up of the dot product of the decision variables (activities) of the model multiplied by their corresponding net utilities. The set of constraints consists of restrictions expressed in a coefficient matrix, with technical coefficients, and on the right-hand side of the equation (RHS), the fixed available resources (2). In addition to the non-negativity conditions (3) for the activities (decision variables) considered.

Figure 1. Location of the Aquifer.
Source: CONAGUA.

The mathematical representation of the theoretical linear programming model can be expressed as follows (Hazell and Norton, 1986).

Objective Function:

$$\text{Maximize} \qquad Z = \sum_{j=1}^{n} C_j X_j \qquad (1)$$

Subject to:

$$\sum_{j=1}^{n} a_{ij} X_j \leq b_i, \quad \text{for } i=1 \text{ to } m \qquad (2)$$

and

$$X_j \geq 0, \quad \text{for } j=1 \text{ to } n \qquad (3)$$

Where: X_j=It is the level of the *j*-th activity of the producer, the number of hectares to be planted with a specific crop *j*. *Cj*=It is the net profit margin (also called net prices) of the *j*-th activity unit (pesos per hectare). A_{ij}=It is the amount of resource (water, land) required to produce one unit of the *j*-th activity. Denoted by m as the number of resources, so i=1 to *m*. b_i=It is the amount of the *i*-th available resource (*e.g.*, hectares, labor days, etc.).

For the construction of the empirical model, the cropping pattern was broken down by agricultural cycle and by module. The following crops were considered: forage oats, barley, maize, alfalfa, roses, grapevines, and second crops such as maize. In total, there were 21 activities. Net prices were calculated using information on rural average prices (RAV), yields, and production costs provided by the ID.

The labels for the variables considered for Module 1 are: forage oats A-W (FOI1), barley A-W (COI1), maize S-S (MPV1), alfalfa (AP1), grapevines (VP1), roses (RP1), and second-crop maize (MSC1). For Module 2: forage oats A-W (AFOI2), barley S-S (COI2), maize S-S (MPV2), alfalfa (AP2), roses (RP2), grapevines (VP2), and second-crop maize (MSC2). For Module 3: forage oats A-W (AFOI3), barley A-W (COI3), carrots A-W (ZOI3), maize S-S (MPV3), alfalfa (AP3), and second-crop maize (MSC3).

The modeling regarding land use in each module was seasonal. Water constraints were organized by production cycle and by module, resulting in a total of 6 water constraints. Water use per crop and cycle was taken from the irrigation schedule of the ID for the 2021-22 agricultural year.

Eight scenarios were constructed. The first considers the available water for the irrigation schedule of the mentioned agricultural year, including both surface water and groundwater. The first model is based on the 2021-22 agricultural year, considered as typical. The second scenario includes a 15% reduction in available water due to moderate drought. The third considers a greater drought with a 25% reduction in water compared to the typical year. The fourth scenario considers a severe drought with no surface water and a 30% reduction in water.

Given the importance of livestock in the region and its connection to agriculture through forage supply, the other four models consider the cultivation of alfalfa at 60% of the amount sown in 2021-22.

Regarding the number of cattle, it is reported that San Juan del Río and neighboring municipalities account for 38% of the total statewide and 13% of the dairy cows (INEGI, 2023).

RESULTS AND DISCUSSION

The results obtained from the different models constructed are presented below. The analysis begins with the effects of the reduction in water availability in the district on the value of the objective function; then, the effects of this availability on the crop pattern are examined. Next, the magnitude of the shadow price of water is analyzed, assuming it is the most scarce input. Finally, results are presented when the model is restricted to consider the planting of alfalfa at 60% of the planned amount for the base year due to the presence of dairy farming in the region.

Table 1 shows the effects of water scarcity on agricultural income in ID 023. The estimation of the effects of a 15% reduction in water availability in the district indicates that it would lead to an 8.6% reduction in agricultural income in ID 023. This is undoubtedly a significant reduction, considering the importance of agricultural activities in the region.

A 25% reduction in water availability would lead to an almost 15% decrease in the income of the population dependent on agriculture in ID 023, while a 30% reduction would result in a 19.1% income loss. Florencio-Cruz *et al.* (2002), Botello-Aguillón *et al.* (2022), and Ramírez Barraza *et al.* (2019) found similar effects on the agricultural income of the district they analyzed. However, the reduction was smaller, as the percentage decrease in water availability was also lower.

These estimates only consider the direct effects on income resulting from the reduction in agricultural activity within the irrigation district. They do not account for the impact on businesses supplying agricultural inputs, nor on related services such as credit and insurance. Additionally, the reduction in labor demand and the wages earned from agricultural work are not included.

It should also be noted that there would be an uncalculated effect on livestock activities in the region, as a significant portion of the irrigation district's land is used for forage crops. A reduction in forage crop production could decrease the net profits of the livestock sector due to the potential scarcity of inputs.

In the four models that consider alfalfa cultivation at 60% of the area sown in each module during the baseline agricultural year, income reductions increase as water scarcity intensifies. Ramírez Barraza *et al.* (2029) found that in the optimal cropping pattern, alfalfa did not appear, despite being present in the district's historical patterns. Forcing its inclusion through a minimum constraint reduced the objective function value. Similarly, Rodríguez *et al.* (2019) demonstrated that with decreasing water availability, alfalfa is the first crop to be removed from the optimal pattern.

The comparison between models with the same water availability, but without and with alfalfa, shows a 4.6% income reduction in the first case. When water availability decreases by 15%, the income reduction reaches 5.7%. With a 25% reduction in water, income decreases by 8.3%, and under severe water scarcity, the reduction is 10.8%. These results are similar to those found by Rodríguez *et al.* (2019).

The most severe drought scenario closely resembles the crops planted in the 2023-24 agricultural year, allowing for an estimation of the economic impact of severe drought on the income of ID 023 users.

Table 2 shows the optimal activities in each model constructed under drought conditions. The activities selected in each model meet the economic optimization criterion, which states that a crop will be included until its marginal income equals its marginal cost. The crops in which the resource is more productive are the ones that will be in the optimal pattern and contribute most to the value of the objective function. In this case, maize SS, second-crop maize, and forage oats. In other cases, crops like strawberries have been favored, as reported by Flores Lázaro *et al.*, (2017).

The activities that were not selected in the optimal solution do not meet this optimization criterion; in other words, in these cases, the marginal cost is higher than

Table 1. Income in the Agricultural Year of ID 023 with Water Scarcity.

Model Water reduction	Value (Millon pesos)	Reduction (%)	Value (Millon pesos) with alfalfa	Reduction (%)
Base	292.76	N/A	279.22	
15% Less	267.71	8.6	252.18	9.7
25% Less	250.43	14.5	229.60	17.8
30% Less	240.86	19.1	214.83	23.1

Note: The models with alfalfa contain 60% of this crop from the area planted in the 2021-2022 agricultural year, under all drought conditions. Source: Prepared by the authors with information from the constructed models.

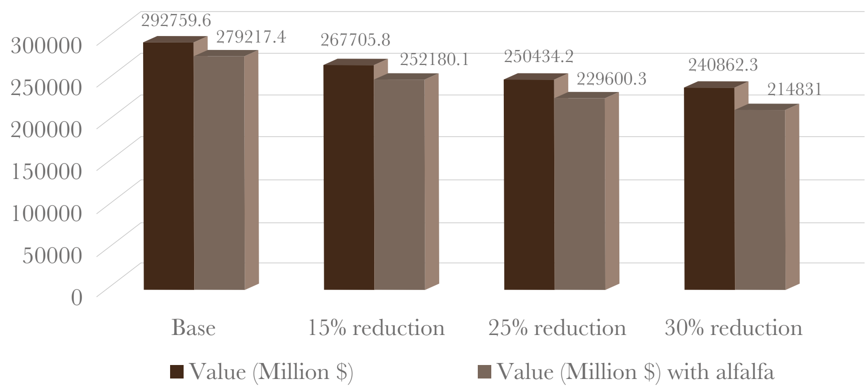

Figure 2. Variations in Agricultural Income of ID 023 in the Agricultural Year Due to Water Scarcity. Source: Prepared by the authors with information from the constructed models.

Table 2. Crops in the optimal solution in the constructed models.

Crop	Base model (ha)	15% Less water (ha)	25% Less water (ha)	30% Less water (ha)
AFOI1	1125	546	160	0
MPV1	2085	1454	1033	849
VP1	17	17	17	17
RP1	6	6	6	6
MSC1	1425	2056	2476	2619
AFOI2	2172	1685	1360	1204
MPV2	1330	656	206	0
RP2	100	100	100	100
VP2	4	4	4	
MSC2	2956	3443	3768	3893
AFOI3	100	0	0	0
ZOI3	50	50	50	50
MPV3	385	314	279	261
MSC3	380	416	354	324

Source: Prepared by the authors with information from the constructed models.

the marginal income. Duality theory states that associated with a primal model whose objective is to maximize a function, provided this function has a solution and is optimal, there is a dual model whose optimization will be minimization. The primal maximizes profits, and the dual minimizes costs (Hazell and Norton, 1986). In this case, the net price of each activity is equivalent to the marginal income, and the concept of "reduced cost" is the marginal cost obtained from the valuation the model imputes for the resources used in its production.

When resources are abundant in the model, there will be surpluses, and they will have a shadow price of zero. In contrast, the scarce resources, the ones that are exhausted, will have a positive value. The crops that use a greater amount of scarce resources in their production will have a higher marginal cost, and consequently, they will not be selected.

For example, in the base model, the activity of barley in the A-W cycle (COI1) has a marginal cost that exceeds the marginal income by \$10,520. In this case, if barley production is insisted upon under the conditions set in the model, the income of the ID will decrease by \$10,520 for each hectare of this crop produced. In the base model, the crop with the highest excess of marginal cost over marginal income is alfalfa AP1 (\$23,280) and AP3 (\$22,570).

When water is scarcer (and thus more expensive), crops that use more of this resource for production will have a higher marginal cost. In the scenario with the greatest water scarcity, the alfalfas in the three modules (AP1, AP2, and AP3) have the highest amounts where the marginal costs exceed the marginal incomes by the following amounts: \$42,038, \$40,195, and \$33,917 per hectare, respectively.

The LP model obtains the marginal productivity of water by extracting the values of the Lagrangian variables from the dual problem solution, and these are known as the shadow prices of water (Hazell and Norton, 1986). This type of model is most suitable for determining the value of water when the resource does not have a market price (Young and Loomis, 2015).

In Table 3, the magnitudes of the marginal productivity of water by cycle are presented. All models record shadow prices with values greater than zero in both production cycles. In the base model, the highest values are recorded in the autumn-winter cycle in the three modules. For module 3, the highest value is recorded at \$3,922 per thousand cubic meters. This means that for each additional thousand cubic meters in module 3 in the autumn-winter cycle, the ID income would increase by \$3,922. This is the interpretation for the rest of the values.

In the models with moderate droughts (15% and 25% less water), the water productivity remains the same as in the base model, except in module 3, autumn-winter cycle, where it increases significantly (\$5,597/mm^3).

When the drought is severe (with a 30% reduction in water and only underground water is used), in all models, the magnitude of the shadow price increases significantly, with values ranging from \$3,467 to \$7,144 per thousand cubic meters. These results align with those reported by Rubiños-Panta *et al.*, (2007). Furthermore, the values are higher when only underground water is used, which is consistent with findings by Florencio-Cruz *et al.*, (2002).

Table 3. Shadow price of water under different drought conditions in the ID023.

Module and cycle	Base model (\$/mm^3)	15% Less (\$/mm^3)	25% Less (\$/mm^3)	30% Less (\$/mm^3)
1 A-W	3448	3448	3448	5819
1 S-S	2054	2054	2054	3467
2 A-W	894	894.4	894	3922
2 S-S	3077	3077	3077	7145
3 A-W	3922	5597	5597	5597
3 S-s	2530	3611	3611	3611

Source: Prepared by the authors with information from the constructed models.

Shadow price values for water have been calculated for various locations at different times, with the aim of making a comparison with those found in this research. These shadow prices per thousand cubic meters were converted into dollars per thousand cubic meters using the exchange rate at the time of each study (BANXICO, 2024). Florencio *et al.* (2002) reported a price of $106.32 per cubic meter for the ID 011. Although they registered lower values for some crops, they also accounted for values higher than those used here, specifically for high-value crops. Martínez *et al.* (2021) found a price of $81 per thousand cubic meters for the ID 100 in Alfajayucan in a 2016 study. Ramírez *et al.* (2019) found a shadow price of $50.63 per thousand cubic meters for the Lagunera region in Mexico. Botello *et al.* (2022) found shadow price values for some irrigation modules of the ID 011 in Celaya, with prices of $195.39 per thousand cubic meters. In all cases, the shadow price is significantly higher than the irrigation fee paid by users.

In the research presented here, as of the exchange rate on April 17, 2024, different shadow price values were found, ranging from the lowest value of $52.62 per thousand cubic meters in the base model for the A-W cycle of module 2, to $420.54 per thousand cubic meters in the extreme drought model for the S-S cycle of module 2.

Given the connection between agriculture in the ID 023 and livestock in the municipality and region, both for beef and dairy cattle, as previously mentioned, versions of comparable models were constructed with the initial ones. The difference is that all of them include 60% of the alfalfa area sown in the year used as a reference.

Figure 3 compares the optimal solution when only groundwater is used for irrigation, with and without sowing 60% of alfalfa. It is observed that the presence of alfalfa causes a significant reduction in the cultivation of forage oats (AW) and maize (SS), as well as in second crops.

In the other models tested with drought conditions, when alfalfa is included, it causes a drastic reduction in the other crops, with a slight gain in the second-crop maize. Similar results were reported by Ramírez Barraza *et al.* (2019).

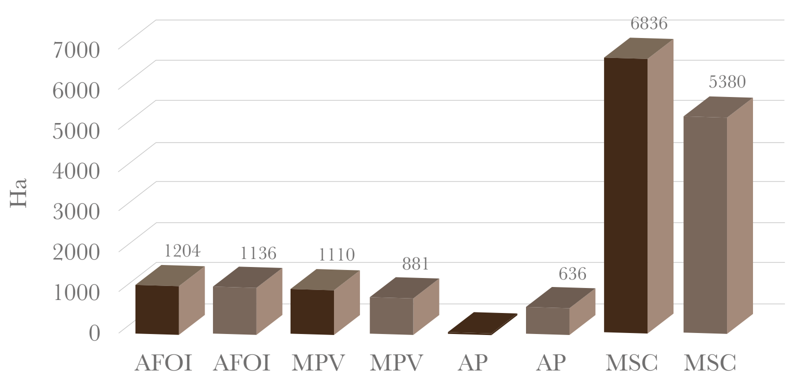

Figure 3. Crops with groundwater irrigation only. Without and with 60% alfalfa.
Source: Prepared by the authors with information from the constructed models.

CONCLUSIONS

It is concluded that the inclusion of high water-consuming crops, such as alfalfa, under drought conditions would only be justified when dairy farming is important in the area. The increase in the shadow prices of water as this resource becomes scarce serves as a signal indicating to which activities the resource should be allocated.

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Colegio de Postgraduados

Guazuma ulmifolia L. biomass: A complementary ingredient in starter piglets diets

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ABSTRACT

Objective: To evaluate the potential of *Guazuma ulmifolia* Lam (*G. ulmifolia* L.) biomass as an alternative ingredient in feed for piglets.

Design/Methodology/Approach: The microbiological characterization of *Guazuma ulmifolia* L. fruit flour was carried out through sequencing of a 16S rRNA gene region. Based on this ingredient, a balanced feed mixture (FMIX) was formulated, consisting of *G. ulmifolia* L. flour, Sorghum, *Glycine max*, and shrimp by-products. The analytical composition of the FMIX was determined, and its nutritional effect was evaluated over a 53-day period in 15 weaned female pigs in the starter phase. The animals were randomly assigned to three experimental groups: G1 (100% commercial diet), G2 (90% commercial diet + 10% FMIX), and G3 (80% commercial diet + 20% FMIX). At 11 weeks of age, complete blood counts were performed to assess potential hematological changes associated with the experimental diets.

Results: Using 16S rRNA gene sequencing, *Bacillus subtilis*, *Cronobacter* spp., and *Enterococcus* spp. were identified in the fruit flour of *Guazuma ulmifolia* L. The bromatological analysis of this flour revealed a crude protein content of 7.12%, crude fiber of 35.40%, and digestible organic matter (DOM) of 47.81%. The analytical composition of the FMIX balanced feed was determined to be 19.98% crude protein, 6.41% crude fiber, and 55.04% DOM. Group G2 showed the highest average weight gain (33.05±8.901 kg) and maintained a hematological profile within physiological ranges, in contrast to groups G1 and G3, which exhibited alterations such as leukocytosis, lymphocytosis, and basophilia. Additionally, thrombocytopenia and neutropenia were observed in group G1. Overall, group G2 demonstrated a slight improvement in weight gain and a more favorable hematological profile, suggesting a potential immunomodulatory and nutritional benefit from the diet supplemented with 10% FMIX.

Limitations on Study/Implications: The climatic conditions (autumn and winter) and the 53-day evaluation period limit the generalization of the results to other seasons and the long term.

Findings/Conclusions: This study showed that the biomass of *G. ulmifolia* L., due to its probiotic microorganism content, is a viable alternative in pig nutrition, as it enhances nutrient absorption, improves gut health, and contributes to better productive performance.

Keywords: *Guazuma ulmifolia* L.; Biomass; Agrifood; Piglets dietary; *Bacillus* spp.; Swine supplements.

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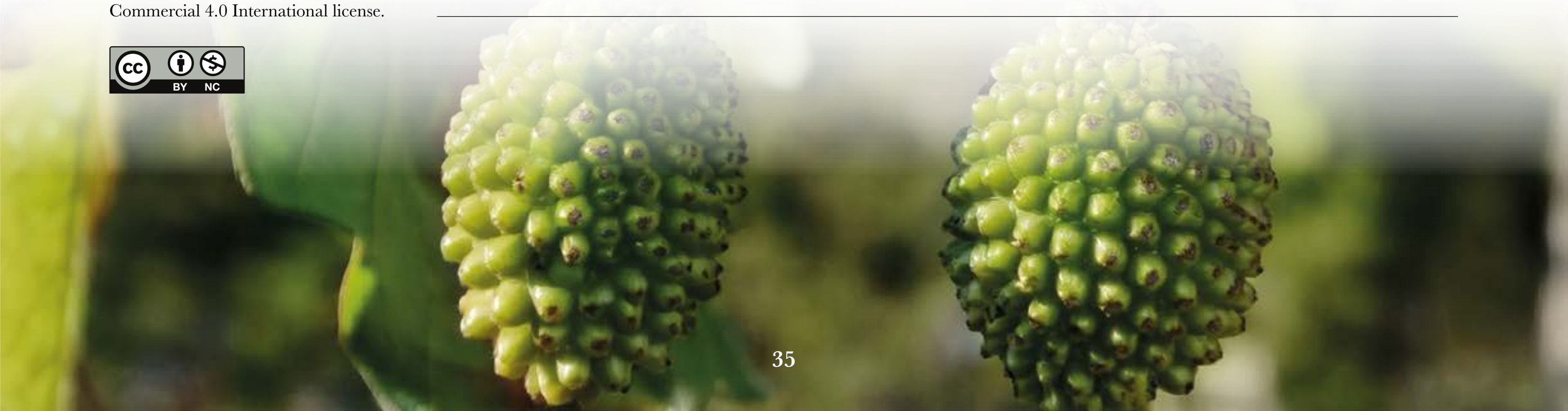

INTRODUCTION

G. ulmifolia Lam, is a tropical tree native to the Americas, including Mexico, and some tropical regions of South America [1-3]. Previous research has explored *G. ulmifolia* L. as a feed supplement for livestock, demonstrating positive effects on intake, digestibility, and nitrogen balance [4]. Studies on sheep have highlighted the nutritional importance of *G. ulmifolia* L. biomass, with higher preference rates and valuable nutritional content observed at different maturation stages [5-6]. Particularly, have demonstrated the productive response of lambs fed with *G. ulmifolia* L. fruits, emphasizing the potential of this tree species as a dietary supplement for small ruminants [6].

G. ulmifolia L. has been recognized for its nutritional value and bioactive properties [7-9], with both its leaves and fruits being considered valuable sources of protein for animal feeds, particularly in the context of swine nutrition [10]. *G. ulmifolia* L. is renowned for its rich concentration of bioactive molecules, particularly within its leaves and fruits [4,11-12]. The leaves are a reservoir of tannins [13], recognized for their antioxidant properties and potential anti-inflammatory benefits, and flavonoids like kaempferol, contributing to antioxidant and anti-inflammatory effects. Also, the essential oil extracted from leaves is predominantly composed of eugenol, a compound recognized for its antiseptic and analgesic properties. Additional key components include spathulenol, β-caryophyllene, sabinene, globulol, γ-terpinene, and α-copaene, each contributing to the oil's overall biological activity [14]. Interestingly, while the fruit lacks steroids [15], these compounds have been detected in the leaves, hinting at potential biological activities. The fruits, sweet and edible, contain mucilage, promoting digestive health, and xanthum gum, a polysaccharide known for its thickening and stabilizing properties, though its impact on gut health when consumed in larger quantities warrants further investigation.

While the inherent nutritional value of *G. ulmifolia* L. is promising, targeted interventions could further enhance its benefits. A research team reported a probiotic strategy using *L. acidophilus* and *Lactococcus lactis* [9], which improved nutrient intake, feed efficiency, gut health, immune response, stress reduction, and the safety of *Coturnix japonica* products for human consumption [10]. Unlike studies using exogenous probiotics, our research employs indigenous strains naturally present in *G. ulmifolia* L. fruit. We evaluated early-stage pigs fed a standard diet versus one enriched with *G. ulmifolia* biomass. Native probiotics such as *Bacillus subtilis*, *Cronobacter* spp, and *Enterococcus* spp., identified via 16S rRNA sequencing, combined with the fruit's nutritional value, may enhance nutrient absorption, gut health, and overall performance through a synergistic and naturally derived formulation.

MATERIALS AND METHODS

Harvesting, cleaning and processing of G. ulmifolia L. biomass

The dried fruit of *G. ulmifolia* L. was harvested in the "Huasteca" zone of the state on the northern region of Veracruz, Mexico (21° 20' 12.7" N 97° 49' 57.2" W). Disinfection of the dried fruit was carried out by washing with neutral soap and a NaClO (Hach, Mexico) solution at a concentration of $10\frac{v}{v}\%$, leaving it to react for 10 minutes to reduce the presence of microorganisms. It was then dried at 60 °C for 36 hours using a conventional oven (Memmert, Germany). Then, the disinfected dried fruit was milled (Insely, USA) until it reached a flour consistency and was stored in sterile plastic containers (Thermo Fischer Scientific, USA) at room temperature for its subsequent formulation.

Microbial isolation in the flour of *G. ulmifolia* L. fruit

From the cleaned fruit sample (WF), serial dilutions (10^{-1} to 10^{-5}) were prepared, and 100 μL of each dilution was spread on nutrient agar plates (Bioxon, Mexico). The plates were incubated at 35 °C for 24 hours (CRM Globe, Mexico). Axenic cultures were characterized by colonial morphology on nutrient agar and by cell morphology using Gram staining (Hycel, Mexico). For cryopreservation, 700 μL of microbial culture was mixed with 300 μL of sterile glycerol (Thermo Fisher Scientific, USA) in nutrient broth and stored at −80 °C in an ultra-freezer (Thermo Fisher Scientific, USA).

Identification and characterization of *G. ulmifolia* L. fruit microorganisms

PCR was carried out using colony PCR technique. A microwave-based rapid lysis protocol was used for DNA extraction. Briefly, each colony was resuspended in 100 μL of TE lysis buffer (Thermo Fisher Scientific, USA.), composed of 10 mM Tris and 1 mM EDTA, and subjected to three cycles of microwave heating (Samsung, South Korea) consisting of 1 min at maximum power of 1.4 kW, followed by a rest for 30 seconds at room temperature and finally 1 min additional at 1.4 kW wattage. The extracted DNA was used directly for PCR. PCR was carried out in a final volume of 25 μL, employing the PCR kit (Invitrogen, U. S. A.). Each reaction contained 2. 5 μL of 10X PCR-$MgCl_2$ buffer, 2 μL of 50 mM $MgCl_2$, 1 μL of 10 mM dNTPs, 1 μL of the direct oligonucleotide P1 (CGGGATC CAGAGTTTGATCCTGGCTCAGAACGAACGCT) (15 pmol/μL), 1 μL of the reverse oligonucleotide P6 (CGGGATCCTACGGCTACGGCTACCTTGTTACGACTTCAC CCC) (15 pmol/μL), 17. 2 μL of DNA extracted directly from the colony, and 0.3 μL of Taq polymerase (5 U/μL) [16]. Amplification conditions in a thermal cycler (Biometra, Germany) proceeded as follows: 94 °C for 5 min (1 cycle); 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min (30 cycles); 72 °C for 10 min (1 cycle). PCR products were analyzed by 1% agarose gel electrophoresis to confirm amplification of the expected fragment. The target band was purified using a DNA gel extraction kit (Zymo Research, USA). Purified samples were analyzed by vertical electrophoresis (Axygen, USA), quantified with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, USA), and sent for sequencing to an external laboratory (LANGEBIO-CINVESTAV, Mexico). Sequences obtained for the identification of microorganisms were analyzed by Basic Local Alignment Search Tool (BLAST), in order to find regions of similarity with sequences from the database of the National Center for Biotechnology Information (NCBI), considering a percentage identity ≥99% [17-18].

Feed formulation and balance with *G. ulmifolia* L. as ingredient

A balanced mixture was formulated using commercial flours of *Glycine max* (soybean) and sorghum, both sourced from local feed stores. Shrimp shells were obtained as waste from shrimp processing in the coastal region of the Tamiahua Lagoon, Veracruz. The shells were dehydrated at room temperature (32.5±2.5 °C) for 72 hours and ground into flour (Insely, China). *G. ulmifolia* L. fruit was processed similarly, as described previously. The nutritional composition of sorghum, soybean, and shrimp meal was verified using literature and swine feed composition tables [19-24]. Ingredients were mixed using an electric horizontal mixer (Covema, Italy).

Bromatological analysis and formulation of *G. ulmifolia* L. biomass

To determine the analytical composition of *G. ulmifolia* meal and the formulated mix, two 500 g samples were sent to the EuroNutec laboratory (Nutec, Mexico): one of *G. ulmifolia* meal and another of the mixture containing *G. ulmifolia*, sorghum, *Glycine max*, and shrimp meal. Nutritional values of reference diets were obtained from commercial products PigTech3 PTBN (BioNova, Netherlands) and Lechoncina PT (Purina, USA).

Study group of pigs and dosage of balanced diet

The effect of the FMIX formulation containing *G. ulmifolia* biomass meal was evaluated in a study involving 15 six-week-old female pigs (10-12 kg), from a genetic line of 50% Landrace, 25% Hampshire, and 25% Duroc (Landrace sow × Hampshire/Duroc boar). Pigs were randomly assigned to three groups (n=5) and housed in separate 4.0 m^2 pens with independent feeders and waterers. Group 1 (G1, control) received a commercial diet: PTBN during week one, followed by LPT. Group 2 (G2) received PTBN during week one and a mix of LPT with 10% FMIX (4 kg) in subsequent weeks. Group 3 (G3) was fed LPT mixed with 20% FMIX (8 kg) from the start. Diets were blended using an electric mixer (Covema, Italy) (Table 1).

Weight measurements in pigs

Weight and thoracic girth measurements were taken every three days during the study period. Each pig was weighed using a commercially available moving platform scale (Torrey, Mexico). To avoid animal movement and stress, the animals were placed in a rectangular plastic box, and the scale was tared and weighed.

Blood collection for swine complete blood count analysis

Fifteen blood samples were collected 23 days after the start by mean the external jugular vein punction using sterile 10 mL plastic syringes with 20G × 32 mm needles (BD, USA). Samples were transferred to 7.2 mL EDTA K2 Vacutainer tubes (purple cap; BD, USA).

Table 1. Table showing the dosage of feed portions for each group of pigs for the duration of the evaluation protocol. Abbreviations: G1, Group 1; G2, Group 2; G3, Group 3; W, Week; F-10%, Feed-10%; F-20%, Feed-20%, F-10%=36 kg of LPT feed mixed with 4 kg of FMIX formula, F-20%=32 kg of LPT feed mixed with 8 kg of FMIX formula.

Group	Experimental period								
	W1	W2	W3	W4	W5	W6	W7	W8	W9
G1	PTBN	LPT							
G2	PTBN	F-10%							
G3	F-20%								

Tubes were stored at 4 °C and sent to an external veterinary diagnostic laboratory (AXIS Laboratory, Mexico) for complete blood count analysis.

RESULTS AND DISCUSSION

Microbial identification from the fruit *G. ulmifolia* L.

Five colonies with different morphology, named WF1, WF2, WF3, WF4 and WF5, were isolated from the washed fruit pulp sample of *G. ulmifolia* L. The cell morphology of these colonies was Gram+bacilli, Gram−bacilli, and Gram+cocci (Figure 1). From the colony PCR test, amplification and purification of the amplified bands of the 16S rRNA gene of 1500 bp were performed and sent for sequencing (Figure 2).

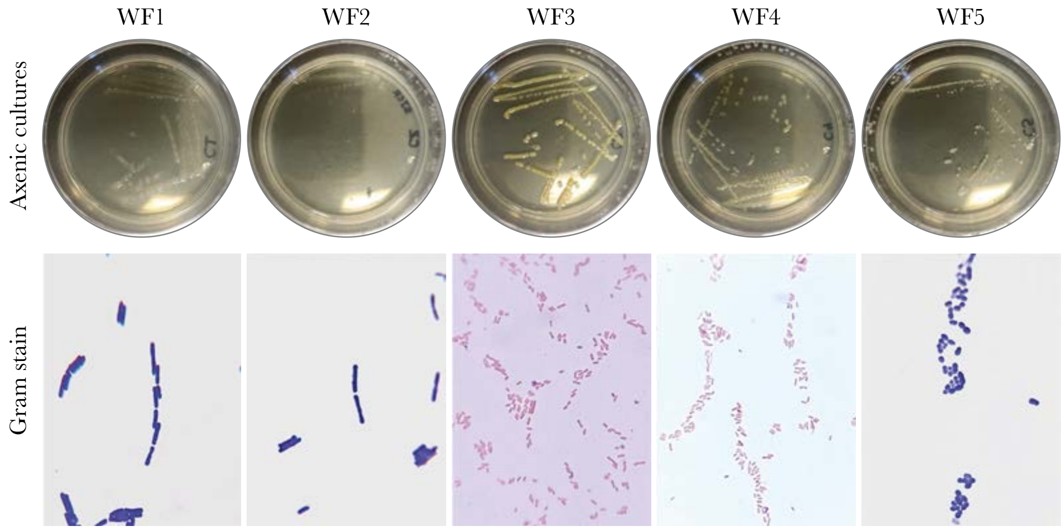

Figure 1. Axenic cultures and Gram staining obtained from the microorganisms present in the sample of *G. ulmifolia* L. WF1) Irregular colony, curled rim, bright white color, convex elevation, mucous consistency and cell morphology are associated with Gram-positive bacilli; WF2) Circular colony, entire rim, bright white color, flat elevation, smooth consistency, and cell morphology are associated with Gram-positive bacilli; WF3) Circular colony, entire rim, bright yellow color, convex elevation, mucous consistency, and the cell morphology are associated with Gram-negative bacilli; WF4) Circular colony, entire rim, translucent cream color, convex elevation, mucous consistency and; WF5) Circular colony, entire rim, bright white color, flat elevation, smooth consistency and the cell morphology are associated with Gram-positive cocci.

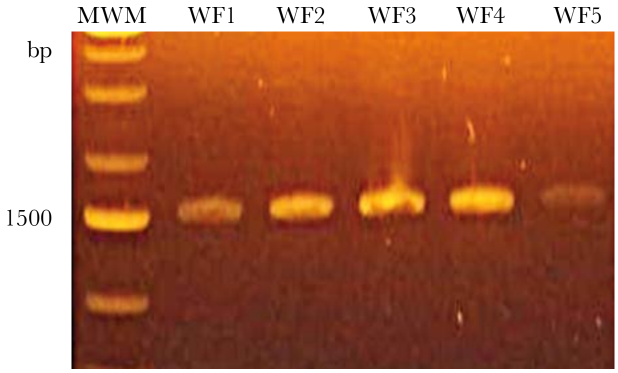

Figure 2. Purification of the amplified 16S rRNA gene band from isolated colonies WF1, WF2, WF3, WF4 and WF5. Abbreviations: MWM) Molecular Weight Marker.

Based on BLAST analysis, and using the NCBI database, samples WF1 and WF2 were identified as *Bacillus subtilis* (Table 2). *B. subtilis* is widely distributed in agrosystems, and one of its main applications is disease control in agricultural crops [25]. In addition, *B. subtilis* has been reported as probiotics, and presents antimicrobial, anticancer and antioxidant properties [26].

On the other hand, samples WF3 and WF4 were identified as *Cronobacter* sp. (Table 2). The *Cronobacter* genus are Gram-negative bacilli belonging to the Enterobacteriaceae family, and they are considered opportunistic pathogens and are frequently spread by contaminated food. This genus is made up of seven species: *C. sakazakii* and *C. malonaticus*, which constitute the majority of clinical isolates from patients, leading to severe neonatal infections and rarely to other diseases, such as conjunctivitis and urinary tract infections; *C. turicencis* and *C. universalis*, which are rarely associated with clinical cases; and *C. dublinensis*, *C. muytjensii* and *C. condimenti* (Group 3), which are environmental commensals and possibly pose little or no clinical significance [27]. Finally, sample WF5 was identified as *Enterococcus* spp. The *Enterococcus* are ubiquitous microorganisms, with Gram-positive cocci cell morphology which are part of the microbiome of terrestrial animals, since they are present in the gastrointestinal tract of these organisms. They can also be found in other environments such as plants and food; due to the fact that they act as initiators for the development of organoleptic properties. Several species such as *E. faecalis* and *E. faecium* are used as probiotics; they also lower serum cholesterol levels and strengthen the immune system. However, these bacteria are also capable of causing serious infections especially in hospitalized individuals, since they can be opportunistic pathogens and some species have acquired virulence factors and antibiotic resistance [28-29].

Bromatological comparative analysis of the proposed formulation

The ingredients that were chosen to formulate the FMIX mixture provide a variety of nutritional properties in swine feed, which were confirmed by the data obtained

Table 2. Identification of microorganisms based on the basic local alignment search tool and the National Center for Biotechnology Information.

Sample	Microorganism	E value	Percent identity	Accesion length	Accesion
WF1	*B. subtilis*	0.0	100	1514	PP917513.1
WF2	*B. subtilis*	0.0	98.69	1286	ON997083.1
WF3	*C. sakazakii*	0.0	99.3	1501	KU696331.1
	C. malonaticus	0.0	99.15	1419	FN401339.1
WF4	*C. sakazakii*	0.0	99.56	755	JQ312046.1
	C. dublinensis	0.0	99.27	4431067	CP012266.1
	C. turicensis	0.0	99.27	1439	MW013478.1
WF5	*E. faecium*	0.0	100	2824615	CP112586.1
	E. durans	0.0	100	1408	MF428861.1
	E. faecalis	0.0	100	1535	MW447606.1
	E. lactis	0.0	100	1344	ON426954.1

from different scientific reports. Sorghum contains an average crude protein of 10.6 % and an organic matter digestibility of about 81.4%, which makes sorghum an excellent alternative ingredient that can successfully substitute corn used in diets for pigs from weaning [19,22-23]. In addition, sorghum grains provide a high content of minerals, vitamins, carbohydrates, and their antioxidant activity [19,21,24]. *Glycime max* meal is commonly known as a high-quality edible oilseed for humans, however, it has a positive impact on animal feed, as it provides amino acids and high levels of vegetal protein ranging from 43-45% [20,24,30-31]. Shrimp waste flour is another ingredient that provides 43.4% crude protein and an organic matter digestibility of 48.31% [24,32]. For the case of *G. ulmifolia* L., its analytical constituents yielded a crude protein content of 7.12%, a digestible organic matter of 47.81% and a crude fiber content of 35.40%. *G. ulmifolia* L. provides an important contribution of fiber for pig nutrition, it also has the role of regulating gastrointestinal transit, helps prevent constipation, and contributes to maintain a balanced intestinal microbial community, which is essential for a healthy digestion [33]. Table 3 shows the concentration of the analytical constituents of each ingredient used for the FMIX formulation.

Taking into account the percentages of the analytical constituents of each ingredient in FMIX shown in Table 3, 20% of *G. ulmifolia* L. flour, 49% of Sorghum flour, 30% of *Glycine max* paste and 1% of shrimp waste were considered for the FMIX formulation. Thus, the theoretical estimate of the analytical constituents is described in Table 4.

The FMIX analytical constituents evaluated by the external laboratory, as well as the analytical constituents for each commercial balanced feed (PTBN and LPT), are shown in Table 5. A similarity was maintained between each parameter, with the purpose of not significantly affecting crude protein, nitrogen free extract and crude fiber, mainly. The parameters described in Table 5 were considered for the final mixtures in the dosage of the groups of pigs. The adjustment of the analytical constituents during the dosing of LPT mixed with FMIX at 10 and 20% can be seen in Table 5, which means that the dosing of 10% FMIX provided 18.19% crude protein and 53.65% digestible organic

Table 3. Comparative of analytical constituents for each ingredient of the formulated flour mixture. GUL: *G. ulmifolia* L. (Data obtained from bromatological analysis by EuroNutec laboratory), S: Sorghun [19,22-23], GMP: *Glycine max* paste [20,24,30], SW: shrimp waste [24]. Abbreviations: ELN, Extract Free Nitrogen-Digestible Organic Matter.

Parmeter	Flour ingredient (%)			
	GUL	S	GMP	SW
Crude protein	7.12	10.60	43.00	43.30
Crude fiber	35.40	2.90	9.00	7.00
ELN	47.81	80.30	22.76	48.31
Moisture	4.19	15.00	8.50	4.49
Fat	2.72	3.06	19.30	1.82
Ashes	2.76	1.60	6.30	24.25
Portions of each ingredient for FMIX				
%	20	49	30	1
g/kg	200	490	300	10

Table 4. Estimates of analytical constituents for FMIX from the percentage values for each ingredient: GUL: 20% *G. ulmifolia* L., S: 49% Sorghum meal, GMP: 30% *Glycine max* paste and SW: 1% shrimp waste. Abbreviations: ELN, Extract Free Nitrogen-Digestible Organic Matter.

Parmeter	Flour ingredient				Calculated FMIX (%)
	20%	49%	30%	1%	
	GUL	S	GMP	SW	
Crude protein	1.42	5.19	12.90	0.43	19.95
Crude fiber	7.08	1.42	2.70	0.07	11.27
ELN	9.56	39.35	6.82	0.48	56.22
Moisture	0.84	7.35	2.55	0.04	10.78
Fat	0.54	1.49	5.79	0.01	7.85
Ashes	0.55	0.78	1.89	0.24	3.46

Table 5. Analytical components of the balanced feeds used for feeding pigs in the initiation stage. Abbreviations: PTBN, PigTech3 Bio-Nova; LPT, Lechoncina PT; FMIX, Formula balanced with *G. ulmifolia* L. flour; ELN, Extract Free Nitrogen-Digestible Organic Matter.

Component	PTBN	LPT	FMIX	LPT+FMIX	
				10%	20%
Crude protein	19.00	18.00	19.98	18.19	18.39
Crude fiber	3.50	5.00	6.41	5.14	5.28
ELN	56.00	53.50	55.04	53.65	53.80
Moisture	12.00	12.00	10.50	11.85	11.70
Fat	2.50	4.00	4.35	4.03	4.07
Ashes	7.00	7.50	3.72	7.12	6.74
Phosporus	No reported	0.80	0.54	0.77	0.74

matter, and for 20% FMIX a contribution of 18.39% crude protein and 53.80% digestible organic matter.

Although the bromatological parameters of each feed show similarities, it is important to consider that in the case of FMIX, the evaluation of digestible organic matter is interesting due to the presence of microorganisms which contribute advantages to the digestive process of pigs and can be considered as probiotics of natural origin.

Weight gain analysis for each group of pigs

Over 53 days, the average weight gain (AWG) for each group of pigs was recorded and finally estimated. It was observed that the trends among the three groups maintained similar behavioral slopes until day 29. Subsequently, the G2 group showed a slight increase in weight gain in relation to the G1 and G3 groups (Figure 3A). Figure 3B shows the final average weight gain (WG) by group at day 53. For the groups that consumed FMIX in their daily ration, G2 had a weight gain of 33.05±8.90 kg and G3 of 29.10±3.61 kg, while the G1 group that did not include FMIX recorded a final weight gain of 28.34±5.00 kg. It should be noted that weight gains were recorded for the three groups, however, when comparing the data among groups, these differences were not significant ($p>0.05$); ruling

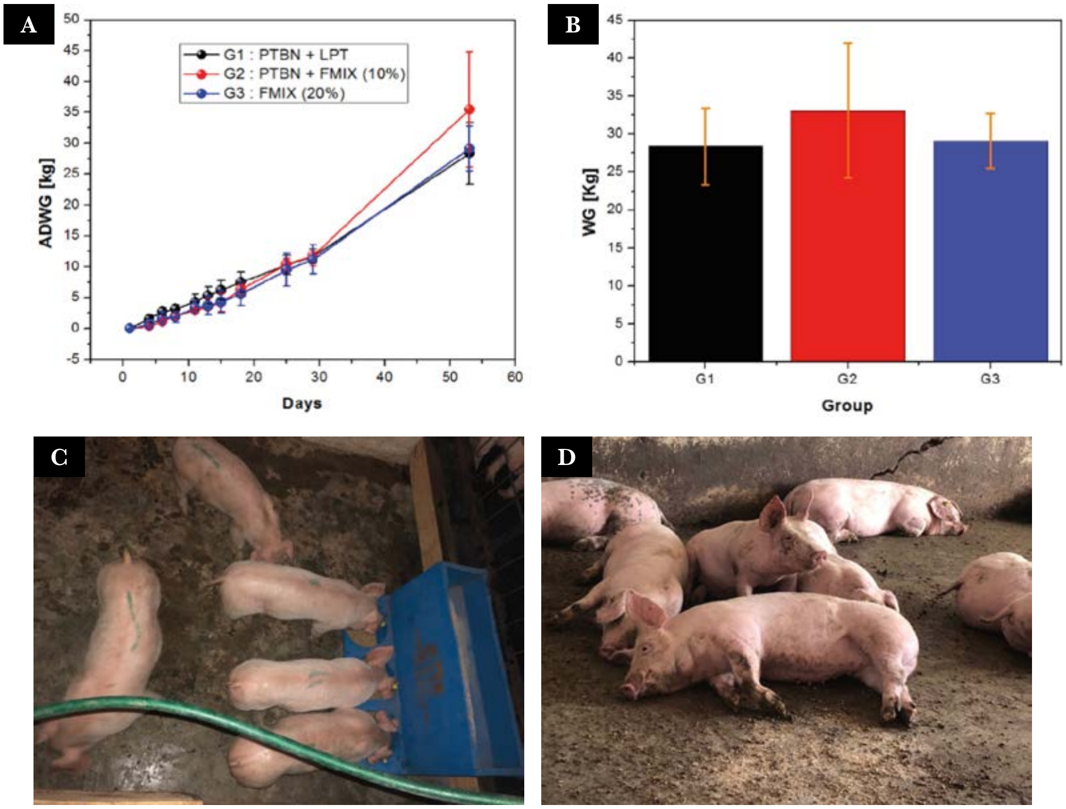

Figure 3. Comparative behavior of weight gain by group of pigs. A) Aver-age daily weight gain by group: G1, G2 and G3, B) Total average weight gain, C) Representative group (G2) of five piglets distributed in a space with water drinkers and a food container, D) Representative group of pigs in a common use space for the three groups after 53 days. Abbreviations: ADWG, Average Daily Weight Gain; WG, Weight Gain.

out any type of negative effect on weight gain, *i.e.*, the weight gain behavior was maintained in the three groups in relation to the progression of growth week by week according to their growth stage reported in different literatures [34-36].

Hemogram analysis per pig and group

Five blood analytes were monitored to assess the overall health of the study animals, such as total leukocytes, lymphocytes, neutrophils, monocytes, and thrombocytes. Table 6 shows the average complete blood count per group. High leukocytes were observed in the G1 group ($24.36\times10^9\pm7.24$/L powered by PTBN) and in group G3 ($20.58\times10^9\pm5.54$/L fed with LPT+FMIX 20%), the leukocyte level was slightly higher in group G1, as three of the five pigs had values higher than the reference level, while in group G3, only two pigs had high values in relation to the maximum reference parameter.

No cases of elevated lymphocytes were observed in group G2 (LPT+FMIX 10%). In contrast, high leukocyte counts in groups G1 and G3 were accompanied by increased lymphocytes, neutrophils, basophils, and monocytes key cells in bacterial infection response [36–37]. Lymphocyte levels exceeded reference values in both G1 ($15.58\times10^9\pm8.78$/L) and G3 ($12.66\times10^9\pm3.21$/L).

Table 6. Complete blood counts by group. Abbreviations: G1, Group1; G2, Group2; G3, Group3.

Analyte	Experimental group			Reference values	
	G1	G2	G3	Min.	Max.
Hematocrit (L/L)	0.40±0.032	0.38±0.01	0.42±0.02	0.32	0.5
Hemoglobin (g/L)	129.80±11.03	127.25±5.93	135.20±5.21	100	160
Erythrocytes (×10^{12}/L)	7.06±0.57	6.97±0.29	7.22±0.30	5	8
VGM (fL)	57.00±1.41	55.00±2.23	58.60±3.91	50	68
CGMH (g/L)	321.00±5.56	328.00±1.00	317.60±5.36	300	340
Protein (g/L)	77.20±4.65	77.75±3.89	78.20±4.32	60	90
Reticulocytes (×10^{9}/L)	-	-	-		<50
Leukocytes (×10^{9}/L)	24.36±7.24	15.50±2.70	20.58±5.54	11	22
Platelets (×10^{9}/L)	456.60±120.28	283.50±134.52	390±69.28	300	700
Neutrophils (×10^{9}/L)	7.94±3.03	6.85	6.30±4.38	4	7.5
Myelocytes (×10^{9}/L)	0	0	0		<0.5
Lymphocytes (×10^{9}/L)	15.58±8.78	7.67±2.17	12.66±3.21	3	13
Monocytes (×10^{9}/L)	0.68±0.27	0.82±0.25	1.32±1.34	0	1.5
Eosinophils (×10^{9}/L)	0.12	0.15±0.11	0.22±0.14	0	2
Basophils (×10^{9}/L)	0.04±0.08	0	0.02±0.04	0	0.03

The mucosa associated lymphoid tissue plays a key defensive role in pigs, as it is the first barrier against pathogens entering through the intestinal, respiratory, and urogenital mucosa. Elevated neutrophil counts observed in all groups suggest immune activation, likely in response to bacterial or fungal agents [38]. Notably, total protein levels and red blood parameters hemoglobin, hematocrit, and erythrocytes remained within normal ranges across all groups. The elevated leukocyte count in group G3 may be related to the presence of *Enterococci*, likely due to the higher concentration of *G. ulmifolia* flour, which increased levels of *Bacillus* spp. and *Enterococci*. Although these microorganisms are used as probiotics, some strains can be opportunistic pathogens [27]. It is also important to note that pigs in the early growth phase are more susceptible to immune disturbances due to dietary transitions involving solid feed [37]. Finally, weight gain per pig and per group remained within normal ranges according to standard growth charts [39-41].

CONCLUSIONS

Based on the bromatological, biological and nutritional properties of the flour from the fruit of *G. ulmifolia* L., it can be considered a potential ingredient in a balanced diet for feeding growing and developing pigs, particularly due to the content and benefits of probiotic microorganisms such as *B. subtilis.*

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Phytochemical profile and phenolic content of traditional milpa vegetables

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ABSTRACT

Objective: To analyze qualitatively the variation of phytochemicals, and to determine the content of total phenolic compounds (TPC) and total flavonoids (TF) in nine leafy vegetables and two fruits traditionally harvested from the milpa in Tlaxcala, Mexico.

Design/methodology/approach: The eleven samples were selected for their relevance in consumption in six localities belonging to four municipalities of Tlaxcala. The identification of phytochemicals by thin layer chromatography (TLC), and the quantification of TPC and TF, determined by UV/VIS spectrophotometry, were performed from methanol and hexane extracts.

Results: Qualitative analysis showed the presence of phenolic acids, flavonoids, saponins, tannins, terpenes and alkaloids in all the samples analyzed, except for tannins in *huazontle* and alkaloids in *apipisco*. TPC and TF were detected in the highest amount in *lengua de vaca* (22.91 mg GAE/g DM and 43.89 mg QE/g DM) and *lengüita* (15.6 mg GAE/g DM and 56.05 mg QE/g DM), while *tlalayote lampiño* (4.35 mg GAE/g DM and 5.06 mg QE/g DM) and *apipisco* (5.21 mg GAE/g DM and 7.5 mg QE/g DM) had the lowest contents.

Implications: The phytochemical analysis of these scarcely documented plants is significant for the pharmaceutical area and potential production of new drugs.

Findings/conclusions: The results of the qualitative profile evidenced the presence of different groups of phytochemicals of interest to further identify, quantify and possibly conduct pharmacological studies of these traditionally consumed vegetables, in addition to their valorization as functional foods.

Keywords: bioactive compounds, edible plants, functional foods, milpa, thin layer chromatography.

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INTRODUCTION

Wild vegetables have carried out an essential role and are frequent in the traditional diet of indigenous peoples and rural communities in many parts of the world. They have an important role for subsistence of many people, since, in addition to being an important source of food, people also depend on the plants in their natural environment for other practical applications. For example, several of them have medicinal properties and are used to treat common ailments due to the presence of bioactive compounds (Sanjay, 2023). Among the more than 50,000 secondary metabolites discovered in plants (Teoh, 2016),

coumarins, lignans, anthocyanins, glycosides, saponins, phenolic compounds, terpenoids, and alkaloids are the phytochemicals that have been widely studied for their benefits to health and their pharmacological potential (Samal *et al.*, 2024).

The state of Tlaxcala is located in the central zone of Mexico and has a total surface area of 3,996.6 km^2, from which more than 59% of its territory is devoted to agriculture, mainly to cultivation of maize (*Zea mays*), bean (*Phaseolus vulgaris*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and potato (*Solanum tuberosum*) (INEGI, 2021); and, where a large part of its agrobiodiversity is preserved thanks to its *in situ* conservation within traditional agricultural systems, such as the milpa system, which integrates a diversity of useful plants that promote food sovereignty and ecological resilience of the region (Altieri and Toledo, 2011).

Recent studies have emphasized the importance of vegetables traditionally collected from the milpa, not only for their nutritional value, but also because of their phytochemical content with bioactive properties (Méndez-Flores *et al.*, 2021). For example, Arrieta-Flores *et al.* (2022) point out that *verdolaga* (*Portulaca oleracea*), a species of common consumption in Tlaxcala, presents high contents of total phenolic compounds (TPC), in leaf (25.4 mg GAE/g DM) and stem (21.8 mg GAE/g DM), compared to what was reported for the same species by Alam *et al.* in 2014 (0.96 to 9.1 mg GAE/g DM). In turn, González-Amaro (2008) identified 109 species of weeds in milpas from three communities in Nanacamilpa, Tlaxcala, two as new reports for quelites, 90 with documented uses, and 19 that do not have a use. This underscores the wealth of local agrobiodiversity available and its potential to contribute to food security and health, which refers that inside the state there are still vegetables in the milpa that are not taxonomically and phytochemically documented. Therefore, those resources could have potential functional properties of interest for the innovation of family and regional agricultural production, as well as for healthcare through their consumption. However, more research about their phytochemical and pharmacological composition is still needed.

Thin layer chromatography (TLC) is a valuable qualitative technique in exploratory studies, such as the one presented here, due to its versatility and efficacy to quickly detect secondary metabolites and bioactive compounds in vegetable extracts. This technique allows the preliminary evaluation of possible bioactive properties (antioxidants, antimicrobial, enzyme inhibitors, among others) to study the relationships and similarities between plants according to their chemical composition, approach known as chemotaxonomy. In addition, TLC is also implemented for the analysis of quality control in medicinal, culinary, cosmetic or psychoactive plants (Zahiruddin *et al.*, 2021; Kowalska and Sajewicz, 2022).

Until now, there is no documentation about the phytochemical composition determined from thin layer chromatography of vegetables associated to milpas in Tlaxcala, and for this reason, the hypothesis of this study maintains that within this traditional agricultural system there are plants and fruits with important diversity of bioactive compounds (such as phenolic acids, flavonoids, saponins, tannins, terpenes, or alkaloids), which could be of interest through their consumption because they have beneficial properties to health, beyond a nutritional benefit. Therefore, this study had the objective of analyzing the qualitative profile of secondary metabolites (phytochemicals) in eleven specimens of vegetables traditionally collected in Tlaxcala, Mexico, and quantifying the content of TPC and TF to evaluate their pharmacological potential and their use as food resource with functional properties.

MATERIALS AND METHODS

Leafy vegetables and fruits were obtained from the milpa in six locations of the state of Tlaxcala, Mexico, where preferably there is no use of agrochemicals. Their collection was carried out prior to the flowering stage during the summer of 2023. The plant species collected, in addition to being identified by their common name, were authenticated taxonomically in the Herbarium of the Benemérita Universidad Autónoma de Puebla (Table 1).

The plant material was dehydrated in a Triad Freeze Dryer Labconco at 0.22 mBar and −81 °C, in a period between 24 and 48 hours depending on the type of tissue. Then, it was pulverized in an electrical Hamilton Beach Fresh Grind™, sieved with N° 40 mesh to obtain a particle size of 420 microns, and stored in amber containers at −20 °C until use.

Qualitative analysis by thin layer chromatography (TLC)

Extract preparation. To 250 mg of freeze-dried sample, 5 mL of solvent were added: methanol or hexane depending on the phytochemical group to be extracted (Table 2). The sample was shaken for 30 seconds in vortex and placed in ultrasonic bath (Ultrasonic Cleaner, modelo AS5150B) for 30 min at a power and degasification of 5. Then, the extract was left in maceration for 12 hours at room temperature and stored under refrigeration at 4 °C until its use (no longer than 24 hours).

Analysis of phytochemical groups. The analyses were conducted on silica gel plates with fluorescence indicator (Silica gel 60, F_{254}) of 20×10 cm (Merck, Catalog 1.05554), to which 25 μL of extract from each sample were added. Preparation of the mobile phase (eluent), as well as the controls and development protocol were performed

Table 1. Nomenclature of eleven types of milpa vegetables collected at six locations in Tlaxcala, Mexico.

Common name	Scientific name	Locality
Lengua de vaca	*Rumex obtusifolius* L.	Tepetitla
Verdolaga	*Portulaca oleracea* L.	Tepetitla
Malva	*Malva parviflora* L.	Tepetitla
Lengua de pájaro	*Calandrinia ciliata* (Ruiz & Pav.) DC	San Felipe Cuauhtenco
Apipisco	*Jaltomata procumbens*	San Felipe Cuauhtenco
Huazontle cimarrón	*Chenopodium album* L.	Tepetitla
Quintonil blanco	*Amaranthus powellii* S. Watson	Jesús Huitznahuac
Quintonil jaspeado	*Amaranthus*	Guadalupe Tlachco
Lengüita	*Calandrinia ciliata* (Ruiz & Pav.) DC	San Lorenzo Techalote
Tlalayote lampiño	*Chthamalia decumbens* (W. O. Stevens) L. O. Alvarado & E. B. Cortez	Hueyotlipan
Verdolaga	*Portulaca oleracea* L.	Hueyotlipan

Source: Prepared by the authors.

Table 2. Thin layer chromatography systems for the identification of phytochemicals in plants and edible fruits from milpas in Tlaxcala, Mexico.

Phytochemical	Solvent	Eluent	Control	Developer
Phenolic acids	Methanol	Ethyl acetate: formic acid: acetic acid: water (10:8:8:1)	Gallic acid 0.5 mg/mL	$Na_2(CO)_3$ 20% and Folin-Cioucalteu 50%
Flavonoids	Methanol	Ethyl acetate: formic acid: acetic acid: water (7.5:1:1:0.5)	Quercetin 0.5 mg/mL	NP 1% and PEG 5%
Saponins	Methanol	Butanol: glacial acetic acid: water (4:1:4)	Saponin 10 mg/mL	Vanillin 1% and H_2SO_4 6%
Tannins	Methanol	Ethyl acetate: formic acid: acetic acid: water (10:8:8:1)	Tannic acid 0.5 mg/mL	$FeCl_3$ 5% in 0.5 N HCl
Terpenes	Hexane	Hexane: ethyl acetate (7:3)	*Syzygium aromaticum* (clove) 0.1 mg/mL	Vanillin 1% and H_2SO_4 10%
Alkaloids	Methanol	Ethyl acetate: methanol: water (3:1:1)	*Camellia sinensis* (black tea) 200 mg/mL	Buchard reagent: I 2%:KI 2%

Adapted from Andrade-Andrade (2016).

following the methodology by Andrade-Andrade (2016), based on Wagner and Bladt (1996), and summarized in Table 2. To ensure the stability of the technique, previous assays were carried out with a control and three repetitions of each sample, according to the phytochemical group to identify, until securing the reproducibility of the results and on clearly defined bands. Observation of the bands was conducted with visible light, except for flavonoids, which were visualized in ultraviolet light at 365 nm (UVP lamp, UVLMS-38, series 3UV^{TM} Lamp). Finally, the displacement of the compounds through the plaque was expressed through calculation of the retention factor (Rf), which involves the distance traveled by the sample over the distance traveled by the eluent.

Quantitative analysis by spectrophotometry

The extracts were prepared in the same way as described for the qualitative analysis, but at a concentration of 20 mg/mL, and the moisture percentage was determined for its expression based on dry matter (DM).

Total phenolic compounds. Their quantification was based on the Folin-Ciocalteau colorimetric method described by Herald *et al.* (2012) with modifications. In a microplate, 75 μL of deionized water and 25 μL Folin-Ciocalteau phenol reagent (1:1 with deionized water) were added to 25 μL of extract, shaken for 10 seconds and rested for 6 minutes at room temperature in the dark. Then, 100 μL of Na_2CO_3 at 7.5% were added, shaken for 10 seconds and left resting for 60 minutes under the same conditions. Finally, its absorbance was recorded at a wavelength of 725 nm in a Varioskan flash UV/VIS (Thermo-Scientific) spectrophotometer, and a standard curve ($y=0.0072x-0.0012$, $R^2=0.9993$) prepared with gallic acid at 1 mg/mL. The results were expressed in mg of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Total flavonoids. They were determined based on the method described by Silva-Beltrán *et al.* (2015) with modifications. The reaction was carried out at room temperature, in the dark, and brief agitation of 8 seconds was carried out after the addition of each reagent: in microplate, 25 μL of extract were mixed with 100 μL of deionized water, 10 μL of $NaNO_2$ (5% w/v) were added, shaken and rested for 5 minutes. Then, 15 μL of $AlCl_3$ (10% w/v) were added and rested for 6 minutes; 50 μL of NaOH 1M were added, shaken and 50 μL of deionized water were added, and shaken again for 15 seconds, rested for 5 minutes, and its absorbance was determined at 415 nm in a Varioskan flash UV/VIS (Thermo-Scientific) spectrophotometer. The results were calculated with the equation of the standard curve obtained with a standard of quercetin dissolved in ethanol at 1 mg/mL ($y=0.0011x+0.036$, $R^2=0.995$) and were expressed in mg of quercetin equivalent per gram of dry matter (mg QE/g DM).

Statistical analysis

The evaluation of qualitative results of TLC was carried out through contingency tables. For the quantification of TPC and TF, an experimental design by completely random blocks was used. The eleven specimens were considered as treatments, each one constituted by four independent blocks (corresponding to four samples of each specimen). For each block, three extracts (repetitions) were carried out and each repetition was determined by triplicate. The data were processed through analysis of variance and means test (Tukey $\alpha=0.05$) with the statistical package SAS version 9.0 (SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

Qualitative analysis by thin layer chromatography (TLC)

The qualitative detection of the different groups of phytochemical components confirmed the presence of phenolic acids, flavonoids, saponins, tannins, terpenes and alkaloids in the eleven specimens analyzed, except for tannins in *huazontle cimarrón* and alkaloids in *apipisco* (Table 3). The saponins were the phytochemical group of greatest presence in this collection of vegetables (50 bands), followed by flavonoids (46 bands), which suggests that these compounds could be key in the bioactive properties of these plants. Thus, the evaluation of their biological properties and studies focused on the quantification of these metabolites are necessary to take advantage of their pharmacological potential in practical applications.

The visualization of the plates developed under visible light and UV light allowed observing a notable diversity of phytochemicals in the extracts analyzed (Figure 1).

Phenolic acids. This phytochemical group was identified in all the plants analyzed, with 1 or 2 bands. The Rf values ranged between 0.61 and 0.87, which suggests the presence of compounds with relatively small molecular weight, for example, hydroxycinnamic acids, common in vegetables and of great interest due to their wide range of antioxidant activity documented *in vitro* (Shahidi and Ambigaipalan, 2015). Within the pattens identified, *lengua de pájaro* and *lengüita* have bands with similar Rf and densities; Rf=0.63 and 0.66; Rf=0.73 and 0.74, respectively (Figure 1, Table 4). According to the density of the bands, it is possible for *huazontle* to have a lower content of phenolic acids, followed by malva and both varieties of *quintonil*. The importance of confirming the presence of phenolic acids

Table 3. Number of bands observed in thin layer chromatography by phytochemical group, from extracts of eleven traditionally collected vegetables in milpas from the localities in the state of Tlaxcala, Mexico.

Specimen	Phe. ac.	Flav	Sap	Tann	Ter	Alk	Total
	Number of bands						
Control	1	1	2	1	1	1	7
(1) Lengua de vaca	1	9	6	1	3	4	24
(2) Verdolaga from Tepetitla	1	6	5	2	3	3	20
(3) Malva	2	6	3	1	4	4	20
(4) Lengua de pájaro	2	2	4	1	4	1	14
(5) Apipisco	1	2	3	1	2	0	9
(6) Huazontle cimarrón	1	2	5	0	3	1	12
(7) Quintonil blanco	1	4	4	1	1	4	15
(8) Quintonil jaspeado	1	4	4	1	2	4	16
(9) Lengüita	2	2	6	1	2	4	17
(10) Tlalayote lampiño	1	2	4	2	3	1	13
(11) Verdolaga from Hueyotlipan	1	6	4	1	4	4	20
Total	15	46	50	13	32	31	187

Phe. ac.=phenolic acids, Flav=flavonoids, Sap=saponins, Tann=tannins, Ter=terpenes, Alk=alkaloids. Prepared by the authors.

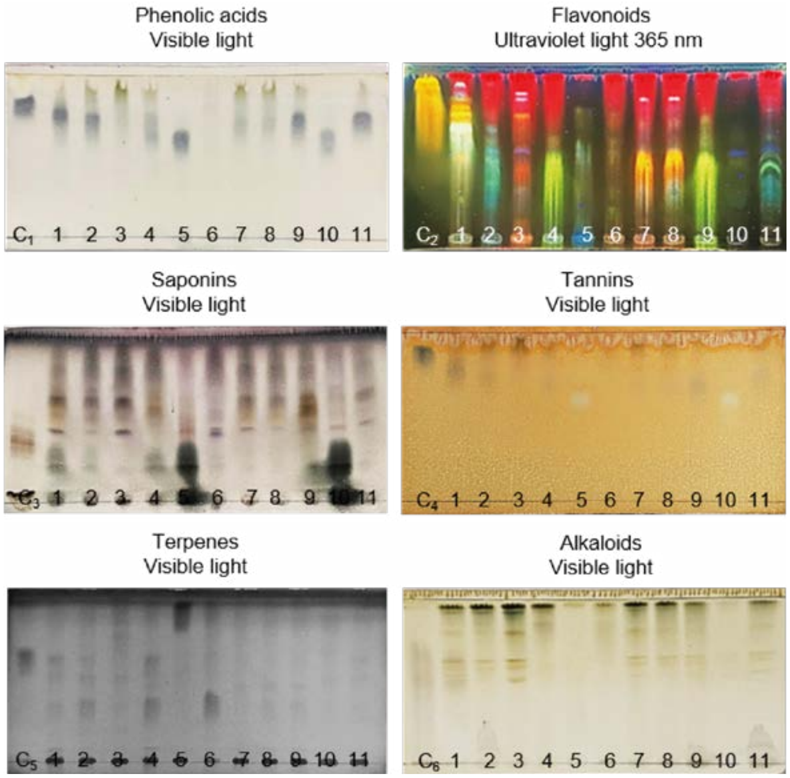

Figure 1. Detection of bands of phytochemicals from thin layer chromatography of edible plant extracts from Tlaxcala, Mexico. C_1=Gallic acid; C_2=Quercetine; C_3=Saponin; C_4=Tannic acid; C_5=*Syzygium aromaticum* (clove); C_6=*Camellia sinensis* (black tea); 1=Lengua de vaca; 2=Verdolaga from Tepetitla; 3=Malva; 4=Lengua de pájaro; 5=Apipisco; 6=Huazontle cimarrón; 7=Quintonil blanco; 8=Quintonil jaspeado; 9=Lengüita; 10=Tlalayote lampiño; 11=Verdolaga from Hueyotlipan. Prepared by the authors.

in these samples lies mainly in that they are multipurpose secondary metabolites that are widely distributed in the plants and represent an important group of phytochemicals with antioxidants, anti-inflammatory, antimicrobial properties, among others (Xu *et al.*, 2008; Shahidi and Ambigaipalan, 2015).

Flavonoids. Because of the light density of the bands, it is possible that the fruits of *apispico* and *tlalayote lampiño*, as well as *huazontle cimarrón*, have a low concentration of flavonoids. However, different tones were detected in the development of this phytochemical group, where the sample of *lengua de vaca* stands out, which presented up to nine clearly defined bands with orange, white, lilac and blue colors; this reflects a wide variety of different flavonoids. In some of the specimens, an intense yellow color was observed which coincides with what was reported by Gomathi *et al.* (2012), although Wagner and Bladt (1996) mention the orange color as characteristic of flavonoids. In this study, it was detected that plants of the same species, such as *lengua de pájaro* and *lengüita*, have a predominant type of flavonoid, responsible for the green-yellow color band, with Rf=0.48 (Table 4). Likewise, the similar profile of both samples of *quintonil* points to the presence of four types of flavonoids with different particle size and characteristic colors, where the bands in orange color stand out in intensity, at Rf=0.49.

Saponins. The size and color of the bands were quite varied, which reflects a great variety of types of saponins. Among them, tones of lilac, pink, blue, green, brown and purple were identified, all in opaque intensity (Figure 1). The phytochemical screening of the samples from both fruits, *apipisco* and *tlalayote lampiño*, reflect a greater possibility of having saponins in a concentration of interest. Although in specific concentrations they are considered as anti-nutrients, they are also molecules of great interest for commercial applications and in the research sphere for their wide range of bioactive properties; they can act as antimicrobial, antihyperglycemic, antiviral, antioxidant, anti-inflammatory, anticancer, immunomodulating molecules, among others, as described by Osbourn *et al.* (2011).

Tannins. In the control, for which tannic acid was used, the bands were identified with a blue tone, but in the samples the bands were purple and white. In the results of this phytochemical group, in the samples from fruits of *apipisco* and *tlalayote lampiño*, the presence of a specific type of tannin, with white bands (Figure 1), and Rf=0.62 stands out (Table 4). In the phytochemical screening of *huazontle cimarrón*, the presence of bands corresponding to this phytochemical group was not identified (Table 4). This result contrasts with what was described by Arora *et al.* (2020), who confirm the presence of tannins in methanol, acetone, and ethyl acetate extracts based on plant material of *huazontle cimarrón* (*Chenopodium album* L.), dehydrated, ground and degreased.

Terpenes. The bands in this group of phytochemicals were detected with purple-blue tones. In general, the bands were faint for most of the samples, but in *lengua de vaca* (1), *verdolaga* (2), *malva* (3), *lengua de pájaro* (4) and *huazontle cimarrón* (6), three bands with greater density were revealed (Figure 1). It should be mentioned that in the study carried out by Arora *et al.* (2020) in *huazontle cimarrón*, they did not detect terpenes in methanol extracts, but they did detect the presence of triterpenes in chloroform and petroleum ether extracts; in this study, the detection of these phytochemicals was carried out in hexane extract (Figure 1). This shows that the presence of terpenes can vary in relation with the extraction solvent used, and it could be due to differences in the polarity and efficacy of solvents to solubilize these compounds, in addition to the differences between treatments of the plant material prior to the maceration of extracts.

Alkaloids. Even when the formation of the bands was dim, possibly due to the structural complexity of the molecules, the results confirm the presence of alkaloids in all the vegetables studied except for *apipisco* (Table 4). For this purpose, the yellow-brown bands identified under visible light were considered to be alkaloids, which agrees with what was reported by Gomathi *et al.* (2012). Their detection in vegetables is very important because they have various applications of interest, such as in dermatology in the medical sphere (as anti-inflammatory, sedative, diuretic, anti-cold, anti-spasmodic, among others) (Lombardo and Ortíz, 2009). In addition, there are plants with high toxicity due to the presence of these compounds, although out of the more than 5000 known alkaloids, the minority are toxic. It should be emphasized that to prevent the ingestion of the plant, these compounds have the property of giving a bitter taste (Villar and Díaz, 2006). Therefore, it

Table 4. Retention factor of the bands identified through thin layer chromatography of extracts from plants and edible fruits collected in milpas in Tlaxcala, México.

Phytochemical	Sample*											
	C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Phenolic acids	0.84	0.77	0.74	0.74 0.87	0.63 0.73	0.62	0.81	0.72	0.72	0.66 0.74	0.61	0.74
Flavonoids	0.95	0.49 0.55 0.60 0.68 0.75 0.80 0.84 0.88 0.93	0.34 0.48 0.54 0.60 0.66 0.92	0.32 0.43 0.53 0.74 0.84 0.88	0.48 0.61	0.54 0.65	0.53 0.74	0.32 0.49 0.72 0.85	0.32 0.49 0.72 0.85	0.48 0.73	0.41 0.64	0.35 0.45 0.50 0.57 0.78 0.87
Saponins	0.37 0.45	0.22 0.28 0.44 0.53 0.64 0.8	0.22 0.43 0.52 0.63 0.8	0.43 0.51 0.8	0.21 0.28 0.57 0.77	0.21 0.27 0.43	0.4 0.44 0.51 0.56 0.84	0.44 0.6 0.77 0.82	0.44 0.6 0.76 0.82	0.19 0.44 0.56 0.63 0.77 0.82	0.21 0.26 0.45 0.63	0.42 0.48 0.53 0.66
Tannins	0.89	0.78	0.76 0.86	0.8	0.71	0.62		0.7	0.73	0.69	0.62 0.74	0.76
Terpenes	0.65	0.34 0.5 0.63	0.36 0.52 0.63	0.36 0.51 0.63 0.74	0.37 0.47 0.52 0.63	0.81 0.93	0.37 0.65 0.85	0.46	0.35 0.46	0.36 0.46	0.51 0.68 0.88	0.36 0.52 0.63 0.88
Alkaloids	0.64	0.5 0.55 0.64 0.81	0.62 0.8 0.84	0.51 0.59 0.63 0.8	0.85		0.81	0.54 0.61 0.65 0.82	0.55 0.61 0.66 0.82	0.55 0.61 0.66 0.82	0.66	0.56 0.63 0.68 0.8

*C=Control, 1=Lengua de vaca, 2=Verdolaga from Tepetitla, 3=Malva, 4=Lengua de pájaro, 5=Apipisco, 6=Huazontle cimarrón, 7=Quintonil blanco, 8=Quintonil jaspeado, 9=Lengüita, 10=Tlalayote lampiño, 11=Verdolaga from Hueyotlipan. Prepared by the authors.

is suggested to approach quantitative studies that determine their concentration and, from this, an evaluation of risks according to the regional patterns of consumption. Likewise, the possible characterization of the alkaloids present.

The detection of phenolic acids, flavonoids, saponins, tannins, terpenes and alkaloids in all the samples analyzed (Figure 1) suggests that these vegetables and fruits under study could have antioxidant properties and benefits for the health of those who consume them, which supports their traditional use in the diet and as medicine. Although, qualitatively, phenolic acids and tannins do not seem to be the most abundant compounds in the samples (Table 4), their constant presence indicates that they could contribute synergically with other secondary metabolites that are more present, such as flavonoids, to potentiate their mechanisms of action and biological effects. Previous studies have shown that phenolic compounds act in an additive and synergic way, improving their bioactive properties (Liu, 2003; Pereira *et al.*, 2009). For example, phenolic acids or tannins can stabilize free radicals, while flavonoids act on other metabolic pathways, which result in a stronger combined effect than each compound separately (Rice-Evans *et al.*, 1996). In addition, two compounds sharing biosynthetic paths and being found together in the plants can help to improve the bioavailability and biological efficacy of both compounds (Shahidi and Ambigaipalan, 2015). In other cases, the presence of phenolic acids (such as gallic acid), increases the antioxidant activity of flavonoids, since they protect them from oxidative degradation (Rice-Evans *et al.*, 1996).

Quantitative analysis

The analysis of variance showed significant differences both for TPC and for TF between the species of plants evaluated. The concentration of TPC varied from 4.3 to 22.9 mg GAE/g DM; within the samples, the leaf vegetable known as *lengua de vaca* showed the highest concentration, followed by *lengüita* (15.6 mg GAE/g DM), *lengua de pájaro* (12 mg GAE/g DM), and both samples of *verdolaga* (Figure 2). In this study, the lowest

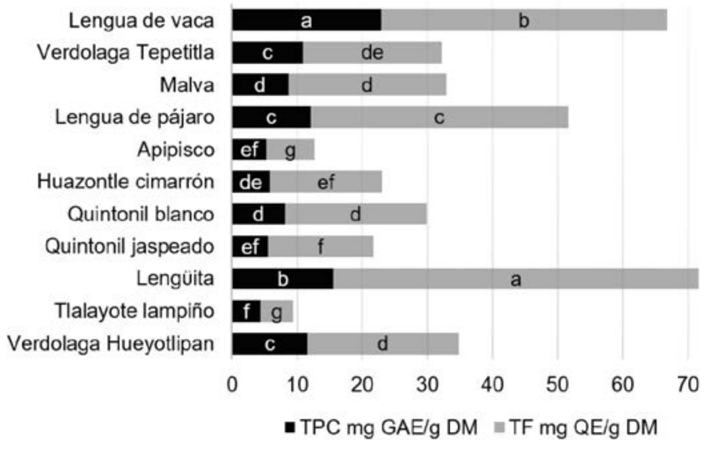

Figure 2. Content of total phenolic compounds (TPC) and flavonoids (TF) in eleven specimens of plants and edible fruits traditionally collected in milpas in Tlaxcala, Mexico. Average values with the same letter in the same phytochemical group were not statistically different (Tukey $p \leq 0.05$). Prepared by the authors.

determinations of TPC corresponded to *apipisco* (5.5 mg GAE/g DM), *quintonil jaspeado* (5.4 mg GAE/g DM) and *tlalayote lampiño* (4.3 mg GAE/g DM), which shows the functional potential of these, and the rest of the species evaluated from the milpa. The results obtained in *verdolaga* (10.9-11.6 mg GAE/g DM) were on average higher than what was reported by Cantú-López *et al.* (2022) in samples from this same species collected in Tepetitla, Tlaxcala (3.7 mg GAE/g DM). In turn, Román-Cortés *et al.* (2018) describe a concentration of TPC of 1.4 mg GAE/g DM for *verdolaga* obtained from the Supply Center in Ecatepec, State of Mexico. *Quintonil* and *huazontle* were also considered as *quelites* of important ancestral use in Mexico, where Román-Cortés *et al.* (2018) detected concentrations of 3.99 and 4.5 mg GAE/g DM, respectively, which are slightly lower levels than the ones obtained in this study for the same plants: *huazontle* (5.77 mg GAE/g DM), *quintonil jaspeado* (5.45 mg GAE/g DM) and *quintonil blanco* (8.11 mg GAE/g DM). In *malva*, the value of TPC (8.6 mg GAE/g DM) detected was nearly double the concentration it had in this species (4.7 mg GAE/g DM) collected in Hidalgo, Mexico, and reported by Sandoval-Gallegos (2022). In this regard, Jiménez-Aguilar and Grusak (2015), in their study of nutritional and phytochemical analysis of four species of green leafy vegetables native to Mexico, Central America and Africa, describe the content of TPC in a range of 2.29 to 5.66 mg GAE/g based on fresh weight; these authors indicate the native species as foods with higher content of TPC, compared to other vegetables of high consumption, such as some spinach, lettuce and cilantro.

It should be mentioned that the studies referred from the literature do not specify the agricultural context where the samples were collected, so the suggestion is to evaluate the impact of traditional and conventional agricultural practices in the phytochemical profile of the vegetables and to complement the information with studies that consider the amount of biomass required to consume to have better use of these bioactive compounds obtained through the diet. For example, in the review by Mercado-Mercado *et al.* (2013) a wide variety of traditional foods used as spices in Mexico was documented, which have great potential of antioxidant properties due to their high content of polyphenols and flavonoids, but, in general, the amount of biomass used tends to be small. This leads us to question up to what point the functional or nutraceutical property of a food depends on the amount that is consumed.

Regarding the content of TF, the highest concentration was identified in *lengüita* (56.1 mg QE/g DM), followed by *lengua de vaca* (43.9 mg QE/g DM), *lengua de pájaro* (39.7 mg QE/g DM) and *malva* (24.3 mg QE/g DM). The concentration of flavonoids obtained in this study in the *verdolaga* (plants 21.3 and 23.3 mg QE/g DM), was at least four times higher than what was found by Cantú-López *et al.* (2022) in samples of this species collected in Huaquechula, Puebla (5.1 mg QE/g DM) and Tepetitla, Tlaxcala (3.3 mg QE/g DM). The determinations of FT carried out by Román-Cortés *et al.* (2018) for *huazontle*, *verdolaga* and *quintoniles*, included 0.47 to 1.14 mg QE/g DM, concentrations quite lower than those obtained in this study for the same species, which varied from 16.23 to 23.23 mg QE/g DM. As presented in the results in this study, and what is suggested by Cantú-López *et al.* (2022), the environment where plants develop plays a defining role in the concentration of TPC and TF, since these variations could be attributed to environmental factors such as water stress, the content of nitrogen in the soil, and other elements that influence the biosynthesis of secondary metabolites. In fact, these authors observed that, under certain stress conditions, the plants increased production of these compounds as a defense mechanism, which could explain the differences obtained in the concentration of both phytochemicals between species of the same genera or between individuals of the same species.

This study provides guidelines to promote future qualitative and bioactivity studies in compounds that these and other available plants in the milpa could contribute, to inquire about their therapeutic potential, as well as foster, validate, and support their conservation and traditional use in the localities of study and others where there is the intention to implement the milpa system.

CONCLUSIONS

The study of the qualitative profile by thin layer chromatography in the nine leafy vegetables and two fruits collected in the milpa system revealed a wide diversity of compounds in the phytochemical groups evaluated, primarily, in the flavonoid and saponin compounds. The results evidence that these dietary resources are not only important from the nutritional point of view for the communities but could also have a pharmacological potential of interest and significant phenolic compounds. Quantification of total phenolic compounds and total flavonoids confirms the presence of these bioactive compounds in relevant concentrations for their continuous study, primarily in *lengua de vaca* (*Rumex obtusifolius* L.), *lengüita* (*Calandrina ciliata* [Ruiz & Pav.] DC) and *verdolaga* (*Portulaca oleracea* L.). This provides guidelines to new perspectives for their use in the diet, preventive medicine and industrial areas, such as cosmetics, among others.

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Colegio de Postgraduados

Evaluation of the effect of nopal meal on meat quality of Japanese quails (*Coturnix japonica*)

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ABSTRACT

Objective: to evaluate the effect of supplementation, with nopal meal in the diet of Japanese quails, on the slaughter variables and quality properties of meat.

Design/Methodology/Approach: 200 female quails (49 d) were used that were randomly assigned to four treatments with five replicates (10 birds per experimental unit). The T1 treatment was the control diet, while the T2, T3 and T4 included different doses of nopal meal in miligrams per kilogram of feed (400, 800 and 1200 mg kg^{-1}).

Results: in the quails evaluated, nopal meal did not modify ($p>0.05$) the weight at slaughter, yield or weights of non-chilled and chilled carcasses. Likewise, the weight of organs ($p>0.05$) was not affected, but the length of the gizzard was modified ($p\leq 0.05$) with the addition of flour. Nonetheless, nopal meal did not influence the weights, both of the chilled and post-cooked legs ($p>0.05$), nor the yield or texture of quail meat. However, the pH decreased, which improved luminosity ($p<0.05$), and increased the intensity of red color, while the intensity of yellow decreased. In addition, water retention capacity was higher in meat of those quails supplemented with 400 mg kg^{-1} of nopal flour in their feed.

Limitations/Implications of the study: according to our results it seems that, with higher concentrations of nopal meal, yields could also be higher. This modification is suggested for further research.

Findings/Conclusions: nopal meal did not influence the weight of the chilled legs, the cooking yield or meat texture. However, it is an additive that lowered the pH of quail meat; thus, it increased the luminosity and accentuated red color in meat, while decreasing the yellow pigments.

Palabras clave: antimicrobials, luminosity, pH, water holding capacity, carcass yield.

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INTRODUCTION

Poultry meat is a high-quality protein source characterized by high digestibility and complete essential amino acid profile. In addition, this meat contains lipids, vitamins and

minerals that are essential for balanced nutrition. In Mexico, chicken meat is the most consumed animal protein, representing 34.2% of total national consumption (Estévez & Miranda, 2022). However, quail is becoming an important animal, due to several advantages among which are rapid growth rate, high feed efficiency and the reduced space needed for rearing (Adom *et al.*, 2023).

Nowadays consumers are increasingly attentive to the quality of the food they consume, especially in regard to meat. Nutrition, environmental conditions and animal health play a crucial role in the final quality of the meat. This quality is evaluated by physicochemical characteristics such as pH, color, texture, water retention, drip water loss, appearance, and sensory properties (Bautista *et al.*, 2016). In this sense, poultry farming has resorted to the use of antimicrobials over time; however, knowledge about the potential risk to human health has led the poultry industry to look for natural alternatives to improve the yield, health and quality of meat in poultry (Abd El-Hack *et al.*, 2022).

Nutraceutical foods are known to modify and maintain normal physiological functions, thus contributing to the health of poultry individuals (Alagawany *et al.*, 2021). Among these food components is the prickly-pear plant (*Opuntia ficus-indica*), called nopal in Mexico, which contains phytochemicals such as terpenoids, phenolics, alkaloids and dietary fiber, with antioxidant capacity, which protects tissues from the damaging effect of free radicals (Aruwa *et al.*, 2018). In quail farming, information on the impact of nopal flour on meat quality in quails is not yet available. Therefore, the objective of this research was to evaluate the supplementation with nopal flour as an additive in feed on carcass yield and meat quality of Japanese quails.

MATERIALS AND METHODS

The experiment was established at the experimental farm of the Faculty of Agronomic Engineering and Zootechnics under the Benemerita Universidad Autonoma de Puebla-BUAP, located in Los Reyes de Juarez (Puebla). The quality evaluation of quail meat was performed at Colegio de Postgraduados Campus Montecillo (State of Mexico), endorsed by the Institutional Committee for the Care and Use of Laboratory Animals of the BUAP.

For the study, 200 female quails with an age of 49 days and a final age of 140 days were used. The birds were housed in 60×90 cm metal cages with five replicates of 10 birds each. The feeding system was in linear feeders and automatic drinkers, providing water and feed *ad libitum*. The design was completely randomized which consisted of four treatments (T1, T2, T3 and T4) with different doses of nopal flour added to feed, 0 (control), 400, 800 and 1200 mg kg^{-1} respectively. These concentrations were supplied in the feed (Table 1) covering the nutritional requirements established for quails, according to Rostagno *et al.* (2017) recommendations.

Once the experiment was over, the birds fasted for 12 h, two birds were randomly selected from each replicate and the live weight (PV, g) was obtained. Subsequently, the quails were humanely sacrificed by cervical dislocation following the Official Mexican Standard NOM-033-SAG/ZOO-2014 regulations (DOF, 2015). Bleeding was made in two minutes by cutting the jugular vein and the carotid artery, then birds were plucked manually.

Table 1. Quail diet composition at the laying stage.

Ingredients	%
Maize (8% CP)	59.00
Soy paste (47% CP)	29.30
Sand	0.52
Non-cooked oil	0.85
DL-Metionine (99%) §	0.41
Lisine (78%) §	0.35
$CaCO_3$ (38%) Þ	7.99
Dicalcium phosphate (18/21%) ¤	1.09
Vitamins and Minerals †	0.15
Salt	0.35
kilograms	100.00
Calculated Analysis (%)	
ME (kcal kg^{-1}) *	2800
Crude Protein (CP)	19.00
Calcium	3.34
Available phosphorus	0.34
Lysine	1.18
Methionine + Cystine	0.96
Tryptophan	0.22
Threonine	0.64

§Purity percentage; Þ38% of calcium; ¤18% of phosphorus and 21% of calcium. †Intake per kg of feed: vitamin A, 7.33 MUI; vitamin D3; vitamin K3; vitamin B1, vitamin B2; vitamin B6; vitamin B12; folic acid; biotin; Calcium D-Pantothenate; nicotinamide; choline chloride; calcium; iron (EDDI, as a source of iodine); zinc; manganese 44 g kg^{-1}; copper; selenium; antioxidant; *ME, metabolizable energy; PC, crude protein.

Carcass yield, Organ length and weight

After the plucking process, the non-chilled carcass was weighed with viscera (CCLC, g) and without viscera (CCLS, g). Non-chilled carcass yield (RCC) was calculated as the percentage difference between the weight of the live bird and the weight of non-chilled carcass plucked and eviscerated. Subsequently, several specific characteristics of the organs were measured using precision equipment, in the proventriculus we measured the width (APRO, mm), length (LPRO, mm) and weight (PPRO, g); also, weight of gizzard without *digesta* (PMO, g), and its width (AMO, mm) and length (LMO, mm). Liver weight (PH, g) was also recorded. Organ length measurements were made with a HER-411 digital caliper (Steren®, Mexico), while weights were determined with a tactile analytical balance (VELAB, AE224C, USA).

Measuring pH in muscle

To determine the pH in muscle, readings were taken in a cross-section that was made in the muscle *Flexor perforans* of the right leg at 5 min *postmortem*. A table potentiometer

(Model SM- 25CW MICROPROCESSOR pH/ mV METER, USA) was used for the measurement. That instrument was previously calibrated with buffer solutions (pH 4.01, 7.00 and 10.01) to ensure the accuracy of the readings. The electrode was inserted at similar points in the muscle and three measurements were made at each point; after each reading, the electrode was cleaned with distilled water. Finally, the average of the measurements was calculated to obtain the final record value.

Weight of chilled carcass, chilled legs and cooking yield of legs

The samples were stored in refrigeration at 4 °C, and at 24 h *postmortem* the weight of the chilled carcass (CF, g) was obtained, and subsequently the yield of the chilled carcass (RCF, %) was determined. Next, the chilled left leg (PF, g) was cut and weighed with an analytical balance (Model AE224C Electronic Balance); then each one was identified for the cooking process. Ten samples per treatment were introduced into a pot with 500 mL of water, they were placed on an electric grill (Voltec basic, China) for 30 min until reaching 100 °C of internal temperature (Honikel, 1987). After cooking, legs were dried with disposable towels, cooled to room temperature and weighed again. Cooking yield (CR, %) was calculated by difference with the initial weight recorded at 24 h *postmortem*.

Meat color

Subsequently, the color of the meat was evaluated, for this the complete piece of breast (*Pectoralis major*) was extracted and, placed on a white plastic board, a colorimeter was used (Model CR-410, Tokyo, Japan), and the variables luminosity (L*), red-green color tendency (a*) and yellow-blue tendency (b*) were determined.

Meat Texture (MT)

The texture of the meat was measured with a texture-meter (TA-XT2, TextureTechnologies® and a Warner-Bratzler® knife), breast samples were taken, cut into fillets 1 cm (width), 1 cm (height) and 3 cm (length). First, the texturometer was calibrated and synchronized with the computer program, once calibrated, the samples were placed on the tray so that the blade made the cut with a speed of 5 mm s^{-1}. The data obtained were recorded in a graph generated by the texture-meter software (Lyon & Lyon, 1996).

Water Holding Capacity (WHC)

To evaluate the water retention capacity, the centrifugation method was used; 5 g of meat were taken, finely chopped and placed in a centrifuge tube (in duplicate), 5 mL of 0.6 M NaCl was added to each sample and shaken with a glass rod for 1 min. Tubes were then left to sit in an ice bath for 30 min, then, they were centrifuged for 15 min at 10 000 rpm. The supernatant was decanted and its volume was measured in a test tube. Finally, the amount of solution retained per 100 g of sample was calculated (Guerrero *et al*., 2002).

Statistical analysis

For this study, a completely randomized design was used, through the GLM procedure SAS®, to determine if there were significant differences between the means of the treatments. Once this difference was proved, the Tukey's test was applied, with a 5% alpha error limit ($p \leq 0.05$). The analyses were performed following the routines described in SAS® user's manual (SAS Institute Inc., 2017).

RESULTS AND DISCUSSION

Nopal fluor concentration did not show a significant effect ($p > 0.05$) on live weight (PV) nor the other carcass variables (CCLC, CCLS, CF, RCC and RCF), which suggests that the additive, in cocentrations up to 1200 mg kg^{-1}, does not modify those variables (Table 2). Badr *et al.* (2019) found that the incorporation of up to 15% prickly pear husk in the diet of broilers increased the final weight, suggesting a positive effect on growth. On the contrary, Moula *et al.* (2019), with 5% and 10% addition of prickly pear cladodes in broiler diets, reported no significant effect on weight. Regarding the yield of carcass, Benteboula *et al.* (2023) found that it decreased when they added a meal, based on prickly pear husk between 20% and 40%, to the diet of broilers. This is, there is variability in the response depending on the type and amount of the additive used.

Results showed that the variables PPRP, LPRO, APRO, PM, AMO and PH were not significantly affected by the concentrations of nopal flour (Table 3). However, a significant increase ($p \leq 0.05$) in gizzard length (LMO) was observed when the 400 mg kg^{-1} concentration of nopal flour was used. It seems that the increase in gizzard length alone does not produce an improvement in slaughter weight. Whereas Betenboula *et al.* (2023) found that when using 10% prickly-pear seed meal in poultry, the weight of the liver, proventriculus and gizzard increased compared to the control. By using such a concentration, the fiber content of the diet increased and stimulated the activity of digestive muscles as a result of the reduction in particle size, which improved digestion through a increased retention time and shortened the grinding time (Tejeda and Kim, 2021).

The effects of nopal flour on the physicochemical properties of quail meat are shown in Table 4, the analyses showed that PF, PDC, RC and MT were not significantly affected ($p > 0.05$) by the treatments. The trait MT, which assesses the ease of cutting during chewing, remained constant regardless of treatment, as it is influenced by the number, diameter, and type of muscle fiber, type of connective tissue, collagen, and amount of intermuscular

Table 2. Effect of the inclusion of nopal flour in the feed on the slaughter variables of Japanese quails.

Treatments	PV (g)	CCLC (g)	CCLS (g)	CF (g)	RCC (%)	RCF (%)
T1	224.46	182.00	167.48	171.12	74.47	74.16
T2	218.04	174.48	159.38	159.30	73.15	73.11
T3	217.60	176.36	159.32	159.52	73.23	73.06
T4	216.26	174.42	159.96	159.95	74.23	74.22
SEM	4.13	3.26	3.19	3.19	0.49	0.50
p value	0.9070	0.8380	0.7768	0.5995	0.7189	0.7580

T1, witness; T2, 400 mg kg^{-1} of nopal flour; T3, 800 mg kg^{-1} of nopal flour; T4, 1200 mg kg^{-1} of nopal flour; PV, live weight; CCLC, non-chilled carcass with viscera; CCLS, non-chilled carcass eviscerated; CF, chilled carcass (24 h *postmortem*); RCC, non-chilled carcass yield; RCF, chilled carcass yield; SEM, standard error of the mean.

Table 3. Organ weights in Japanese quails fed with different added concentrations of nopal flour.

Organs	T1	T2	T3	T4	SEM	p value
PPRO (g)	0.80	2.60	1.36	1.90	0.36	0.3717
LPRO (mm)	17.94	18.94	19.21	18.88	0.36	0.3559
APRO (mm)	8.37	8.14	8.35	8.01	0.37	0.6197
PMO (g)	3.14	3.30	3.46	3.36	0.37	0.6088
LMO (mm)	21.66^{ab}	22.48^{a}	22.31^{ab}	21.07^{b}	0.37	0.0411
AMO (mm)	19.13	18.09	18.45	18.10	0.38	0.3097
PH (g)	4.74	5.14	4.92	4.36	0.38	0.4093

T1: control; T2, 400 mg kg^{-1} of nopal flour; T3, 800 mg kg^{-1} of nopal flour; T4, 1200 mg kg^{-1} of nopal flour; PPRO, proventricule weight; LPRO, proventriculus length; APRO, proventriculus width; PMO, gizzard weight; LMO, gizzard length; AMO, gizzard width; PH, liver weight; SEM, standard error of the mean. Means with different letters between columns are significantly different ($p \leq 0.05$).

fat (Wright *et al.*, 2018). The pH was higher in the control treatment (T1) ($p \leq 0.05$) and decreased with the concentrations of nopal flour. Jankowiak *et al.* (2021) mentioned that a lower pH is correlated with an increase in luminosity (L*) since proteins are denatured and as a consequence the light-scattering properties increase, which coincides with this research; when the pH was lower the meat was brighter due to the effect of nopal flour (Table 4).

The luminosity (L*) of the meat increased ($p \leq 0.05$) in the T3 and T4 treatments, while it decreased with the lowest concentration of nopal flour (400 mg kg^{-1}) and in the control. On the other hand, the intensity of red (a*) was only lower ($p \leq 0.05$) in T2 and was higher in treatments T1, T3 and T4, which could be important since quail is a red meat. Cori *et al.* (2014) found that higher myoglobin content is associated with increased redness in quail meat, chicken meat, and hen meat. Regarding the intensity of the yellow color (b*), it was higher ($p \leq 0.05$) in the control and decreased due to the addition of nopal flour. Treatment

Table 4. Physicochemical characteristics of quail meat supplemented with nopal flour.

Variables	T1	T2	T3	T4	SEM	p value
PF (g)	7.03	7.03	6.80	6.94	0.13	0.9229
PDC (g)	5.55	5.59	5.24	5.39	0.12	0.7425
RC (%)	78.88	79.70	77.32	75.23	1.04	0.4525
MT	14.06	12.15	14.91	12.92	0.49	0.1971
pH	7.27^{a}	6.45^{c}	6.40^{c}	6.72^{b}	0.04	<0.0001
L^{*}	49.64^{b}	50.49^{b}	52.75^{a}	52.61^{a}	0.20	<0.0001
a^{*}	13.26^{a}	12.16^{b}	12.90^{a}	13.11^{a}	0.09	<0.0001
b^{*}	4.09^{a}	2.67^{b}	3.24^{ab}	2.91^{b}	0.15	0.0042
WHC (mL per 100g)	25.61^{b}	36.03^{a}	27.45^{b}	27.72^{b}	1.09	0.0024

T1, control; T2, 400 mg kg^{-1} of nopal flour; T3, 800 mg kg^{-1} of nopal flour; T4, 1200 mg kg^{-1} of nopal flour; PF, chilled leg weight (24 h postmortem); PDC, leg weight after cooking; RC, cooking yield; MT, meat texture; pH, hydrogen potential; L*, luminosity; a*, red; b*, yellow; WHC, water holding capacity; SEM, standard error of the mean. Means with different letters between columns are significantly different ($p \leq 0.05$).

T2 was the one that had the greatest reduction in this intensity (34.72%), while T3 and T4 reduced yellow intensity by 20.79% and 28.86%. Treatment T2 was the one with the highest WHC, which could cause a dilution in yellow pigmentation (Roza *et al*., 2018). This decrease in yellowing may not negatively influence quail meat quality, since it is red; unlike chicken meat, which is white.

The WHC in quail meat was lower ($p \leq 0.05$) in T1, T3 and T4, while meat with the lowest concentration of nopal flour (400 mg kg^{-1}) was the one in which the proteins of the muscle tissue retained the most water quantity. That is, it was the juiciest, which means that at this additive concentration, the antioxidant compounds of the nopal positively influenced the muscle fibers to increase WHC. This finding coincides with Aquino *et al*. (2020) who, by adding antioxidants to the diet of rabbits, also increased WHC. This property is related to the dryness or juiciness of the meat during chewing and the latter is related to meat tenderization (Warner, 2023). The WHC is affected when the pH reaches 5.4, which is the isoelectric point of the myosin protein. This produces retraction of muscle fibrils, thus decresing juiciness of the meat (Sánchez *et al*., 2019). In our research pH ranged from 7.27 to 6.40, therefore the decrease in water holding capacity is not attributed to pH.

CONCLUSIONS

The addition of nopal flour in the quail diet did not modify the weight at the time of slaughter. Neither the yield and weight of both non-chilled and chilled carcasses. It also did not affect the proventriculus weight nor its width, as well as those of gizzard and liver.

In regard to meat quality, nopal flour did not influence the weight of chilled legs, cooking yield or meat texture. However, it is an additive that decreases the pH of the meat, increasing luminosity and accentuating the red color, while the yellowing decreases. In addition, with a concentration of 400 mg kg^{-1} of nopal flour, the water retention capacity increased, resulting in juicier meat.

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Colegio de Postgraduados

Valuation and differentiation attributes in pulque distillate identified by means of discrete choice experiments

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ABSTRACT

Objective: To identify consumer preferences and willingness to pay by assessing the differentiating attributes of distilled pulque.

Design/methodology/approach: The discrete choice experiments methodology (DCE) was used to estimate the willingness to pay (WTP), based on surveys of pulque distillate consumers. The information analysis was carried out with the XLSTAT 2023 software through logistic regression of the willingness to pay data on a choice response with a confidence interval of 95% and with 207 iterations.

Results: The most valued attributes were a low price (MXN $400), artisanal production, and certification. However, a negative marginal WTP was found for origin (Tlaxcala, Mexico), artisanal production, and certification, indicating that some consumers do not perceive clear benefits from these attributes.

Limitations on study/implications: One of the main limitations was the dissemination of this distilled beverage; the highest participation was observed in the central region of the country.

Findings/conclusions: Affordable pricing is the most influential factor for consumers. It is recommended to develop segmentation and communication strategies that highlight the cultural and identity value of pulque distillate, especially regarding certification and artisanal production.

Keywords: attributes, pulque, distilled beverage, choice experiment.

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INTRODUCTION

Pulque is the quintessence Mexican beverage because it has a long tradition since pre-Hispanic times (Erlwein *et al.*, 2013); it is made from mucilage fermentation, popularly known as aguamiel, which is obtained by scraping from the "heart" of the *Agave salmiana* plant after extraction of the apical meristem and rest of 6 to 12 months. The fermentation process is carried out in establishments called *tinacales*, where the *aguamiel* is poured into containers made of materials that range from plastic to those designed in bovine leather, and it is mixed with the "seed" or *pie de pulque* (initial ferment); from that moment, the

fermentation and transformation of *aguamiel* into pulque begins (SADER, 2015). A range of byproducts are obtained from both *aguamiel* and pulque (Astudillo *et al.*, 2022), one of them being the pulque distillate.

In distilled beverages, certain aspects such as the class of distillate and production process make them a difficult and confusing product for consumers to select (Kallas *et al.*, 2012). Therefore, it is important to understand the relationship between properties and attributes of the pulque distillate and their relationship with the consumer's perception.

Intrinsic signals such as flavor, year of distillation, variety of agave (or varieties), and alcohol content; as well as the place of origin, brand, price, awards obtained, containers, and labels are relevant in the agroindustrial market (Cervantes, 2021). As consequence, the variety of distilled alcoholic beverages available in the market makes consumer choices more complex than those of other food products.

There are tools designed to evaluate the preferences of consumers; one of the multi-attribute valuation methods is that of Choice Experiments (CE). This methodology facilitates a more accurate evaluation of esteem for a product, improving the accuracy with which the economic value is determined that consumers are willing to pay for an article that is characterized by its range of tangible and intangible qualities (Barrera-Rodríguez *et al.*, 2019).

Choice experiments have been used to evaluate the preferences of consumers and the willingness to pay for genetically modified foods (Hu, Veeman, and Adamowicz, 2005; James and Burton, 2003; Burton, Rigby, Young and James, 2001), organic foods (Rousseau and Vranken, 2013), meat (Linhai-Wu, Shuxian-Wang, Dian-Zhu, Wuyang-Hu and Hongsha-Wang, 2015; Loureiro and Umberger, 2007; Tonsor, Schroeder, Fox and Biere, 2005), and fresh fruits (Sepúlveda, Ureta, Hernández and Solorzano, 2018; Wongprawmas and Canavari, 2017), among others.

The Choice Experiment is based on the theory of random utility (McFadden, 1974; Thurstone, 1927) and the theory by Lancaster (Lancaster, 1966). The first suggests that individuals associate a utility with each choice alternative, so consumers choose the one that offers them the most utility (McFadden, 1974). Conversely, Lancaster (1966) proposes that the utility of a good can be broken down into the utilities generated by the different attributes of the products.

The research on pulque distillate in this study is a combination of attributes that include information about the place of origin, type of process, certification, distinctions, and price. Therefore, the preferences and willingness to pay by consumers were identified and evaluated by means of the differentiating attributes of pulque distillate.

MATERIALS AND METHODS

A survey was carried out with 207 participants, generated by Microsoft Forms, which was distributed through social networks. The criteria that were considered were: older than 18 years old, connoisseurs or consumers of pulque distillate, and willingness to participate in the study. For an infinite population, the maximum variance was assumed, with a confidence level of 95% and an error margin of 7% (Martínez-García and Martínez-Caro, 2008).

The survey was composed of three sections: 1) in the first part, participants were asked if they were connoisseurs of the beverage, 2) the willingness to pay for a 750 mL bottle of pulque distillate was evaluated by means of discrete choice experiments where the selection of an alternative of preference was proposed, and 3) sociodemographic data about the consumers were asked (place of origin, age, sex, education, occupation and economic income). The selection of attributes and levels was carried out through a bibliographic review, as well as by approaching producers of the pulque distillate, to determine the attributes that they considered important. The attributes of origin, production process, certification, awards and price were selected (Table 1) as the most important for the study.

The attribute of origin was represented by the state where the distillate was elaborated, which was evaluated in four levels: made in the State of Mexico, Hidalgo, Puebla or Tlaxcala. The attribute production process represented the activities and material used for the transformation of aguamiel into pulque and its distillation, including two levels: artisanal and industrial. The attribute of certification was evaluated in two levels: with certification and without certification. The attribute of awards referred to whether the beverage had received any national or international distinction, such as the medals given in the Spirits Selection of the Concours Mondial de Bruxelles, and it was evaluated in two levels: with medal and without medal. Lastly, the attribute price to be paid for a 750 mL bottle was evaluated at three levels: $400.00 MXN, $700.00 MXN, and $1,000.00 MXN.

The experiment included 16 simulation cards, which were calculated (Table 2) through a Fractioned Factorial Design, using the IBM SPSS Statistics Version 29.0.2.0 software. The cards were grouped into pairs with the aim of obtaining eight sets, where the consumer chose between option A, option B, and included the option of "Would not buy any" (Figure 1).

The choice data were analyzed using the Multinomial Legit Model (MLM), which is based on the theory of random utility (McFadden, 1974), and for this purpose, the statistical analysis was conducted with the XLSTAT Version 2023.2.1414 software.

RESULTS AND DISCUSSION

Table 3 provides a summary of the sociodemographic characteristics of participants in the study. A greater willingness to participate was seen from men, with 58.94%, compared to 41.06% from women. The predominant age range was 18 to 29 years old (39.61%), followed by the group that includes the range of 30-39 years with 32.37%, highlighting that these two groups were the most representative.

Table 1. Attributes and levels of the discrete choice experiment.

Attribute	Attribute Levels
Origin	Mexico, Hidalgo, Puebla, Tlaxcala
Production process	Artisanal, Industrial
Certification	Certificate, No certificate
Awards	Award, No award
Price	MXN$ 400, MXN$ 700, MXN$ 1000

Table 2. Cards obtained through SPSS.

ID	Origin	Process	Certification	Award	Price
1	Tlaxcala	Artisanal	NC	A	$ 1000
2	Mexico	Industrial	NC	NA	$ 400
3	Tlaxcala	Industrial	C	NA	$ 400
4	Tlaxcala	Industrial	NC	A	$ 700
5	Mexico	Artisanal	C	A	$ 700
6	Hidalgo	Artisanal	NC	NA	$ 700
7	Tlaxcala	Artisanal	C	NA	$ 400
8	Hidalgo	Industrial	NC	NA	$ 1000
9	Puebla	Artisanal	NC	A	$ 400
10	Mexico	Artisanal	NC	NA	$ 400
11	Puebla	Industrial	C	NA	$ 700
12	Hidalgo	Industrial	C	A	$ 400
13	Mexico	Industrial	C	A	$ 1000
14	Hidalgo	Artisanal	C	A	$ 400
15	Puebla	Artisanal	C	NA	$ 1000
16	Puebla	Industrial	NC	A	$ 400

NC: No certificate, C: Certificate, NA: No award, A: Award.

Figure 1. Example of choice card for pulque distillate.

The population surveyed reported 63.28% with university education level, while 31.40% declared having studied high school. The National Occupation and Employment Survey reported that the average monthly salary in the fourth trimester of 2023 was $5,750.00 (Secretaría de Economía, 2024); regarding the survey respondents, 56.52% have income in the group of $5,000 to $10,000 pesos Mx, while 43.47% declared having

Table 3. Sociodemographic characteristics of consumers (N=207).

Variable	Central	Western	Southern	Total
Gender				
Male	32.37	9.66	16.91	58.94
Female	22.71	8.21	10.14	41.06
Age				
18-29	17.87	8.21	13.53	39.61
30-39	19.81	5.31	7.25	32.37
40-49	12.08	3.86	4.83	20.77
50-59	5.31	0.48	1.45	7.24
Educational level				
Upper Secondary Education	14.98	6.76	9.66	31.4
Higher Education	37.68	11.11	14.49	63.28
Postgraduate Education	2.42	0.00	2.90	5.32
Occupation				
Rural farmer / Agriculturist	0.97	0.48	0.97	2.42
Government employee	1.45	0.00	0.48	1.93
Student	8.21	3.38	6.28	17.87
Industrial worker	19.81	3.86	8.70	32.37
Self-employed	11.59	4.35	6.76	22.7
Other	13.04	5.80	3.86	22.7
Monthly income				
MXN$ 5,000 to MXN$ 10,000	29.95	11.59	14.98	56.52
MXN$ 10,000 to MXN$ 15,000	19.32	4.83	9.66	33.81
MXN$ 15,000 to MXN$ 20,000	2.90	1.45	0.48	4.83
Greater than MXN$ 20,000	2.90	0.00	1.93	4.83

income higher than $10,000 pesos MX. Regarding occupational categories, 32.37% indicated they work as laborers in various companies, while 22.7% declared being independent workers.

From Table 4, the goodness of fit provides several indicators of the model obtained and the variability of the data. The most relevant value is that of χ^2 and R^2 (McFadden) associated with the logarithm rate, which is equivalent to Fisher's F test of the linear model.

In this case, the probability is lower than 0.0001 (Table 5), and it can be concluded that the variables contribute a significant amount of information; in addition, R^2 (McFadden)=0.368, which is a value within the range of 0.10-0.50, considered acceptable in social studies (Ozili, 2022). According to Melo-Guerrero *et al.* (2020), a value of R^2 (McFadden) obtained between 0.2 and 0.4 would be equivalent to R^2 of 0.70-0.90 in ordinary least squares, which indicates a good fit.

The logit model was used to estimate and calculate the coefficient for each variable and to obtain the significance at a value of $Pr>\chi^2$ for those attributes that help to explain the punishment or overprice from consumers. Table 6 shows the attributes and levels analyzed; from these, the price of $400 MXN, origin Tlaxcala, artisanal process, with certification,

Table 4. Statistics of goodness of fit.

Statistic	Independent	Complete
Observations	4968	4968
Sum of weights	4968.000	4968.000
DF	4967	4957
−2 Log-Likelihood	6324.405	3997.252
R^2 (McFadden)	0.000	0.368
R^2 (Cox and Snell)	0.000	0.374
R^2 (Nagelkerke)	0.000	0.519
AIC	6326.405	4019.252
BIC	6332.916	4090.870
Iterations	0	12

Table 5. Hypothesis test.

Statistic	DF	χ^2	p-value
−2 Log-Likelihood	10	2327.153	<0.0001
Score	10	2094.052	<0.0001
Wald	10	1296.979	<0.0001

Table 6. Standardized coefficients for WTP.

Attribute	Coefficient	Standard Error	p-value	Lower Limit (95%)	Lower Limit (95%)
Interception	−2.822	0.107	<0.0001		
Hidalgo	0.000	0.000			
Puebla	0.034	0.168	0.838	−0.294	0.363
Tlaxcala	1.237	0.171	<0.0001	0.902	1.572
Mexico	0.419	0.153	0.006	0.119	0.719
Industrial	0.000	0.000			
Artisanal	0.910	0.097	<0.0001	0.719	1.101
C	2.378	0.288	<0.0001	1.813	2.943
NC	0.368	0.290	0.205	−0.201	0.937
A	−0.318	0.214	0.137	−0.737	0.101
NA	−1.280	0.200	<0.0001	−1.672	−0.889
400	2.274	0.120	<0.0001	2.040	2.509
700	0.155	0.124	0.213	−0.089	0.398
1000	0.000	0.000			

and without medal were the characteristics that were statistically significant and because of which consumers are willing to pay extra or receive compensation.

The marginal WTP is commonly expressed as the negative rate of the coefficient of the attribute unrelated to the price and the price coefficient. This willingness to marginal payment is reported in Table 7, and it shows that consumers expect to receive economic

Table 7. Marginal willingness to pay for pulque distillate.

Attribute	Coefficient	Marginal WTP
Hidalgo	0.000	0.00
Puebla	0.034	−0.02
Tlaxcala	1.237	−0.54
Mexico	0.419	−0.18
Industrial	0.000	0.00
Artisanal	0.910	−0.40
C	2.378	−1.05
NC	0.368	−0.16
A	−0.318	0.14
NA	−1.280	0.56

compensation when the distillate is originally from Tlaxcala (−$0.54 MXN), is obtained through artisanal process (−$0.40 MXN), or has certification (−$1.05 MXN); or they expect to pay extra when it does not have any medal such as the CMB ($0.56 MXN).

Facing this situation, some of the factors that affected the consumer's perception and influenced the WTP are ignorance from participants, because they were not familiar with the terms used or considered them irrelevant since it was a study where both connoisseurs and consumers were considered; some of the participants do not belong to the segment that values these attributes. The consumers who valued most the price valued less the artisanal process or the certifications.

CONCLUSIONS

The study showed that consumers have different levels of willingness to pay because of the attributes of the pulque distillate, highlighting that not all the attributes are equally valued. This emphasizes the importance of understanding the priorities and perceptions of the market and the consumers. The most influential attributes were the price, the origin (especially Tlaxcala), the process of artisanal production, and the certification. However, characteristics such as awards did not obtain a significant positive valuation, which could be related to lack of awareness or perception of irrelevance from consumers. Considering these aspects, more participants from different regions and sociodemographic profiles could be incorporated, with the purpose of providing a more representative view of the market, as well as implementing communication strategies that explain the value of attributes such as certification, artisanal process, and awards which could increase the consumer's valuation.

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Colegio de Postgraduados

Economic valuation of drinking water quality in Aguascalientes, Ags, Mexico

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ABSTRACT

Objective: To estimate the economic valuation of inhabitants of Aguascalientes, Mexico, to improve the quality of the drinking water service.

Design/methodology/approach: The contingent valuation method was used through a questionnaire that incorporated socioeconomic variables and questions about willingness to pay. It was applied to a sample of n=160 users, selected with simple random sampling in the municipality of Aguascalientes.

Results: The results show that heads of family are willing to pay on average $189 pesos monthly to contribute to improving the quality of the drinking water service; likewise, variables such as days when they receive water, perception of the fee, age, improvement of the quality, and sufficiency of the water they receive were identified as relevant in the willingness to pay. Factors such as marital status, occupation, and gender were not relevant for such willingness.

Findings/conclusions: There is high acceptance among heads of family towards initiatives to improve the drinking water quality, which indicates a significant awareness regarding the importance of preserving the quality of the water resource and a tangible willingness to participate in its recovery.

Keywords: Contingent valuation, willingness to pay, water policy.

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INTRODUCTION

Factors such as climate change associated with the increase in population, the use of water for waste dissolution, and the contamination generated by anthropogenic activities contribute to its scarcity and deterioration, making it inappropriate for most uses (Naciones Unidas, 2024).

Distribution problems, as well as difficulties related to the quality and quantity of water, continue to affect rural zones and regions with high water pressure. The limited availability of this resource is closely linked to poverty, with repercussions in health, food production, and gender equity, which finally lead to its exclusion (Soares, 2021).

Something to emphasize is that the main consumers of water in Mexico are agriculture with 76% and public supply with 14%, while the remaining 10% is distributed between industry and thermoelectric plants (INEGI, 2020a).

In 1995, the municipality of Aguascalientes had a population of 582,827 inhabitants, whereas by 2020 the population was 948,990 inhabitants. This shows that demographic growth in 30 years is a direct factor on the overexploitation of aquifers (INEGI, 1995; INEGI, 2020b).

In October 2023, the municipality acquired the service of drinking water and sewage. Based on an integral analysis about legal, technical, commercial and financial aspects, the Integral Waters Model of Aguascalientes (Modelo Integral de Aguas de Aguascalientes, MIAA, 2023) emerged, to operate the services.

CONEVAL places Aguascalientes as the number one state in the Mexican Republic in drinking water coverage at the national level (99.1%), followed by Colima (99%) and Tlaxcala (98.9%) (Alva, 2021). In contrast with the national average of 73% of the households with drinking water piping, Aguascalientes has 93.7% households with this service (INEGI, 2017); however, it faces specific challenges in terms of availability and quality of the drinking water. Located in a semi-arid region, its main source of supply comes from the Aguascalientes Valley and underground wells, which have shown signs of overexploitation and deterioration of the water quality (CONAGUA, 2024).

The growing water demand, together with the contamination of important bodies of water in the state, such as the San Pedro River, have generated worry over the sustainability of the resource and the need to improve infrastructure and management of drinking water in the state. Based on this, the economic valuation of inhabitants in Aguascalientes, Mexico, was estimated, to improve the quality of the drinking water service.

MATERIALS AND METHODS

The state of Aguascalientes is made up of 11 municipalities and covers a surface area of 5,615.7 km^2, which represents 0.3% of the national territory. Its population amounts to 1,425,607 inhabitants, equivalent to 1.1% of the national total (INEGI, 2020c). Based on the census carried out by INEGI (2020b), the municipality of Aguascalientes had a population of 948,990 inhabitants.

The zone where the study was conducted is found along the Aguascalientes Valley (0101), which belongs to the Administrative Hydrological Region VIII Lerma-Santiago-Pacífico and stretches almost completely within the municipality of Aguascalientes, with a territorial extension of 5,617 km^2. The region's climate is mainly semi-dry, with mean annual temperature of 17.4 °C and average precipitation of 526 mm (INAGUA, 2023).

Willingness to pay (WTP) by the heads of households of the municipality for water quality improvement in the drinking water service was estimated through the method of contingent valuation (CVM). This technique has the aim of quantifying individual WTP in face of changes in the quantity or quality of goods and services, as well as identifying the influence of socioeconomic variables on that willingness. According to Young and Loomis (2014), the CVM is especially adequate to value non-commercial benefits of water, such as the ones that are analyzed in this study. For data collection, a simple random sampling for finite populations was used, using the formula suggested by Aguilar (2005), and considering the variables of level of confidence and margin of error suggested by Cochran (1985), expressed in the following way:

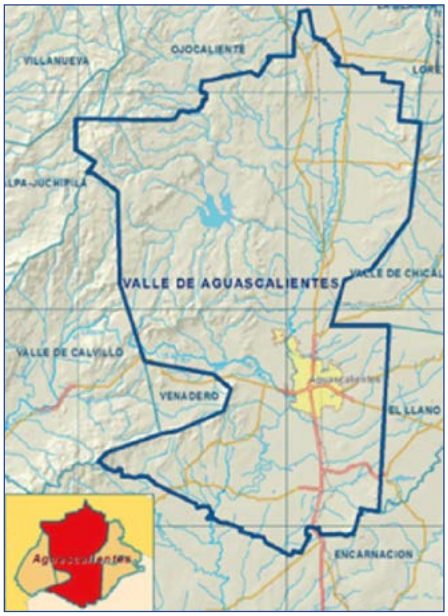

Figure 1. Zone of the Aguascalientes Valley. Source: CONAGUA.

$$n_0 = \frac{Z^2 Npq}{(N-1)d^2 + Z^2 pq} \quad (1)$$

Where: n_0 represents the size of the sample (96 surveys), while N corresponds to the total size of the population (948,990 inhabitants). The level of confidence used was 95% (Z=1.96). Parameter d reflects the absolute level of accuracy (0.1), p corresponds to the proportion of the phenomenon estimated in the population referenced (0.5), and q denotes the proportion of individuals in the reference population that does not present this phenomenon (0.5).

A pilot sample was defined in form of open question, where the participants were requested to indicate the monetary amount that they would be willing to pay without predefined options.

The objective of this phase was to establish the superior and inferior limits of WTP. From the results obtained, the final version of the questionnaire was made with a simple dichotomous format where the questions about willingness to pay included response options, which allowed to reduce possible biases. Based on the data from the pilot test, a series of fees was established that were proportionally distributed between the surveys applied. The values considered were: 50, 100, 150 and 200 pesos monthly.

The survey was made up of four sections and 26 questions. The first section refers to data of control and classification of the questionnaires, ensuring that the questionnaires are consistent and the data are structured adequately for their analysis. The second section of the survey seeks to obtain information about aspects related to the water resource, how they perceive the quality, and the sufficiency of drinking water. In the third part, interview respondents are explained the problems associated with using poor quality water for human consumption. Once this scenario is explained, there are questions about the monetary willingness to pay for a project to improve the quality of the drinking water service, and in case it is negative, interviewees are asked about the reasons. In the last section, the socioeconomic information of the survey respondent is gathered.

The final sample used in the study consisted of n=180 validated questionnaires, after having dismissed 20 for various reasons that are explained next. During the validation process, it was identified that eight interview respondents presented a behavior of protest when asked about their WTP; that is, although they answered affirmatively, they refused to indicate a specific amount from the options proposed in the questionnaire. Also, six questionnaires were excluded because the interviewees showed strategic behavior by declaring exceedingly high amounts. The six remaining questionnaires were eliminated due to mistakes made while completing them, by the staff in charge of applying them. However, n=160 questionnaires were considered to carry out the study.

The information gathered from the total number of questionnaires was captured in a MS Excel spreadsheet, while the econometric analysis of the database was carried out using the NLogit software through the method of maximum authenticity to estimate the probability of willingness to pay. Different models were evaluated with the objective of defining which variables more accurately explain WTP related with improving the quality of water from the San Pedro River. For this purpose, a Logit type model was used.

The econometric model that WTP describes to improve the quality of the river's water can be expressed in the following way:

$$P(SI) = \beta_0 + \beta_1 AMOUNT + \beta_2 AGE + \beta_3 PERCUO + \beta_4 DAYS + \beta_5 CAL + \beta_6 SUF + \varepsilon \tag{2}$$

In this equation, P(SI) is a dichotomous dependent variable that indicates the probability of the answer being YES=1 or NO=0, while the other variables are independent (see Table 1).

Calculating the average WTP was carried out using the following formula:

$$WTP_{media} = \frac{\sum_{i=1}^{n} \dfrac{\beta_0 + \beta_2 AGE + \beta_3 PERCUO + \beta_4 DAYS + \beta_5 CAL + \beta_6 SUF}{\beta_1 AMOUNT}}{n} \tag{3}$$

RESULTS AND DISCUSSION

The results are presented in the following order: 1) profile and characteristics of the survey respondents, and 2) characteristics of the model. The surveys were conducted completely in the municipality of Aguascalientes, because most of the population in the state resides the capital municipality. Initially, results from the main descriptive statistics corresponding to the most relevant variables from the study were presented (Table 2).

Table 1. Definition of the variables used in the study.

Variable	Definition	Unit	Scale
WTP	Willingness to pay (NO=0, YES=1)	Dichotomous	Ordinal
AMOUNT	Amount available for payment	Pesos	Discreet
AGE	Age of interviewee	Years	Discreet
PERCUO	Perception of water rates (Low=0, Adequate=1, High=2)	Dichotomous	Ordinal
DAYS	Days per week with access to drinking water (1 to 3=0, 3 to 5=1, Every day=2)	Dichotomous	Ordinal
CAL	Perception of water quality (Poor=0, Fair=1, Good=2)	Dichotomous	Ordinal
SUF	Sufficiency of water supply (NO=0, YES=1)	Dichotomous	Ordinal

Source: Prepared by the authors.

Table 2. Descriptive statistics of the research variables.

Variable	Average	Standard deviation	Maximum value	Minimum value	Coefficients
WTP	0.6062	0.4901	1	0	9.3241
AMOUNT	125	56.0772	200	50	−0.0147
AGE	41.5688	13.1233	73	18	−0.0600
PERCUO	0.9687	0.6483	2	0	−3.1602
DAYS	0.7625	0.8047	2	0	1.7394
CAL	0.9062	0.8452	2	0	−1.2690
SUF	0.3687	0.4839	1	0	−3.1179

Source: Prepared by the authors.

The information was processed with the NLOGIT software using the maximum likelihood method to estimate the probability of willingness to pay. Different Logit-type models were evaluated with the objective of identifying the variables that best explain WTP due to the improvement of water quality of the public service.

In discrete binary selection models, it is common to use an average analogous to the R^2 coefficient of determination, with the aim of evaluating the general degree of adjustment of the model. This statistic is known as pseudo-R^2 by McFadden, and it is calculated in the following way (Valdivia *et al.*, 2009):

$$pseudo\ R^2 = 1 - \frac{LnL}{LnL_0} \tag{4}$$

Where: LnL represents the likelihood function under the restriction that all the parameters are equal to zero, and LnL_0 corresponds to the same function evaluated for the model without restrictions, calculated through the following expression:

$$LnL_0 = n\left[PLnP + (1-P)Ln(1-P)\right] \tag{5}$$

Where: *P* represents the proportion of affirmative responses obtained in the survey regarding the question about WTP, and *n* corresponds to the size of the sample. Making the pertinent calculations, the result was that: $LnL_0=-107.26$; when *LogL* was solved, the value was: $LnL=-48.90$.

Substituting the data in (5), a pseudo-R^2 of $0.5440 \approx 0.54$ was obtained. Therefore, the model was considered acceptable, since in an adjustment higher than 0.1, it is considered satisfactory (Bateman, cited by Valdivia *et al.*, 2009).

The model of estimated probability $[P(SI)]$ was:

$$P(SI) = 9.3241 - 0.014AMOUNT - 0.06AGE - 3.16PERCUO + 1.7394DAYS - 1.269CAL - 3.1179SUF$$

To analyze each parameter of the model, the marginal effects were considered, which are a measurement of the instantaneous effect in face of unitary change in an independent variable, which it has on the prediction of the probability that P_i is equal to one, when all the variables remain constant.

Assuming the logistic distribution as (6): This way, the marginal change rate in the probability of occurrence of the event in face of changes in the explicative variables was (7) (Gujarati, 2004):

$$P_i = \frac{e^{z_i}}{1+e^{z_i}} \tag{6}$$

$$\frac{dP_i}{dX_i} = \beta_1 P_1 (1-P_1) \tag{7}$$

After codifying the information of the surveys in the NLOGIT software, the marginal effects were obtained (see Table 3); based on the exit of the software, the most influential variable in WTP was perception of the fee (PERCDECUO). That is, the perception that survey respondents have about the perception of the fee is the factor that most reduces the probability of them being willing to pay it; therefore, if they consider that the fee is unfair or high, this impacts their decision strongly, with 63.6%. However, as the amount of the fee increases, the probability of response decreases by approximately 0.297% for each monetary unit. On the other hand, for each additional year in age, the probability of positive response decreases 1.2%; that is, the older survey respondents are less prone to pay. Likewise, a worse perception of the water quality reduces the probability of payment by 25.5%, which could be because they do not want to pay for a service of bad quality. In contrast, if they consider that water is not enough, their WTP decreases by 62.5%; that is, if they do not receive enough, they are not willing to pay. However, for each additional day when they receive water, the probability increases by 34.9%; that is, with greater continuity in the service, the WTP will be higher.

Table 3. Marginal effects.

Variable	Comments
CONST	1.87557
AMOUNT	−.00297
AGE	−.01208
PERCUO	−.63569
DAYS	.34989
CAL	−.25527
SUF	−.62554

Source: Prepared by the authors.

As presented in Figure 2, perceptions about the cost of the drinking water service directly influenced people's WTP. Those who perceived the fee as "high" had a negative average WTP of −84.66, which indicates a lower willingness to carry out additional payments. On the other hand, those who considered that the fee was "low" showed a significantly higher average WTP, of 454.49, which reflects greater willingness to contribute economically. In the case of the people who qualified the fee as "average", the average WTP was 178.12, representing an intermediate point between the two extreme opinions. This translates into how individual perceptions of the cost can notably modify the attitude towards additional payments.

The responses reflected that the average WTP varies according to the frequency of access to drinking water. Those who indicated having limited access to drinking water during 1 to 3 days per week showed higher average WTP, with a value of 235.89. On the other hand, the people who reported having access 3 to 5 days per week reflected a lower average WTP, of 152.77. Finally, those who have daily access to drinking water represented the lowest average WTP, of 135.87.

This suggests that the people with significant restrictions on the supply are more motivated to contribute economically, probably due to the perception of greater need to improve access to the resource. In contrast, those with more regular supply have lower willingness to make additional payments, possibly because they perceive that their basic needs are already being covered.

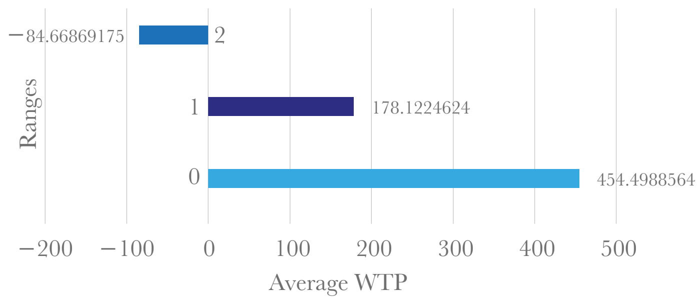

Figure 2. Perception of the average fee.
Source: Prepared by the authors.

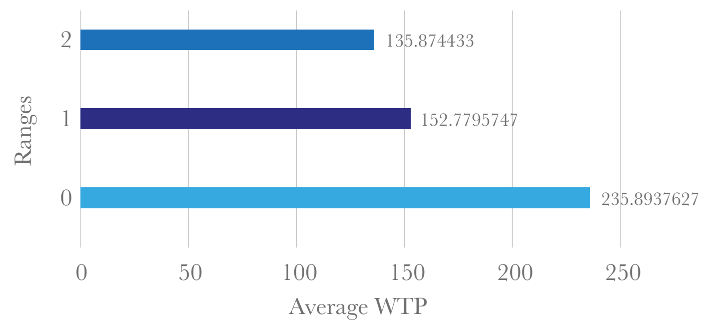

Figure 3. Average WTP of the variable Days.
Source: Prepared by the authors

The data obtained indicated that perception of the quality of drinking water significantly influences WTP. The people who consider that drinking water is of poor quality present the highest average WTP, with a value of 308.16. This could reflect a greater preoccupation over improving the quality of the water resource. On the other hand, those who perceived water as being of good quality have a much lower average WTP, of 91.70, possibly because they are already satisfied with the service and do not feel the urgent need to contribute to its improvement. Those who grade it as having "average" quality show an average WTP of 126.37, between both extremes.

This can be translated into a higher willingness to pay when people perceive significant problems in water quality, which makes a connection between perception of the resource and interest in investing in its improvement.

The average WTP was calculated through:

$$WTP_{media} = -\frac{\alpha}{\beta} = -\frac{2.7927}{-0.0147} = 189.39 \approx 189$$

Where: α is the sum of the coefficients of independent variables multiplied by their average values, including the ordinate of the origin 0 (β_0), and β corresponds to the coefficient of the variable PRECIO, which has a negative sign.

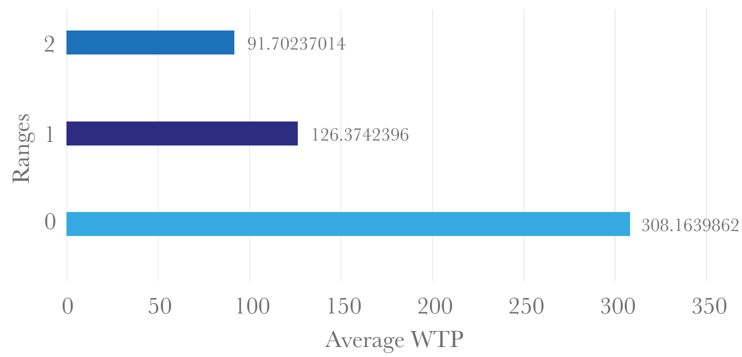

Figure 4. Average WTP of the variable Quality.
Source: Prepared by the authors.

The amount 189 indicates the compensated variation or willingness to pay in Mexican pesos that each of the heads of households from the municipality of Aguascalientes would be willing to offer monthly for improvement of the quality of the drinking water service.

When the results are extrapolated to the totality of the inhabitants of the three communities used in the study, a monthly income flow of $179,359,110 destined to financing the project was estimated.

The results obtained are consistent with previous studies, which have applied the method of contingent valuation, such as the study conducted in the National Molino de Flores Park (Tudela *et al.*, 2011), where an annual conservation value of USD $384,000 was estimated and an entry fee of $24 pesos MX. As in that study, it was identified that variables such as income and environmental perception influence WTP. In contrast, variables such as marital status, occupation and gender did not show a significant relationship, which suggests that WTP responds more to the direct experience with the service and perception of the resource, than to general demographic characteristics.

On the other hand, when comparing with the study conducted in Valle de León, Guanajuato (Valdivia *et al.*, 2022), where monthly WTP was estimated to be $182 pesos MX, also using the method of contingent valuation and a Logit-type model. Both cases show similar values, which suggests that, despite regional and demographic differences, there is a common sensitivity around water quality and the willingness to finance improvements. In León, the total estimated annual value is $1,034 million, representing a solid basis to evaluate investments in infrastructure, which could be replicated in contexts such as Aguascalientes if similar scales and conditions are considered. The estimation of the economic value of the drinking water service offers a useful quantitative basis to support the formulation of policies related to the use of water management. The willingness to pay obtained could represent a potential source of financing to implement such policies and actions to improve the quality of the public service.

CONCLUSIONS

Inhabitants in the municipality of Aguascalientes perceive and are aware of the problem of water contamination and scarcity, so 60.6% of the interview respondents pointed out that they agree with the WTP to improve the quality of the drinking water service. The variables that explain further the probability of an affirmative response to WTP were the days that they receive water, their perception of the fee, age, improvement of the quality and sufficiency of water that they receive, which are relevant in WTP. In contrast, factors such as marital status, occupation and gender were not relevant with such willingness. The economic valuation by heads of households was $189 pesos monthly. When results are extrapolated to the heads of family of the municipality of Aguascalientes, it is estimated that total willingness to pay would reach $179,359,110 pesos MX monthly.

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Colegio de Postgraduados

Effect of storage time on the physicochemical characteristics of apple paste

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ABSTRACT
Objective: To produce apple paste (ate) and evaluate its acceptance and physicochemical changes during storage.
Design/Methodology/Approach: Golden Delicious apples were processed to obtain the apple paste. The product was molded, cooled, cut, packaged, and stored at room temperature. A sensory analysis was conducted with 29 panelists to assess consumer acceptance.
Results: Soluble solids, titratable acidity, moisture content, texture, and color were measured at time 0 and after 1, 2, and 3 months. The apple paste maintained its quality after 3 months of storage, received no negative feedback, and achieved 89.65% acceptance.
Findings/Conclusions: To maximize the apple harvest in the state of Chihuahua, it is essential to raise awareness of the importance of adding value to apples through the production of paste.

Keywords: Apple, paste, storage, shelf life, sensory analysis.

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INTRODUCTION

The state of Chihuahua is the leading apple producer in Mexico, with a production of 624,696 tons (SIAP, 2020). The apple-growing region in Chihuahua includes the municipalities of Cuauhtémoc, Álvaro Obregón, Bachíniva, Guerrero, La Junta, and Namiquipa, which account for the highest apple production (Arredondo Bustillos *et al.*, 2021; Ramírez Legarreta *et al.*, 2011). Apple commercialization is mainly in fresh form, and its distribution is primarily domestic.

During apple production, losses of up to 25% are estimated due to pre- and postharvest handling and storage conditions. When apples do not meet quality standards —because they are overripe or show mechanical damage (caused by insects or hail)— they are classified as cull fruit, which is allocated to the agroindustry at low cost and, in some cases, discarded because transportation and redistribution are not economically viable (FAO, 2011). This fruit can be utilized through processing to obtain high-quality, nutritious, and safe products.

Added value in the agricultural sector consists of transforming raw materials into products with higher commercial value while maintaining quality and extending shelf life (Moctezuma López *et al.*, 2011). Apples are highly versatile fruits, making them suitable for a wide range of processes and products. One simple, profitable, and easy-to-implement alternative is the production of apple paste (ate in Spanish), a traditional sweet highly valued in national and regional markets. Ate is a fruit paste prepared by cooking fruit pulp with sugar until a thick mass is obtained, which solidifies upon cooling (Medellín-Cruz *et al.*, 2019). The desired consistency of apple paste is achieved due to the high pectin content naturally present in the fruit. When fruit pulp is combined with sugar and heated, pectin forms a three-dimensional gel network, resulting in a thick and firm texture (Said *et al.*, 2023). Apple paste is a microbiologically stable product because sugar acts as a preservative; by dissolving in the water present in the fruit, it reduces the amount of free water available for microorganisms. This hinders the growth of bacteria, yeasts, and molds, which require water to proliferate (Jay *et al.*, 2005; Jakubczyk *et al.*, 2021). Ate or fruit paste is mainly produced with fruits such as guava and quince; however, apples have the desired characteristics to serve as raw material for the production of this sweet. Nevertheless, little information is available on apple paste and its properties, which may be influenced by storage time. Therefore, the aim of this study was to produce apple paste, evaluating its acceptance and the effect of storage on its physicochemical properties.

MATERIALS AND METHODS

Raw material

Golden Delicious apples were used, obtained directly from an orchard in the Cuauhtémoc region, Chihuahua.

Study area

This study was conducted at the Faculty of Agrotechnological Sciences, Cuauhtémoc campus, in collaboration with the Technological Institute of Cd. Cuauhtémoc.

Apple processing

The process began with the cleaning and disinfection of equipment, utensils, and the production area. Apples were disinfected using sodium hypochlorite (10 ppm) and then transferred to the sorting table, where pieces unsuitable for consumption were discarded. The chopped apples were weighed, crushed, and processed in a semi-industrial pulper to obtain apple puree, which was stored in 20-kg barrels at −18 °C. Once the apple puree was obtained, the production of apple paste proceeded.

Apple paste production

Approximately 40 kg of apple puree were poured into a semi-industrial kettle with an automatic stirrer and heated with steam. The puree was heated for 30 minutes, after which 22.5% sugar was added. Following one hour of stirring, 0.5% pectin and 0.1% citric acid were incorporated. The apple puree reached a temperature of 90 °C, sufficient to eliminate pathogenic bacteria. During the cooking process, soluble solids were continuously monitored using a refractometer until a value of 68 °Bx was reached. Once the desired point was achieved, the mixture was poured into trays and allowed to rest for 24 hours. After this period, the product was unmolded, cut, packaged, weighed, and labeled, and subsequently stored in a cool, dry environment at room temperature. Some images of the process and the final product are shown in Figure 1.

Yield determination

The yield of apple paste was calculated as the ratio between the weight of the finished product and the weight of the raw material, according to Equation 1:

$$Yield(\%) = \frac{(Weight\ of\ final\ product)}{(Weight\ of\ raw\ material)} \times 100 \quad (1)$$

Sensory evaluation

An affective sensory test was conducted to assess the acceptance of apple paste. Affective tests allow researchers to determine whether consumers accept a product for consumption, would purchase it, or how much they like it (Severiano-Pérez, 2019). The evaluation was performed using freshly prepared apple paste in the sensory analysis laboratory of the Technological Institute of Cd. Cuauhtémoc, following the guidelines established for this type of test. The analysis was carried out by a panel of 29 young consumers, both men and women (aged 18-20), without prior training, who evaluated the paste according to their level of liking. A 5-point hedonic scale was used, with the levels of liking shown in Table 1.

Figure 1. Images of the apple paste production process.

Table 1. Hedonic scale used for the sensory analysis of apple ate.

1	2	3	4	5
Dislike very much	Dislike moderately	Neither like nor dislike	Like moderately	Like very much

Moisture content of the samples was determined using the AOAC method 934.06. Acidity, expressed as citric acid, was determined by titration with NaOH according to the method reported by Salvatori *et al.* (1998). Total soluble solids were analyzed using a digital refractometer (Atago, USA). Texture was measured using a Brookfield Texture Analyzer, model CT3, with a 7 mm diameter flat metal probe, via direct reading. Firmness was expressed in Newtons. For color, CIELab parameters (L*, a*, and b*) were determined to calculate the color difference (ΔE) using a Minolta CR-400 colorimeter and Equation 2,

$$\Delta E=\left(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2}\right)^{1/2} \quad (2)$$

where: $\Delta L^{*}=L^{*}$ sample $-$ L^{*} control, $\Delta a^{*}=a^{*}$ sample $-$ a^{*} control, and $\Delta b^{*}=\mathrm{b}^{*}$ sample $-$ b^{*} control.

All measurements were carried out in triplicate, and the results are presented as mean values.

Statistical analysis

Analysis of variance (ANOVA) and Tukey's test were performed at a 95% confidence level to identify significant differences between treatments, using Minitab 19 software (Statistical Software, USA).

RESULTS AND DISCUSSION

Yield

Figure 1 shows the apple paste obtained, which exhibited a firm and homogeneous consistency with amber hues characteristic of the cooking process. These features are similar to commercial quince and guava products. Although it is possible to clarify the color using chemical additives, this study opted for producing a more natural product, avoiding excessive use of acidulants and synthetic colorants. A yield of 55% was obtained, due to weight loss during the cooking process from water evaporation. In addition, the pulping process, which allows partial removal of the peel and seeds, also contributes to reduced yield. The value obtained falls within the range reported for fruit pastes (Kader, 2002).

Physicochemical properties of apple paste

The physicochemical parameters of freshly prepared apple paste and at different storage times are shown in Table 1. No significant differences were observed in total soluble solids, indicating that the sweetness of the paste remains constant during storage. The soluble solids values are similar to those reported for pastes made from other fruits, as reaching 67 °Bx is a process requirement to achieve the desired consistency. Acidity showed only minimal changes, suggesting that the product's acidic taste is also preserved. The acidity values (∼1%) are slightly lower than those reported for quince past (Najman *et al.*, 2023). The results obtained for total soluble solids and titratable acidity are consistent with those reported by Haroon *et al.* (2024) for apple jam.

The texture of the paste increased during storage, which could be related to moisture loss processes, as observed by Pérez-López *et al.* (2016) in processed fruit products. This result is consistent with a 10% decrease in moisture content of the samples (Table 2), which likely contributed to the increase in product firmness. Lower moisture content implies a reduction in water activity, decreasing the likelihood of microbial growth and consequently extending the shelf life of apple paste (Jakubczyk *et al.*, 2021). Color changes in the paste became more pronounced from the second month and intensified during the third month, which could be attributed to oxidation or non-enzymatic browning reactions, common in sugar-rich foods (Lyu *et al.*, 2018). However, the characteristic amber-brown color remained appreciable in the product. The shelf life of apple paste produced without preservatives can extend up to three months or more; however, it is essential to evaluate its physicochemical, microbiological, and sensory characteristics after this period to ensure safety and quality.

Sensory analysis

Figure 2 shows images of the sensory analysis laboratory where the evaluation of freshly prepared apple paste was conducted. The images illustrate the process from sample preparation to the individual booths used by panelists to perform the sensory tests.

The results of the sensory analysis carried out with a panel of 29 participants are presented in Figure 3. A 5-point hedonic scale was used, ranging from "dislike very much" to "like very much."

The results indicate a high sensory acceptance of apple paste, with 26 out of 29 panelists expressing liking for the product: 14 reported "like very much" and 12 "like moderately." Only 3 participants remained neutral ("neither like nor dislike"), and none expressed dislike. This absence of negative responses suggests that the formulation is sensory balanced, which is consistent with the findings of Mesías *et al.* (2021), who observed that more natural products tend to achieve higher acceptance. Overall, apple paste demonstrated a

Table 2. Physicochemical properties of apple paste at different storage times.

Parameters	Storage time (months)			
	0	1	2	3
Soluble solids (°Bx)	$67.36^{a}\pm0.70$	$67.13^{a}\pm0.90$	$69.96^{a}\pm0.25$	$67.00^{a}\pm0.80$
Titratable acidity (%)	$1.00^{abc}\pm0.03$	$0.80^{ab}\pm0.14$	$1.07^{ac}\pm0.02$	$0.96^{abc}\pm0.01$
Moisture content (%)	$39.29^{a}\pm1.00$	$39.27^{a}\pm1.85$	$35.47^{b}\pm0.86$	$35.77^{b}\pm0.64$
Firmness (N)	$4.77^{a}\pm0.11$	$5.81^{a}\pm0.15$	$8.54^{b}\pm0.61$	$10.33^{c}\pm1.42$
Color difference (ΔE)	--	0.751^{a}	3.179^{b}	4.088^{c}

Different letters indicate significant differences ($p\leq0.05$) in the parameters with respect to storage time (Degrees of freedom: treatments=3, groups=8).

Figure 2. Images of the sensory analysis conducted on apple paste

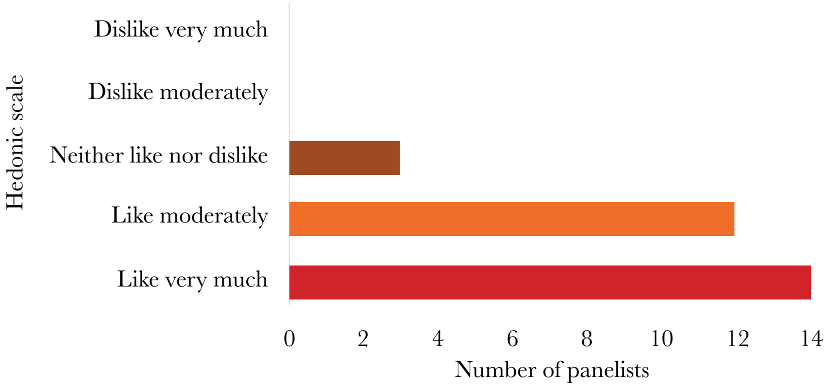

Figure 3. Results of the sensory evaluation of apple paste using a hedonic scale

favorable sensory profile, with potential for market acceptance, particularly among young consumers.

CONCLUSIONS

Apple paste was obtained with flavor, color, and texture characteristics that met the expectations of 89.65% of the panelists, with no negative evaluations, reinforcing the quality of the product. Apple paste retained its flavor characteristics during three months of storage, while texture, moisture content, and color showed minimal changes that did not affect product quality. The processing of apples to produce paste resulted in a yield of 55%, making it a viable alternative to add value to apples that do not meet commercial quality standards. This strategy not only contributes to the reduction of postharvest losses but also represents an opportunity for the development of productive and entrepreneurial projects in rural communities, promoting the full utilization of local resources.

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Colegio de Postgraduados

Impact on the leaf moisture and chlorophyll content in *Bursera bipinnata* (DC.) Engl. from the extraction of resin

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ABSTRACT

Objective: To establish the variables of the resin extraction method that have the greatest influence on the leaf moisture and chlorophyll content in *Bursera bipinnata*, through correlation analysis and multiple linear regression, to determine the physiological impact.

Design/methodology/approach: The experimental site was developed in the Los Sauces micro watershed, in Morelos, Mexico, with a random sample of $n=70$ trees. The explicative variables corresponded to the extraction method. The response variables were the Normalized Difference Moisture Index (NDMI) and the Green Chlorophyll Index (GCI).

Results: The resin production is correlated with the NDMI in October ($r=-0.287$; $p=0.016$). The incision depth was correlated with the GCI in August ($r=-0.438$; $p=0.001$) and October ($r=-0.300$; $p=0.012$). The resin production and the incision depth were factors associated with the NDMI in October ($R^2=0.09$; $p=0.041$). Meanwhile, the incision depth and the total number of resin tapping faces were factors associated with the CGI in August ($R^2=0.204$; $p=0.05$) and October ($R^2=0.106$; $p=0.023$).

Limitations on study/implications: The results are limited to the period and sampling site; it is recommended to replicate the study in other ecological conditions.

Findings/conclusions: Finally, it is necessary to limit the incision depth, as well as the number of resin tapping faces, especially under drought conditions, since this would favor their sustainable exploitation.

Keywords: Copal, non-timber forest products, physiological impact, NDMI, GCI.

INTRODUCTION

The collection of non-timber forest products (NTFP) is an activity of economic, social and ecological importance in Mexico, especially in dry tropical forests. Among NTFP, resins obtained from the genus *Bursera*, such as copal, represent a strategic resource for rural communities. *Bursera bipinnata* (DC.) Engl. stands out from this group because of the volume and quality of the resin it produces (Medina-Lemos, 2008).

The species of *Bursera* present the capacity to exude resin immediately after tissue damage, phenomenon that constitutes a compositional defense mechanism against herbivores and pathogens (Becerra *et al.*, 2001). This resin contains bioactive compounds such as terpenoids and lignans, whose biosynthesis implies a high energetic cost for the plant (Marcotullio *et al.*, 2018; Pallardy, 2008). On the other hand, resin production as a defense mechanism is activated by stress signals in face of tissue damage, induced by reactive oxygen species (ROS), electrical signals, calcium (Ca^{2+}), and jasmonic acid (Choi *et al.*, 2017).

The traditional extraction of resin from *B. bipinnata* is conducted during the rainy season, through repeated incisions in trunks or branches, with metallic tools, and collected through in maguey leaves or plastic bottles (Montúfar-López, 2016). Although this practice has cultural and economic importance, its intensive execution can compromise key processes such as growth, reproduction and regeneration of the tree, in addition to exposing the plant to exogenous stress factors (Huot *et al.*, 2014).

Various studies have documented negative physiological effects in resin-producing species subjected to frequent extraction, including reductions in nutrient content, carbohydrate reserves, and leaf area, as well as in the reproductive capacity (Abad-Fitz *et al.*, 2022; Khan *et al.*, 2018; Mengistu *et al.*, 2012; Montúfar-López, 2016).

In *B. bipinnata*, the frequency and depth of the damage during extraction could affect vascular tissues, altering water and nutrient transport, which could generate physiological stress that limits photosynthesis, post-stress recovery, as well as growth. In face of this scenario, the objective was to determine how the variables of the resin extraction method impact the leaf moisture and chlorophyll content in *Bursera bipinnata* using multiple linear regression models. The results will allow optimizing the collection practices, reducing the physiological impact on the trees, and fostering a sustainable management of this resource in dry tropical ecosystems.

MATERIALS AND METHODS

Study area

The study was carried out in the Los Sauces micro watershed, located in the Cuautla River sub watershed, in the southeast of the state of Morelos, Mexico. This micro watershed covers a surface of 384 ha, with an altitudinal range of 1230 to 1580 meters, subhumid semi-warm climate [(A)C(w1)], mean annual temperature of 22.8 °C, and average precipitation of 819.2 mm (Servicio Meteorológico Nacional, 2024). The predominant soils are haplic Phaeozem, with a dark superficial horizon rich in organic matter, clayey subsoil, shallow depth, and high stoniness. The relief corresponds to volcanic mountain with steep slopes, where vegetation is dominated by secondary, shrubby and arboreal tropical dry forest, with dispersed agricultural areas (INEGI, 2015).

Experimental site. The study was conducted in the Los Sauces *ejido*, in Tepalcingo, Morelos, at an altitudinal range of 1368 to 1476 meters, covering 29.21 ha. The dominating vegetation corresponds to secondary shrubby tropical dry forest.

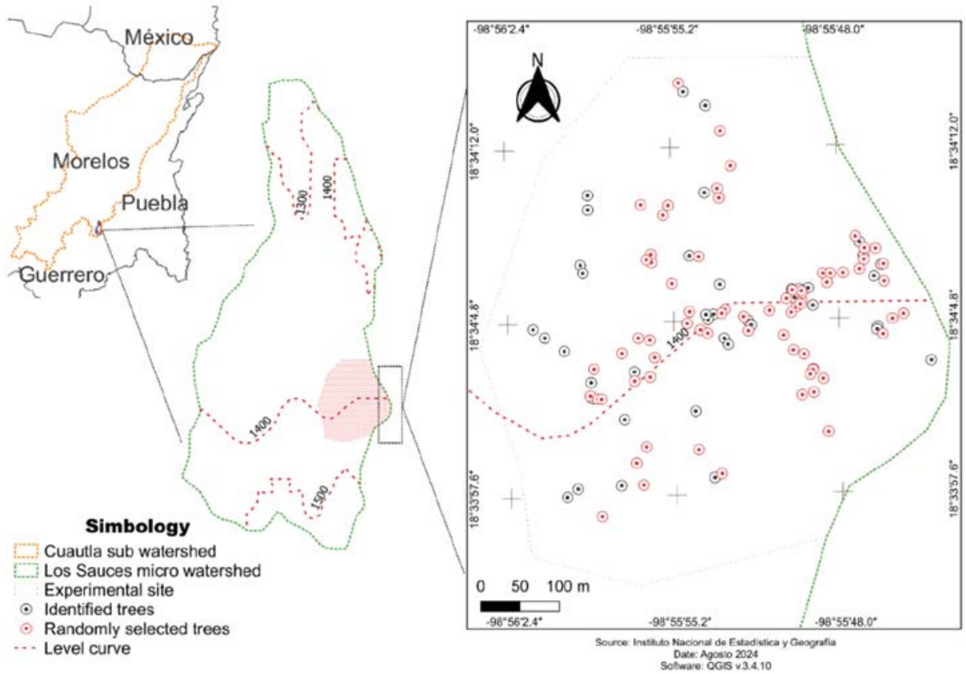

Figure 1. Location of the experimental site and the 70 sampling units (*Bursera bipinnata* trees) inside the Los Sauces micro watershed, Cuautla River sub watershed, state of Morelos.

Response variables

Two spectral indices were selected as indirect indicators of the physiological stress in *Bursera bipinnata* associated to resin extraction: the Normalized Difference Moisture Index (NDMI) and the Green Chlorophyll Index (GCI).

The NDMI, measured for August (NDMIA) and October (NDMIO), was calculated from near-infrared (band 8) and shortwave infrared (band 11) reflectance. This index is sensitive to changes in the hydration of foliage and cell structure of the mesophyll (Gao, 1996), allowing the detection of variations related to water stress induced by tissue damage. In *B. bipinnata*, resin extraction can compromise the functioning of vascular tissues, affecting the transport of water and nutrients to the canopy, as well as the photosynthetic capacity (Bhattacharya, 2021).

The GCI, recorded in the same months (GCIA and GCIO), was used as a proxy of the chlorophyll content and, therefore, of the photosynthetic capacity of the leaves (Gitelson *et al.*, 2005). Reduced levels of chlorophyll reflect a reallotment of resources towards the production of defense compounds after tissue damage, which can jeopardize light harvesting and carbon fixation (Guo *et al.*, 2012; Huot *et al.*, 2014; Khan *et al.*, 2018). Therefore, a decrease in GCI values can be interpreted as a physiological response to stress induced from resin extraction.

Explicative variables

The explicative variables considered in the model are directly related to the resin extraction technique in *B. bipinnata*:

- **Resin tapping faces**: The number of resin tapping faces on the stem (NFS), the branches (NFB), and the total from both (NFT). Each face represents a set of incisions carried out alternately throughout the period of exploitation. These incisions immediately release resin, at the same time triggering a stress response involving calcium, reactive oxygen species, and electrical signals (Choi *et al.*, 2017). These signals induce the biosynthesis of more resin.
- **Resin tapping face height (RFH)**: It is the height at which each resin tapping face is made on the tree. Previous studies have shown that the location of the incisions can influence the amount and quality of resin produced (Cherenet *et al.*, 2020), as well as the physiological response to tissue damage.
- **Incision length and depth**: They were recorded as mean length (MIL) and mean depth (MID). These dimensions define the degree of impact on the tissues, from the peridermis, the phloem and the xylem, responsible for the transport of water, nutrients and photo assimilates (García-Pineda, 1988). Deeper and more extensive incisions can induce more intense defensive responses but also jeopardize the vitality of the tree.
- **Mean face area (MFA)**: Calculated as indicator of the average size of the resin tapping zones. A higher MFA is associated with an increase in the flow and quality of resin, but also with greater damage of the vascular tissues and reallotment of resources for recovery (Sabo *et al.*, 2022).
- **Total resin tapping area (RTA)**: It represents the sum of the areas of all the faces made on the tree during the extraction cycle. This variable quantifies the intensity of use and accumulated damage, which can affect processes such as growth, tissue regeneration, adaptation and survival (Huot *et al.*, 2014).
- **Total resin production (PROD)**: Resins are complex mixtures; in the case of *B. bipinnata* they contain mainly terpenoid and lignan compounds, of high metabolic cost (Pallardy, 2008). This variable reflects the defense ability of trees in the presence of tissue damage. Its intensive extraction is related to the reduction in population observed in the region of the High Balsas (Abad-Fitz *et al.*, 2022; Montúfar-López, 2016).

Database and variable measurement

In the experimental site, *B. bipinnata* trees were georeferenced, selected for their use by copal extractors during the period of August to October 2023. Each tree was considered as a sampling unit. Initially, 111 trees were recorded, from which a simple random sample of 70 units was selected, using the sample function of the R software (R Core Team, 2024).

The geographical coordinates of each tree were obtained through a global positioning system (GPS) device. The *resin tapping face height* was measured from the ground level with a measuring tape, averaging the height of each face in the tree. The *incision length* was determined with the same measuring tape, taking the average of the incisions located in the center of each face. The *incision depth* was measured with calipers, considering the average of the incisions also located in the middle of each face.

The *area of each resin tapping face* was calculated through the trapezoid formula, using the measurements of the bases and the height of the faces, obtained with measuring tape. The mean value of these areas was used to calculate the *total resin tapping area per tree*, by multiplying the average by the total number of faces found. The *total production* of resin was weighed at the end of the exploitation cycle with a triple beam balance, recording the total weight of resin collected per tree.

The *physiological variables*, corresponding to the NDMI and the GCI, were estimated from Sentinel-2 L2A satellite images. Calculation of the indices was carried out in the QGIS 3.10 software with a raster calculator. The NDMI was calculated with the formula:

$$NDMI = NIR - SWIR1 / NIR + SWIR1$$

where: *NIR* corresponds to the near-infrared reflectance band (band 8) and *SWIR*1 to the shortwave infrared band (band 11). The index results in values between −1 and 1: values close to −1 indicate bare soils or without plant cover; values close to 0 (−0.2 to 0.4) reflect possible water stress; while values higher than 0.4 indicate a dense and healthy canopy (EOS Data Analytics, 2024; Sentinelhub, 2024).

The GCI was calculated through the formula:

$$GCI = NIR / Green - 1$$

where: *NIR* represents the near-infrared band (band 8) and *Green* the reflectance of the green band (band 3). This index presents values between 0 and 10; high values indicate higher content of chlorophyll and greater photosynthetic capacity, while low values can be indication of physiological stress and low photosynthetic activity.

Data analysis

To identify the best explicative model of physiological stress in *B. bipinnata*, a two-stage statistical analysis was conducted: linear correlation and multiple linear regression, using the R software (R Core Team, 2024). The variables related to the extraction methods were treated as explicative, while the NDMI and GCI indices evaluated in August and October were used as response variables.

In the first stage, the Pearson correlation coefficient $\left(r_{xy}\right)$ was applied to determine the linear relationship between pairs of variables. The magnitude of the correlation was classified in function of its absolute value, from very weak (<0.25) to very strong (≥ 0.75), evaluating the significance with a confidence level of 95% and contrasting the hypothesis of independence through the *t*-Student distribution with $n-2$ degrees of freedom (McGibney, 2023).

Then, multiple linear regression (MLR) models were adjusted to evaluate the joint effect of the explicative variables on the physiological indices. The model was structured according to:

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \ldots + \beta_p X_{pi} + \varepsilon_i$$

where: Y_i is the response variable, β_p the model's coefficients, X_{pi} the explicative variables, and ε_i the random error (Cohen *et al.*, 2003). Standardized coefficients (β) were calculated to estimate the relative importance of each predictor and the adjusted determination coefficient $\left(R^2_{adjusted}\right)$ was used as a measure of goodness of fit. In addition, the assumptions of the model were verified through graphic analysis and statistical tests: linearity, independence, normality (Shapiro-Wilk and Q-Q graphs), and homoscedasticity (Breusch-Pagan test and residue graphs) (Cohen *et al.*, 2003).

To avoid problems of multicollinearity, correlations between the explicative variables $(|r| > 0.70)$ and the variance inflation factor $(VIF > 4)$ (Schober *et al.*, 2018) were analyzed. The redundant variables were rejected to improve the accuracy of the model (Akinwande *et al.*, 2015). These measurements were evaluated through corrplot (Wei and Simko, 2021) and usdm (Naimi *et al.*, 2014) packages in R.

RESULTS AND DISCUSSION

Database and variable selection

The database was established by 70 *Bursera bipinnata* trees, whose geographic location was georeferenced. As predicting variables, the following were considered: resin tapping faces on the stem, resin tapping faces on the branches, total resin tapping faces, face height, incision length, incision depth, mean area of the face, total resin tapping area, and resin yield. The response variables were: $NDMI_{August}$, $NDMI_{October}$, GCI_{August}, and $GCI_{October}$.

Table 1 shows that the median number of resin tapping faces on the stem was 0, and the number of resin tapping faces on the branches and number of total resin tapping faces was 1. On average, the face height was 1.56±0.66 m, the incision length was 15.64±3.57 cm, and the depth 5.97±2.40 mm. The mean face area was 56.77±20.15 cm^2, while total resin taping area reached 90.72±52.69 cm^2. The mean resin production per tree was 38.56±50.44 g.

Table 1. Descriptive statistics of the resin tapping method.

Variable	Units	Median	Minimum	Maximum	Range
Resin tapping faces on the stem	Amount	0.00	0.00	2.00	2.00
Resin tapping faces on branches	Amount	1.00	0.00	4.00	4.00
Total resin tapping faces	Amount	1.00	1.00	4.00	3.00
Variable	**Units**	**Mean**	**Minimum**	**Maximum**	**Standar deviation**
Resin tapping face height	m	1.56	0.40	3.72	0.66
Incision length	cm	15.64	5.00	25.00	3.57
Incision depth	mm	5.97	3.00	19.00	2.40
Mean resin tapping face area	cm^2	56.77	18.00	109.25	20.15
Total resin tapping area	cm^2	90.72	18.00	285.00	52.69
Resin yield	g	38.56	0	190	50.44

Table 2 shows that the NDMI decreased from 0.30±0.02 in August to 0.11±0.05 in October, and the GCI went from 4.76±0.46 to 3.79±0.47, which suggests a progressive reduction in the moisture content and the photosynthetic capacity of the foliage.

Correlation coefficient

The Pearson correlation coefficient analysis indicated that no productive variable presented a significant association with the NDMI in August (Table 3). However, in October, resin production showed a weak negative correlation, although significant, with this index ($r=-0.287$; $p=0.016$), suggesting that a higher extraction could be related to a lower availability of water in the canopy (Table 4).

Regarding the GCI, incision depth was the only variable that presented a significant negative correlation in August ($r=-0.438$; $p=0.001$) and October ($r=-0.300$; $p=0.012$). This finding shows that deeper incisions can be associated with a reduction in the photosynthetic capacity, possibly due to a greater allotment of resources to defense mechanisms (Table 5 and Table 6).

Multiple linear regression model

Two multiple linear regression (MLR) models were constructed to analyze the effect of the productive variables on the physiological indices NDMI and GCI in *Bursera bipinnata*. Both models were defined from the results of the correlation analysis and fulfilled the

Table 2. Descriptive statistics of physiological variables.

Variable	Units	Mean	Minimum	Maximum	Standar deviation
$NDMI_{August}$	Dimensionless	0.30	0.25	0.35	0.02
$NDMI_{October}$	Dimensionless	0.11	−0.004	0.24	0.05
GCI_{August}	Dimensionless	4.76	3.71	5.86	0.46
$GCI_{October}$	Dimensionless	3.79	2.39	4.80	0.47

Table 3. Correlation between productive parameters and NDMI in August.

Variable	Pearson Correlation Coefficient	Sig.
Resin tapping faces on the stem	−0.055	0.652
Resin tapping faces on branches	−0.057	0.639
Total resin tapping faces	−0.113	0.353
Resin tapping face height	0.014	0.906
Incision length	0.006	0.964
Incision depth	−0.085	0.485
Mean resin tapping face area	−0.006	0.963
Total resin tapping area	−0.013	0.915
Resin yield	−0.179	0.139

** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

Table 4. Correlation between productive parameters and NDMI in October.

Variable	Pearson Correlation Coefficient	Sig.
Resin tapping faces on the stem	0.030	0.788
Resin tapping faces on branches	−0.169	0.163
Total resin tapping faces	−0.211	0.080
Resin tapping face height	−0.051	0.674
Incision length	0.047	0.698
Incision depth	−0.90	0.457
Mean resin tapping face area	−0.010	0.937
Total resin tapping area	−0.103	0.398
Resin yield	−0.287*	0.016

** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

Table 5. Correlation between productive parameters and GCI in August.

Variable	Pearson Correlation Coefficient	Sig.
Resin tapping faces on the stem	−0.094	0.438
Resin tapping faces on branches	0.150	0.214
Total resin tapping faces	0.147	0.225
Resin tapping face height	0.057	0.641
Incision length	−0.192	0.112
Incision depth	−0.438**	<0.001
Mean resin tapping face area	−0.226	0.060
Total resin tapping area	−0.021	0.861
Resin yield	0.079	0.513

** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

Table 6. Correlation between productive parameters and GCI in October.

Variable	Pearson Correlation Coefficient	Sig.
Resin tapping faces on the stem	−0.111	0.365
Resin tapping faces on branches	0.161	0.184
Total resin tapping faces	0.151	0.213
Resin tapping face height	0.023	0.850
Incision length	−0.065	0.594
Incision depth	−0.300*	0.012
Mean resin tapping face area	−0.173	0.152
Total resin tapping area	0.018	0.881
Resin yield	0.153	0.207

** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

statistical assumptions of normality, homoscedasticity and independence of the residues, guaranteeing the validity of the inferences.

Normalized Difference Moisture Index (NDMI). In the month of October, the model included the following predictors: resin production (PROD) and incision depth (MID). Only the resin production showed a significant effect (p=0.016), which explains 9% of the variability in the canopy moisture (adjusted R^2=0.063). The negative coefficient indicates that a greater extraction of resin is associated with a decrease in the leaf moisture content $\left(\hat{\beta}_1 = -0.287\right)$, suggesting a relationship between intensive exploitation and water stress in the canopy (Table 7).

Green Chlorophyll Index (GCI). For the month of August, the model incorporated the incision depth (MID) and the total number of resin tapping faces (NFT). The incision depth was the only significant predictor (p<0.05), explaining 20.4% of the variation observed (adjusted R^2=0.180). A negative effect was identified $\left(\hat{\beta}_1 = -0.420\right)$, which means that deeper incisions negatively affect the chlorophyll content, probably due to a higher allotment of resources to defense mechanisms, as well as from the damage to vascular tissues (Table 8).

For the month of October, only the incision depth showed a statistically significant effect (p=0.014), with negative coefficient $\left(\hat{\beta}_1 = -0.289\right)$. The model explained 10.6% of the index variability (adjusted R^2=0.079), which suggests that deep incisions carried out at the end of the season also compromise the photosynthetic activity (Table 9).

Table 7. Model on the effects of the productive parameters on NDMI in October.

	Estimate	Standard Error	T-Value	Pr>|t|
INTERCEPT	0.000	0.115	0.000	1.000
PROD	−0.287	0.116	−2.463*	0.016
MID	−0.089	0.116	−0.772	0.443

Residual standard error: 0.967 on 67 degrees of freedom.
Multiple R-squared: 0.090 and adjusted R-squared: 0.063.
F-statistic: 3.334 on 2 and 67 degrees of freedom, p-value: 0.041.
Variance Inflation Factor (VIF): PROD: 1.000; MID: 1.000.
*** Correlation is significant at the 0.001 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

Table 8. Model on the effects of productive parameters on GCI in August.

	Estimate	Standard Error	T-Value	Pr>|t|
INTERCEPT	0.000	0.108	0.000	1.000
MID	−0.428	0.109	−3.925***	<0.05
NFT	0.114	0.109	1.051	0.297

Residual standard error: 0.905 on 67 degrees of freedom.
Multiple R-squared: 0.204, and adjusted R-squared: 0.180.
F-statistic: 8.614 on 2 and 67 degrees of freedom, p-value: 0.05.
Variance Inflation Factor (VIF): MID: 1.005; NFT: 1.005.
*** Correlation is significant at the 0.001 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

Table 9. Model on the effects of productive parameters on GCI in October.

	Estimate	Standard Error	T-Value	Pr>|t|
INTERCEPT	−0.000	0.114	0.000	1.000
MID	−0.289	0.115	−2.502*	0.014
NFT	0.129	0.115	1.114	0.269

Residual standard error: 0.959 on 67 degrees of freedom.
Multiple R-squared: 0.106, and adjusted R-squared: 0.079.
F-statistic: 3.98 on 2 and 67 degrees of freedom, *p*-value: 0.023.
Variance Inflation Factor (VIF): MID: 1.005; NFT: 1.005.
*** Correlation is significant at the 0.001 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

The results indicate that resin tapping in *B. bipinnata* has important physiological effects on moisture and chlorophyll content in the canopy, estimated from the NDMI and GCI indices.

Relationship between NDMI and resin yield

The correlation analysis and the multiple linear regression model evidenced a negative relationship between the NDMI and resin production during the month of October. As resin production increases, the moisture content in the canopy decreases, which suggests that the intensity of tissue damage from extraction affects the water supply, from the root system to the leaves. In previous studies in *Boswellia* and *Acacia* have proven that the increase in intensity of tissue damage generates an increase in the yield of resin or gum (Negussie *et al.*, 2021; Sabo *et al.*, 2022), but the physiological effect from the damage is not established. In this study, the average incision depth, which was 5.97±2.40 mm, showed impacts on peridermis, conducting and non-conducting phloem, which is where the resin producing channels are located (García-Pineda, 1988). Likewise, the damage from incision depth reached the xylem, tissue responsible for the ascending transport of water to satisfy the demand for transpiration and cell expansion (Couvreur *et al.*, 2018).

In addition to this, the tissue damage generated by resin extraction could contribute to the loss of water in the main stem and the branches. As exposed by Oliva-Carrasco *et al.* (2015), since the water stored in the trunks can contribute between 6% and 28% to the daily water budget of large trees. In extreme cases, the water stored in the main stems can supply up to 50% of the total daily transpiration (Scholz *et al.*, 2008). Therefore, the reduction in the supply from loss can contribute to the water deficit of the canopy leaves.

This water stress was worsened under conditions of low water availability in the soil, as happened in the sampling period, characterized by a deficit in precipitation. The mean value recorded of NDMI (0.11±0.05) indicated severe stress as consequence of the low volume of accumulated rainfall, which was 26.6 mm between July and October of 2023, in contrast with the 417.48 mm expected for the same period (Servicio Meteorológico Nacional, 2024). This water deficit, combined with the damage in vascular tissues and the loss of water from tissue damage, reduces both the content and the water potential in the plant, which makes the adequate distribution of water in the tissues difficult (Zhao *et al.*, 2021). In addition this compromises fundamental processes such as cell growth, photosynthesis, respiration rate and other key enzyme processes (Bhattacharya, 2021).

Relationship between GCI and incision depth

The chlorophyll content (GCI) showed a significant negative correlation with the incision depth in both periods evaluated. This evidence shows that an increase in incision depth causes a decrease in canopy chlorophyll content. On the other hand, if it is considered that GCI is a reliable estimator of the green leaf area index (LAI), which is related to the proportion of photosynthetically active leaf area by surface unit of the soil (Nguy-Robertson *et al.*, 2014), it is deduced that the GCI also reflects a leaf area reduction as the incision depth increases. Therefore, if an incision affects the vascular tissues and the transport of water and mineral nutrients through the xylem is altered, as in the case of nitrogen, the composition and function of chloroplasts is collaterally disturbed, specifically the primary reactions of photosynthesis and carbon fixation reactions (Evans and Clarke, 2019).

In addition to this, the tissue damage generated from resin extraction is marked by reactive oxygen species (ROS), calcium (Ca^{2+}), and electrical signals, known as trio signaling (Choi *et al.*, 2017). These signals activate chemical messengers such as jasmonic acid (JA, Koo, 2018), which performs a crucial role in the reallotment of resources, including carbon, hydrogen and nitrogen, towards defense, which contributes to the reduction of essential components for photosynthesis, such as the light harvesting and carbon fixation complexes (Guo *et al.*, 2012; Huot *et al.*, 2014). This contributes to senescence and reduction of the leaf area, as indicated by the GCI values of this study in both periods. This has been proven in *Boswellia papyrifera*, where the continuous and intensive extraction of resin caused a significant decrease in the levels of essential nutrients like nitrogen, carbon and nitrogen, as well as a reduction in the leaf area of the crown, favoring the defensive capacity in detriment of growth and development (Khan *et al.*, 2018; Mengistu *et al.*, 2012).

Physiological effects of the accumulated damage

The multiple linear regression showed that resin production explained 9% of the variation in NDMI, while the incision depth explained 20% and 10.6% of the variability in GCI in August and October, respectively. These results evidenced that intensive exploitation has cumulative physiological impacts in *B. bipinnata*, by jeopardizing water availability and photosynthetic activity in the canopy.

Thus, the reduction in leaf area, consequence of the decrease in water supply and reallotment of resources towards defense, negatively affects the productivity of *B. bipinnata*, since the leaves carry out a crucial role in carbon fixation through photosynthesis and respiration (Binkley *et al.*, 2013). Similarly, the senescence of leaves leads to lower production and storage of non-structural carbohydrates (NSC), which are fundamental for transport of resources, energetic metabolism, cellular osmoregulation, and synthesis of defense compounds, as well as post-stress recovery (Piper and Paula, 2020). In the study by Mengistu *et al.* (2013) for species of the genus *Boswellia*, it has been proven that the continuous and intensive extraction of resin decreases the concentration of non-structural carbohydrates, leading them to possible exhaustion. In addition to this, the recovery of NSC can take several years due to the continuous extraction of resin, since it compromises the defensive capacity of trees in the presence of conditions of biotic or abiotic stress (Wang *et al*., 2021). This could contribute directly to the mortality of the *B. bipinnata* trees used if resin tapping is not conducted in a sustainable manner.

CONCLUSIONS

The intensity of resin extraction from *Bursera bipinnata* directly impacts the physiological stress of the trees. The incision depths affect the conducting tissues (xylem and phloem) that reduce the moisture content in the canopy, situation that can be aggravated in contexts of environmental water deficit. The decrease in chlorophyll content, associated with the loss of leaf area and the reallotment of resources towards defense mechanisms, compromises the photosynthetic capacity of the trees. Both effects alter the reserves of non-structural carbohydrates (NSC), fundamental for post-stress recovery, thus increasing the vulnerability of individuals in the presence of adverse biotic and abiotic factors. To promote sustainable management of *B. bipinnata*, the recommendation is to limit the depth of incisions to less than 6 mm, decrease the frequency of extraction, establish rest periods between seasons of exploitation, and reduce or avoid extraction under restrictive environmental conditions (drought). The implementation of these practices will favor the physiological health of the trees, allow keeping their productivity in the long term, strengthen the resilience of dry tropical ecosystems facing the effects of climate change, and contribute to the design of sustainable forest management strategies in local communities.

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Colegio de Postgraduados

Evaluation of Different Scarification Methods for Chile Maax Seeds (*Capsicum annum* L. var. *glabriusculum*)

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ABSTRACT

Objective: To evaluate different scarification methods for maax pepper seeds under humid tropical conditions.

Design/methodology/approach: Maax chili pepper seeds collected in greenhouses at the Colegio de Postgraduados Campus Campeche were evaluated. These seeds were subjected to six scarification methods by immersion in a solution of coconut water (*Cocos nucifera* L.) (8 and 12 h); hydrogen peroxide (H_2O_2) (5 and 10 minutes); and sulfuric acid (H_2SO_4) (1 and 5 minutes), plus a control treatment. The evaluation was carried out under greenhouse conditions in plastic planting trays.

Results: The germination percentage and germination time of maax pepper seeds improved with all pre-germination treatments, advancing the onset of germination by up to three days compared to the control. The most effective treatments were seed immersion in coconut water for 8 and 12 h. Early seedling growth of *maax* pepper exceeded that of the control by 26%, 25%, 18%, and 14% in plant height, stem diameter, leaf width, and number of leaves, respectively.

Study limitations/implications: *Capsicum annuum* L. var. *glabriusculum* seeds have difficulty germinating if they are not in their natural state and are not ingested by a bird.

Findings/Conclusions: Due to its plant hormone and other nutrient content, coconut water is a suitable pre-germination treatment for breaking seed dormancy and promoting early growth of maax chili peppers under tropical conditions.

Keywords: *Capsicum annum* L. var. *glabriusculum*; Scarification; Seeds.

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INTRODUCTION

Mexico hosts a wide variety of chili peppers that differ in size, color, shape, and flavor, among which is the maax chili (González-Cortés *et al.*, 2015). This ecotype is also regionally known as *piquín*, *chiltepín*, *chilpaya*, *chiltepillo*, *chiltepec*, *chile de monte*, *chile parado*, *pájaro pequeño*, *amomo*, *pico de paloma*, and *pico de pájaro*, among others, and is a highly valued product (Megchún-García *et al.*, 2024). *Maax* chili is a non-domesticated species

widely consumed in states such as Veracruz, Tabasco, Campeche, Quintana Roo, Yucatán, and Chiapas, although it is broadly distributed throughout Mexico, and currently all indigenous groups interact with this species (Balderas *et al.*, 2023; Solís-Montero *et al.*, 2023). It is rich in nutrients; its fruits contain vitamin A and antioxidants and also possess medicinal properties. Consequently, indigenous communities traditionally used it to treat various ailments such as asthma, cough, sore throat, toothache, and others. Furthermore, it is widely used in various traditional Mexican dishes (Balderas *et al.*, 2023).

Currently, commercial demand has increased due to its exotic characteristics, and it is now available in both national and international supermarkets (Ramírez *et al.*, 2018; Alcalá-Rico *et al.*, 2023). However, to meet this demand, almost all supply comes from wild harvesting, which negatively impacts its distribution and abundance. This species grows at elevations up to 1,300 m above sea level, under the shade of trees and shrubs. However, *maax* chili is characterized by low germination rates, even when seeds are mature and environmental conditions are favorable. This limitation is known as physiological dormancy, in which one or more internal seed conditions prevent germination (Brondo-Ricárdez *et al.*, 2020). Among these factors, seeds of this species possess a hard, waxy, impermeable cuticle and contain natural inhibitors, resulting in germination rates below 5%. Therefore, seed germination represents a major constraint for intensive commercial cultivation. The natural mode of propagation of this chili occurs when mature fruits are ingested primarily by birds, and the seeds are subsequently dispersed through their droppings (Megchún-García *et al.*, 2024). Therefore, to overcome these low germination rates, various methods and techniques have been used to break seed dormancy in *Capsicum* spp., including: pre-soaking for 5 h at 21 °C and pre-drying at 22 °C, 32 °C, and 37 °C; exposure to incandescent and infrared light; potassium nitrate at 0.2%; indole-3-acetic acid at 1,000 ppm; gibberellic acid (GA_3) at 10-100 ppm; kinetin at 10-100 ppm; and removal of seed coat structures (Mireles-Rodríguez *et al.*, 2015).

When dormancy is shallow, it can be broken using chemical treatments, gibberellic acid, or warm or cold stratification. However, when dormancy is intermediate, it can only be broken after a prolonged period of cold stratification, and gibberellic acid may or may not be effective in overcoming dormancy (Loayza *et al.*, 2023).

Additionally, other solutions can be used to overcome dormancy in *maax* chili seeds, such as coconut water, which contains a variety of hormones with cytokinin-like activity, including isoprenoid-type cytokinins that aid in cell division, as well as aromatic-type cytokinins involved in post-germination processes. Bertolini *et al.* (2014) demonstrated that a culture medium containing copper chelate and coconut water promoted the development and survival rate of *Rossioglossum grande* (Lindl.) Garay & G.C. Kenn. protocorms and reduced chlorosis after 60 days. Similarly, Arana-Paredes *et al.* (2017) reported that using coconut water as a culture medium for Dianthus caryophyllus resulted in higher germination percentages, as well as increased germination rate and index. Loayza *et al.* (2023) demonstrated that ultra-drying (>3% moisture) and treating seeds with sulfuric acid, gibberellic acid, or potassium nitrate are the most effective methods for germination of *C. chilensis*. Therefore, the following research question arises: Which is the most effective method to induce and achieve higher germination of maax chili seeds under humid tropical conditions? Accordingly, the objective of this study was to evaluate different scarification methods for *maax* chili seeds and determine their germination percentage under humid tropical conditions.

MATERIALS AND METHODS

The research was conducted in the Plant Physiology Laboratory and experimental field at the Colegio de Postgraduados, Campus Campeche, located on the Haltunchén-Edzná road, km 17.5, Sihochac, Champotón, Campeche, Mexico.

Seed Treatment

Mature red maax chili fruits were collected from greenhouses at the same institution (Figure 1). Seeds were extracted by decantation, assisted by blending the mature chilies at low speed. The seeds were then placed in a container of water to remove non-viable seeds, which remained floating. Afterwards, the seeds were collected using a sieve and spread on brown paper. The seeds were rinsed three times with sterile water and then evenly distributed in a glass jar. They were subsequently treated with 10 ml of coconut water for 8 and 12 h, sulfuric acid (H_2SO_4) for 1 and 5 min, or hydrogen peroxide (H_2O_2, 3%, JENFARMA ZAGAL) for 5 and 10 min, plus a control consisting of seeds without hydration. These treatments and immersion times were selected because Ruiz-Carranza *et al*. (2024) demonstrated their effectiveness in breaking physical dormancy and promoting germination, including in woody species such as *Ebenopsis ebano*, *Havardia pallens*, *Parkinsonia aculeata*, *Prosopis laevigata*, and *Vachellia farnesiana*.

Sowing

The seeds were sown in 200-cell plastic trays using a substrate composed of garden soil mixed with sugarcane bagasse vermicompost in a 3:1 ratio, prepared on the same campus. One pre-treated seed was placed in each cell at a depth of 0.5 cm. After sowing all seeds,

Figure 1. Mature fruits of *Capsicum annuum* L. var. *glabriusculum* collected for experimentation.

potable water was sprayed using a plastic watering can. The trays were then placed under shade at an ambient temperature of 30 °C. Irrigation was performed every 3 days during the first 3 weeks and thereafter once per week.

Statistical Analysis

A completely randomized design was used with seven treatments plus a control, each with four replicates. The treatments were as follows: T1=unscarified seed (Control), T2=Coconut water (8 h), T3=Coconut water (12 h), T4=H_2O_2 (5 min), T5=H_2O_2 (10 min), T6=H_2SO_4 (1 min), and T7=H_2SO_4 (5 min).

The study variables were the number of germinated plants, plant height at 15 and 30 DAS (days after sowing), stem diameter, leaf width, and number of leaves. Data was analyzed using the SAS statistical software. An analysis of variance (ANOVA) was performed, and when significant differences were detected, Duncan's multiple range test was applied for mean comparison.

RESULTS AND DISCUSSION

Germination percentage

The evaluation of scarification methods for *maax* chili seeds began with seven treatments; however, the H_2SO_4 treatments showed no effect, as they resulted in low or no seed germination. Therefore, the analysis was based only on coconut water and H_2O_2 treatments, which were considered more effective for these seeds. Nonetheless, some reports on *E. ebano* seed germination indicate that H_2SO_4 was even more effective than other treatments. The difference observed in this experiment may be attributed to the seed coat of *E. ebano*, which is thicker and harder than that of *maax* chili (Ruiz-Carranza *et al.*, 2024).

Similarly, the results of the scarification evaluation of *maax* chili seeds showed higher germination percentages with H_2O_{22} (75%) and coconut water (70%) treatments (P>0.05), exceeding the control treatment by an average of 2% to 25%. In addition, the control treatment required more days to germinate, confirming the positive effect of H_2O_2 and coconut water treatments (Table 1). Likewise, Herrera-Aguilar *et al.* (2017) reported that the application of the commercial soluble powder product Biogib and gibberellic acid

Table 1. Mean comparison by treatment for germination percentage and days to germination of *maax* chili seeds (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods.

Treatments	Germination (%)	Germination (days)
T1=Unscarified seed (Check),	50 b	10
T2=Coconut water (8 h),	70 a	7
T3=Coconut water (12 h),	70 a	8
T4=Hydrogen peroxide H_2O_2 (5 min);	75 a	8
T5=hydrogen peroxide H_2O_2 (10 min);	75 a	6
T6=Sulfuric acid H_2SO_4 (5 min);	Low germination	
T7=Sulfiric acid H_2SO_4 (10 min)	Low germonation	

Treatments with the same letter are not significantly different (Duncan, α=0.05).

promoted seed germination, resulting in higher germination rates at 21 days of evaluation.

In addition, Sandoval-Rangel *et al.* (2018) noted that other factors such as management, production, collection, extraction method, selection, conditioning, drying, and storage of the seed also influence the germination and viability of *maax* chili seeds.

The results confirmed the usefulness of coconut water as a treatment to stimulate seed germination and reduce the number of days to germination, thereby shortening the staggered germination period.

In this way, coconut water represents a viable option as a germination stimulant for *maax* chili seeds in coastal areas. This effect can be attributed to its content of diverse hormones with cytokinin-like activity, such as isoprenoid-type cytokinins involved in cell division processes and aromatic-type cytokinins associated with post-germination processes. As previously mentioned, this cytokinin is a phytohormone that, among other functions, promotes dormancy release and seed germination by stimulating cotyledon cell elongation in response to light (Campos-Ruíz *et al.*, 2014).

Therefore, the addition of coconut water may provide seeds with sufficient hormone levels to break the dormancy observed under natural conditions. It should also be noted that the coconut palm is typical of tropical regions, making coconut water a natural resource for promoting *maax* chili seed germination. Moreover, it is an economical product, with an average price of 30 pesos per liter, compared to hydrogen peroxide at approximately 300 pesos per liter. Coconut water is thus considered not only safer to handle, due to its natural origin, but also more economically accessible than hydrogen peroxide. Actions of this kind should be implemented because the primary habitat of *maax* chili is being lost, highlighting the need to establish germination and production protocols for this ecotype (Solís-Montero *et al.*, 2023).

Plant Growth

When evaluating the effect of seed scarification treatments on germination, their influence on seedling growth was also assessed ($P>0.05$). A positive effect of the pre-germination treatments was observed on plant height, stem diameter, leaf width, and number of leaves. Plant height was greater with scarification treatments compared to the control, with 8-hour coconut water immersion showing the highest values, followed by the 12-hour coconut water treatment, at 15 and 30 DAS (Figure 2). Although the type of seed scarification was evaluated in *maax* chili, it is always recommended to use organic substrates for growth and development, as noted by Palma-López *et al.* (2020), since they lead to higher production yields.

Similar results were found for the basal stem diameter of the seedlings. Seedlings from scarified seeds exhibited greater stem diameter, particularly those treated with coconut water for eight hours (Table 2). These results indicate that the hormonal composition of coconut water, specifically cytokinins, promoted vegetative growth, providing an advantage for plant production under nursery conditions.

Regarding leaf width and number of leaves, similar to plant height and stem diameter, scarification treatments on *maax* chili seeds had positive effects, observable from 15 DAS when using coconut water ($P>0.05$) (Figures 3 and 4). Authors such as Origenes and

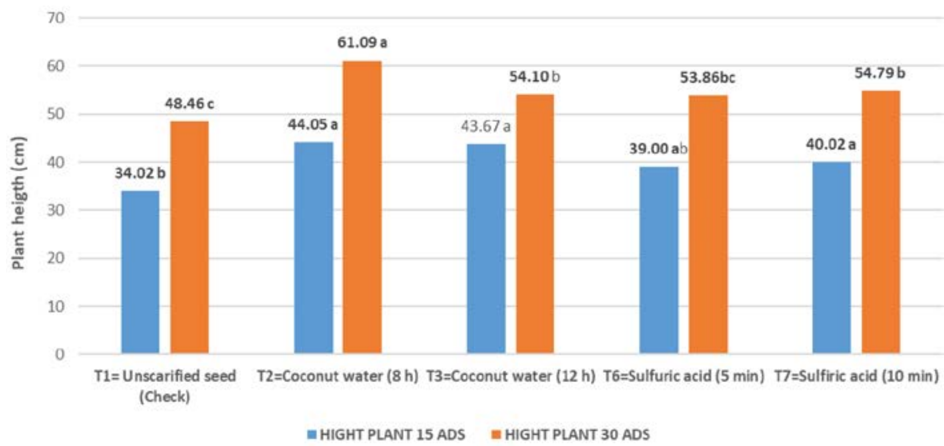

Figure 2. Mean comparison by treatment for plant height of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods. Treatments with the same letter are not significantly different (Duncan, α=0.05).

Table 2. Mean comparison by treatment for seedling stem diameter of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods.

Treatments	15 DAS	30 DAS
T1=Unscarified seed (Check),	0.42 c	0.82b
T2=Coconut water (8 h),	0.66 a	1.03 a
T3=Coconut water (12 h),	0.53 bc	0.95 ab
T6=Sulfuric acid H_2SO_4 (5 min);	0.47 c	0.95ab
T7=Sulfiric acid H_2SO_4 (10 min)	0.63 ab	1.00 ab

Treatments with the same letter are not significantly different (Duncan, α=0.05). DAS=days after sowing.

Lapitan (2020) also found that coconut water promoted the greatest height and average number of leaves in *Diospyros discolor* seedlings.

Leaf width was enhanced by scarification of *maax* chili seeds with H_2O_2 during the first 15 DAS, followed by coconut water treatments with 8- and 12-hour seed immersion. At 30 DAS, the effect of all scarification treatments was statistically similar (Duncan, α=0.05) (Figure 3).

Regarding the number of leaves, similar effects were observed at 15 DAS, with the best treatment being 10-minute H_2O_2 scarification, followed by coconut water scarification ($P>0.05$). At 30 DAS, the 8-hour coconut water treatment on maax chili seeds proved to be the most effective (Figure 4).

The effect of coconut water observed can be attributed to its richness in plant hormones such as gibberellins and cytokinins (González-Cortés *et al.*, 2015). These characteristics are associated with faster initial seedling growth, reflected in plant height, stem diameter, number of leaves, and leaf width. Although coconut maturity can also be a determining factor in promoting germination, Quinto *et al.* (2009) reported higher germination percentages using tender coconut water in *Tabebuia rosea* seeds, indicating that lower maturity is associated with higher nutrient concentration. However, in this study, coconut

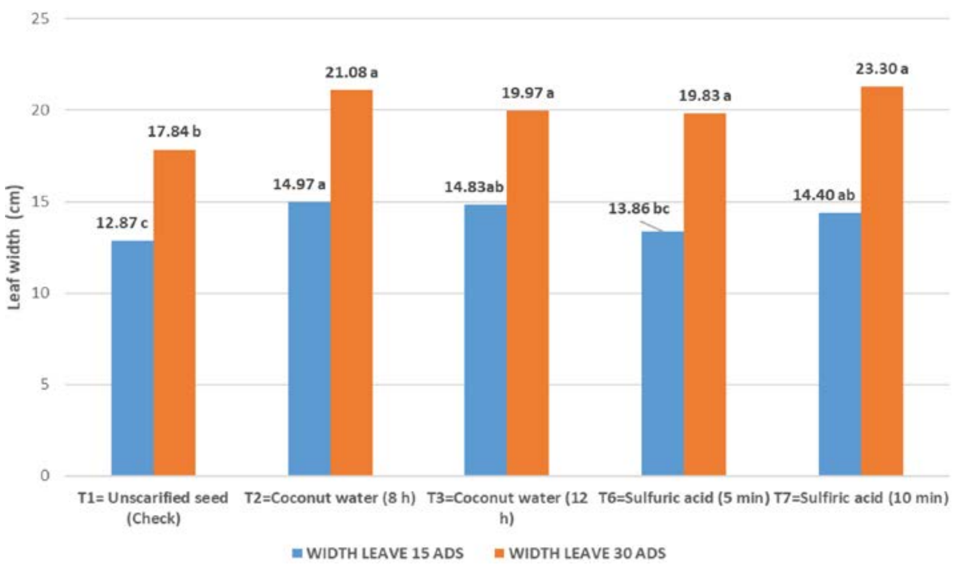

Figure 3. Mean comparison by treatment for seedling leaf width of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods. (Treatments with the same letter are not significantly different, Duncan, α=0.05).

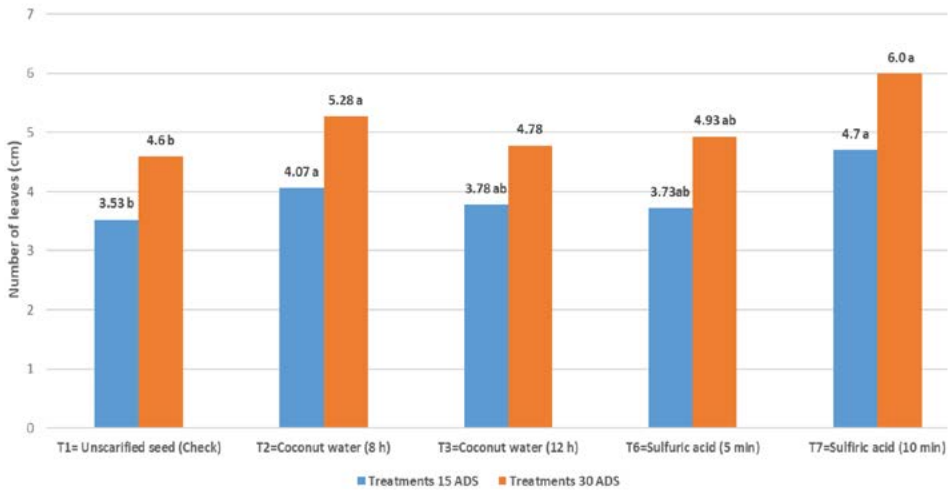

Figure 4. Mean comparison by treatment for seedling number of leaves of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods. (Treatments with the same letter are not significantly different, Duncan, α=0.05).

maturity was not evaluated as a variable. Furthermore, it is important to consider that there is considerable variation among *maax* chili ecotypes. According to various studies, some ecotypes have hard seed coats that require acidic substances to promote germination, whereas others have softer seed coats that can germinate when treated with plant hormone-containing substances, such as coconut water (phyto-regulators), as in this case.

CONCLUSIONS

Scarification of *maax* chili seeds by immersion in coconut water proved to be the most effective method for promoting germination and stimulating early seedling growth.

Under the study conditions, the results suggest that the dormancy of the evaluated *maax* chili seeds was primarily physiological rather than physical, although variations in seed coat characteristics due to genotype may induce physical dormancy. Therefore, it is recommended to conduct similar experiments evaluating the effect of these treatments with variations in coconut maturity and across different *Capsicum annuum* varieties and genotypes.

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Colegio de Postgraduados

Analysis of the *Macrobrachium rosenbergii* prawn trade in markets and restaurants in Mexico

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ABSTRACT

Objective: To identify the links that integrate the production-distribution-consumption chain of the Malaysian prawn *Macrobrachium rosenbergii* in Mexico.

Design/methodology/approach: The survey method, semi-structured interview, and open-ended questions were used to learn about the origin and destination of the product, installed technological capacity, production volumes, purchase and sale prices, supply and demand characteristics, as well as the perception of product quality, which was carried out through the "mystery customer" technique.

Results: Imports are the origin and driving force of the *M. rosenbergii* prawn market in Mexico, mainly in the markets of Mexico City (La Nueva Viga) and Zapopan, Jalisco (Mercado del Mar), which concentrate more than 80% of the domestic market through a network of importers, wholesalers, retailers and restaurateurs, mainly. Aquaculture production is incipient, with two farms and two programs promoted by academic institutions.

Limitations on study/implications: This study was a first effort to understand the structure and components of the *M. rosenbergii* prawn market in Mexico. It focuses on descriptive work that will serve as a basis for future research.

Findings/conclusions: There are international marketing companies that stockpile the product and introduce it into the domestic market; this import reaches large supply centers and is then distributed to retail markets and supermarkets. This marketing chain is a motivating factor for inducing domestic production of the *M. rosenbergii* prawn.

Keywords: Aquaculture, supply, demand, quality, prices.

INTRODUCTION

Breeding and trade of Malaysian prawn (*Macrobrachium rosenbergii*), also known as giant freshwater prawn, has attained relevance in the aquaculture sector globally (New and Kutty, 2010). In Mexico, despite having been introduced more than 50 years ago, its production is still limited and faces technological and commercial challenges (Asiain-Hoyos *et al.*, 2022). Malaysian prawn is a product present in the local market (CONAPESCA, 2021) in different

regions of the country, and it is imported from Vietnam, Bangladesh and Malaysia (FAO, 2020). Many times, it is a substitute for native prawns such as *M. acanthurus* and *M. carcinus* in the Gulf of Mexico coast, and *M. americanum* and *M. Tenellum* in the Pacific coast (García-Guerrero *et al.*, 2013). These species have increasingly less presence in the markets. There are naturally other freshwater crustaceans with various ethnic uses (Vázquez-García *et al.*, 2004), but the ones mentioned before are important for their commercial value in local markets.

Despite its scarce production in the country (INAPESCA, 2018), the Malaysian prawn has considerable potential because of its flavor, texture and size, which make it attractive both for the gourmet market and for popular consumption.

In seafood markets (López-Tostado, 2013), *M. rosenbergii* can be found in window displays together with other species of crustaceans such as marine shrimp (*Litopenaeus* spp.), crabs (*Callinectes* spp.), among others, and a wide diversity of marine and freshwater fish. In the supermarkets, they are in the sections of select products (Kotler and Armstrong, 2017), with high commercial value, since *M. rosenbergii* reaches a value of \$500.00 pesos MX kg^{-1}. In restaurant menus in touristic zones of important cities like Veracruz, Boca del Río, Puebla, Mexico City, among others, its value reaches \$1,200.00 pesos MX kg^{-1}. In gourmet services (Porter, 2010), it has been found proportionally in weight per portion at \$3,000.00 pesos MX kg^{-1}.

However, aquaculture production is limited; the price of *M. rosenbergii* at the pond ranges between \$500.00 pesos MX kg^{-1} and \$700.00 pesos MX kg^{-1}.
The objective of this study is to identify the market structure (Resico, 2011) of Malaysian prawn (*Macrobrachium rosenbergii*), analyzing the links that make up its production, distribution and consumption chain, as well as the interrelation between the stakeholders involved (Cayeros-Altamirano *et al.*, 2016). Understanding the offer and demand of the Malaysian prawn in the country clarifies the viability of the market to drive its breeding, and therefore, the growth of aquaculture, an activity that generates employment and participates in the market economy.

MATERIALS AND METHODS

The study was carried out from October 2023 to October 2024, considering as study region the states of Mexico, Puebla, Veracruz and Jalisco. The main seafood markets were included such as La Nueva Viga, which is part of the Central Supply Market in Mexico City; Mercado del Mar in Zapopan, Jalisco; the popular seafood market located at 18 Oriente in the historical Center of the city of Puebla; and Plaza del Mar in the city of Veracruz. In addition, malls and restaurants that sell seafood, including prawns, were visited.

Surveys with shopkeepers (tenants)

Open interviews were applied (Tonon, 2009) with tenants (shopkeepers) to identify the trading companies that introduce the *M. rosenbergii* prawn to Mexico, and to characterize the product in terms of price and presentation (Dvoskin, 2004).

Interviews with producers

The method of interviews with open questions was used (Vega-Budar and Taguenca-Belmonte, 2012) to identify the socioeconomic and technological characteristics of the farms devoted to the production of *M. rosenbergii*, considering their geographic location, annual production, breeding technique, and characteristics of the product.

Interviews with traders

The technique of semi-structured interview was used (Ríos-Martínez, 2019) to understand the volume of imports, origin, and destination of the product, as well as prices, quality conditions, and characteristics both of the offer and the demand, through interviews with previously identified wholesale and retail traders.

Visits to restaurants

Restaurants were visited in the cities of Mexico, Puebla and Veracruz. The technique of "mysterious customer" was applied (Block *et al.*, 2022), through which questions were formulated as a diner to understand the origin of the product, prices and quality. In addition, the menus from ten restaurants visited in those cities were reviewed.

Self-service stores

Visits were conducted to self-service stores (Islas, 2007), detecting the presence of the *M. rosenbergii* prawn in window displays, and obtaining the information from exhibitors and applying open interviews to the persons in charge of the seafood departments in the stores.

Surveys with consumers

Surveys were applied to consumers (Schiffman and Kanuk, 2005) in the markets visited, with the purpose of knowing their socioeconomic characteristics, frequency of purchase, preferences, and the product prices.

Analysis of the trade chain

With the information gathered, a model of the trade chain was built (Stern, 1998), based on the general systems theory (Ríos and Santillán, 2017). A quota-controlled non-probabilistic sampling was used, considering the participation of each agent in the production-distribution-transformation-consumption chain, through the "snowball" method. This strategy allowed an agent to lead to establishing contact with other informant agents in each of the chain links.

Descriptive statistics were used, backed by field observations, since the small number of agents that make up the market structure did not allow a robust statistical analysis.

Data processing

A database was built with the information gathered in the field, which was analyzed using the Statistica software.

RESULTS AND DISCUSSION

International packaging companies of *M. rosenbergii* in the Mexican market

International trade of crustaceans has, as part of its marketing strategy, packaging the product with the aim of conserving, transporting and trading in presentations that are ready for storage and exhibition of the product by wholesale and retail traders (Simoes *et al.*, 2019). In Vietnam, packaging companies of prawn play a key role in the export chain (Son *et al.*, 2005); they purchase the prawns from fishermen and local farmers settled on the Mekong River delta, a crucial region for the aquaculture production of this country. Then, the *M. rosenbergii* prawn is frozen and packed, allowing its conservation and later distribution to different countries of the world, mainly the United States (USA) and the Popular Republic of China (PRC). Despite this regional activity, some of the prawn exported from Vietnam is stockpiled from other nearby countries like Bangladesh (30-40%) (Kibria *et al.*, 2022). One of the reasons why it is necessary to stockpile for export is because of the decrease in local product, as a result of contamination of the Mekong River (Sneddon and Fox, 2012) and overfishing (Clayton *et al.*, 2003), aspect that has reduced the natural populations of the prawn. This way, the global supply chain is ensured despite the environmental challenges. The product that reaches Mexico is through companies that provide their services to Asian packagers of prawn (Table 1); that is, there are basically three companies that supply the national market primarily through La Nueva Viga Market in Mexico City (López-Tostado, 2013) and the Seafood Market in Zapopan (Palomino-Núñez *et al.*, 2022), Jalisco, which concentrated 80% of the national seafood market.

M. rosenbergii farmers in the incipient national market

Based on the information gathered through desk research and field work, three important producers were identified (Reta-Mendiola *et al.* 2021), the first in the state of

Table 1. International packaging companies of Malaysian prawn (*M. rosenbergii*) detected in the national seafood markets in Mexico.

Company	Country of origin	Sizes per pound (lb)	Package (g)	Market /Company
Calcutta Seafoods Private Limited	India	2 prawns per pound 4 prawns per 2 pounds box	907	Plaza del mar, Veracruz / Pescados y Mariscos Pérez
Divron Bioventures	Sri Lanka	4 por libra	>400 250-400 150-250	La Nueva Viga/ El Galión
NewgenInter Trading Co., Ltd.	Thailand		>400 250-400 150-25	La Nueva Viga/Plaza del Mar, Veracruz.
Factory viet asia foods Co.	Vietnam		>400 250-400 150-250	La Nueva Viga/ El Chirgo/ Mercado de Pescados y Mariscos, Puebla/Mercado de San Juan,Ciudad de México

Source: Prepared by the authors based on field research.

Quintana Roo in the municipality of Othón P. Blanco, the second in the state of Veracruz in the municipality of Soledad de Doblado, and the third in the state of Oaxaca in the municipality of San José Chacalapa. The first has an intensive modernized production system to produce post-larvae and juveniles to fatten *M. rosenbergii*. The average annual production is 300 kg of prawn, which is traded directly in the restaurants of the Riviera Maya (Jouault and García de Fuentes, 2020) at an average price of \$400 pesos MX kg^{-1} (\$20.12 USD) for females of weight that ranges between 30 and 40 g, while for the males (blue claws), the price is \$650.00 pesos MX kg^{-1}(\$32.70 USD) with average weight of 70 g.

The presentation of both is packaged and frozen in 1 kg bags. The prices are kept constant throughout the year, the seasons of greatest demand are the ones that generally agree with the vacation period, mainly of foreign tourism. The main competitor is product imported from Vietnam, Bangladesh and Malaysia; the prices are similar, but consumers prefer national product because of its freshness and flavor, in addition to proximity to its supply, so the farm as productive unit presents a competitive advantage.

The second producer, located in the municipality of Soledad de Doblado, Veracruz, also uses a modernized and intensive production system for prawn, in addition to post-larvae production which is traded inside the state of Veracruz and the states of Oaxaca and San Luis Potosí. The sale of prawn is done at the farm gate, with an average price of \$650.00 pesos MX kg^{-1} (\$32.70 USD); consumers visit the farm to purchase the product considering freshness and flavor as the main characteristics that they demand from the product; this purchase is generally done on weekends since it is considered as a meal for parties. In addition, buying at the farm gate causes an additional intangible satisfier in the consumers by being in contact with the natural environment in a recreational environment. The average production of this farm is 100 kg per year; the average sale weights are 35 g for females and 70 g for males.

The third producer was found in the municipality of San José Chacalapa, Oaxaca. The unit is based on a technological transference project to produce Malaysian prawn in the zone, coordinated by the Universidad del Mar. Its production system is extensive in rustic ponds with an average harvest of 12 kg per 10×20 m pond; the harvest is used for self-consumption among the same farmers who participate in the project. It is considered that the price can reach \$700.00 pesos MX kg^{-1} (\$37.80 USD) with good acceptance within the project's influence area.

Another effort for technology transfer is taking place in the region of Sotavento and the High Mountains of the state of Veracruz (Reta-Mendiola *et al.*, 2019) including the municipalities of Paso del Macho, Tierra Blanca, Cuitláhuac and Tezonapa, through Colegio de Postgraduados Campus Veracruz, which managed to involve 10 tilapia (*Oreochromis* spp.) producers to accept using part of their infrastructure to breed prawn in association with the fish. The average sale price attained in these municipalities ranged from \$400.00 pesos MX kg^{-1} (\$21.60 USD) to \$700.00 pesos MN kg^{-1} (\$37.80 USD), with an average weight of 30 g, sold at the farm gate.

Of the four producers located in the country, only two are within the productive sector while the others are academic projects for innovation and technology transfer;

therefore, it is necessary to promote and develop the activity, because of the ease of association with other species, the possibility to diversify production, and the attractive market price.

Wholesale traders

La Nueva Viga (Mexico City) is the main distribution center of fishing products in the country; it distributes products from all national ports and imports from the four continents, which places it as the second largest market in the world, exceeded only by the market in Tokyo. It has a surface area of 90,000 m^2, with 202 wholesale warehouses, 55 retailers, and 165 commercial shops. Four of these warehouses already have certifications and 80 more are in process of obtaining them. An approximate daily volume of 1,500 tons of seafood is handled, which is equivalent to 60% of the national fishing production. In this market, around 300 species of fresh and frozen products are traded, plus 100 more species of imported frozen products (López-Tostado, 2013). In the case of Malaysian prawn, only five shopkeepers sell it in two-pound or master packaging, which is equivalent to two boxes of prawns with 12 two-pound boxes, respectively. The wholesale trader who sells most at the supply center of La Nueva Viga is a distributor company that handles an average wholesale sale price of $350.00 pesos MX kg^{-1} ($18.97 USD) starting at 10 masters, which have 12 one-kg or two-pound boxes. They trade up to 500 masters per month during high season, which coincides with the vacation period, and a public price of $400.00 pesos MX ($21.60 USD) per box.

Retail traders

The city of Puebla is one of the most economically important cities in southeastern Mexico. The seafood market, known as Mercado Popular de la 18 Poniente in Puebla, receives products coming mainly from La Nueva Viga and to a lesser extent from other states. During a field visit, a single establishment was identified that sells the Malaysian prawn (*M. rosenbergii*) from Vietnam, which shared space in commercial window displays with other high-value species like squid, crab claws, smoked oysters, and lobster; the prawn is traded at a public price of $650.00 pesos MX ($32.91 USD) per two-pound box (eight pieces) or boxes with 4-6 pieces depending on size.

Using the commercial name of prawn, some native species can be found such as acamaya (*M. carcinus*) and Australian lobster (*Cherax quadricarinatus*), which compete as substitutes.

Plaza del Mar in Veracruz receives fishing products and freshwater crustaceans with commercial value, among which the following stand out: *M. acanthurus*, *M. carcinus*, *M. americanum* and *M. tenellum*, from regional fishing and from the states of Tabasco, Campeche, Tamaulipas, Nayarit, Sinaloa, and Colima. Only two shopkeepers were identified who traded *M. rosenbergii* prawn, with a stock of 10 masters in the warehouse per month; the product is imported from Vietnam and Bangladesh with a similar average price and product presentation to what is found in Puebla's market. The interview respondents agreed that it is a luxury product for special occasions, for the weekend and/or a party, and not accessible for all social classes, since their main sales are for middle-class customers and restaurants.

In the neighboring Veracruz-Boca del Río zone, an establishment was found devoted to the sale of Malaysian prawn; the presentation is the same as in the markets mentioned before, in box packaging presentation (box with eight pieces) but with the difference that this establishment carries out the sale per piece, focused on customers that cannot acquire the product by box ($650.00-$700.00 pesos MX).

Restaurants

With the technique of "mysterious customer", seven restaurants were visited, two in Mexico City, two in the city of Puebla, and two in the city of Veracruz. It was found that they offer Malaysian prawn dishes with average weight of 80 g in different presentations, the most popular being with garlic sauce, butter, or fine herbs, with prices that range from \$600.00 pesos MX kg^{-1} (\$30.19 USD) to \$1,800.00 pesos MX kg^{-1} (\$90.58 USD), depending on the type of establishment. It is considered that diners who prefer this product are middle and/or high class, since it is regarded as a gourmet product, which is consumed at events and/or on special occasions.

Self-service stores

Considering that Malaysian prawn is a gourmet product, based on the information gathered in the field, a visit was conducted through self-service stores in the cities of Veracruz (2 stores), Puebla (2 stores), and Mexico City (4 stores) devoted to the sale of specialized products. The prawn was found in presentations of four to seven pieces in plastic domes at prices that range between \$650.00 pesos MX kg^{-1} (\$32.91 USD) and \$680.00 pesos MX kg^{-1} (\$34.49 USD). However, through observation, it was possible to detect that the quality was not the same as in the other members of the market structure given that they presented a deteriorated appearance, not fresh, and of low quality (Tacon *et al.*, 2020). This is possibly because of the time of refrigeration, and which can be inferred that it is not the main way for consumers to acquire the prawn, given that freshness is an important aspect that they consider at the time of buying.

General profile of buyers of Malaysian prawn in Mexico

According to Del Río-Zaragoza *et al.* (2022), the profile of seafood consumer can be characterized and classified. Based on surveys, as well as triangulating the information with various agents that integrate the structure of the Malaysian prawn market in Mexico, a consumer's profile could be created. The gender of the consumer is unimportant, with age between 35-65 years, so it can be inferred that consumers present some economic stability with monthly income >$20,000 pesos MX ($1,006.0 USD), so it is thought that there are no budget restrictions for food purchasing. They present an educational level of undergraduate and/or graduate studies, and they are from middle, middle-high class in urban zones. According to New and Kutty (2010), there are emotional codes to identify the purchasing decisions of the consumer, and in this study, it is detected that the consumer decides to make his/her dishes buying the prawn in gourmet supermarkets or online stores; or sometimes, when they decide to try dishes, they attend restaurants. They consider that the Malaysian prawn is a dish for special occasions; they show high willingness to pay taking into consideration the quality and freshness of the product; there is greater preference for live presentations (movable way of trading, with control systems for water quality) (Table 2).

Commercialization structure of *M. rosenbergii* in Mexico

According to Vilaboa-Arróniz *et al.* (2009), there are diverse agents who participate in the commercialization of a product, shaping a system with operative structure and function. These agents are interrelated, in different combinations, to establish the distribution channels of the product. Figure 1 shows the commercialization structure of Malaysian prawn *M. rosenbergii* in Mexico. The elements considered are the following: production as initial element, which considers international and national market movements; wholesale and retail traders which reach distribution centers such as seafood supply centers, malls and restaurants, until reaching the final consumer.

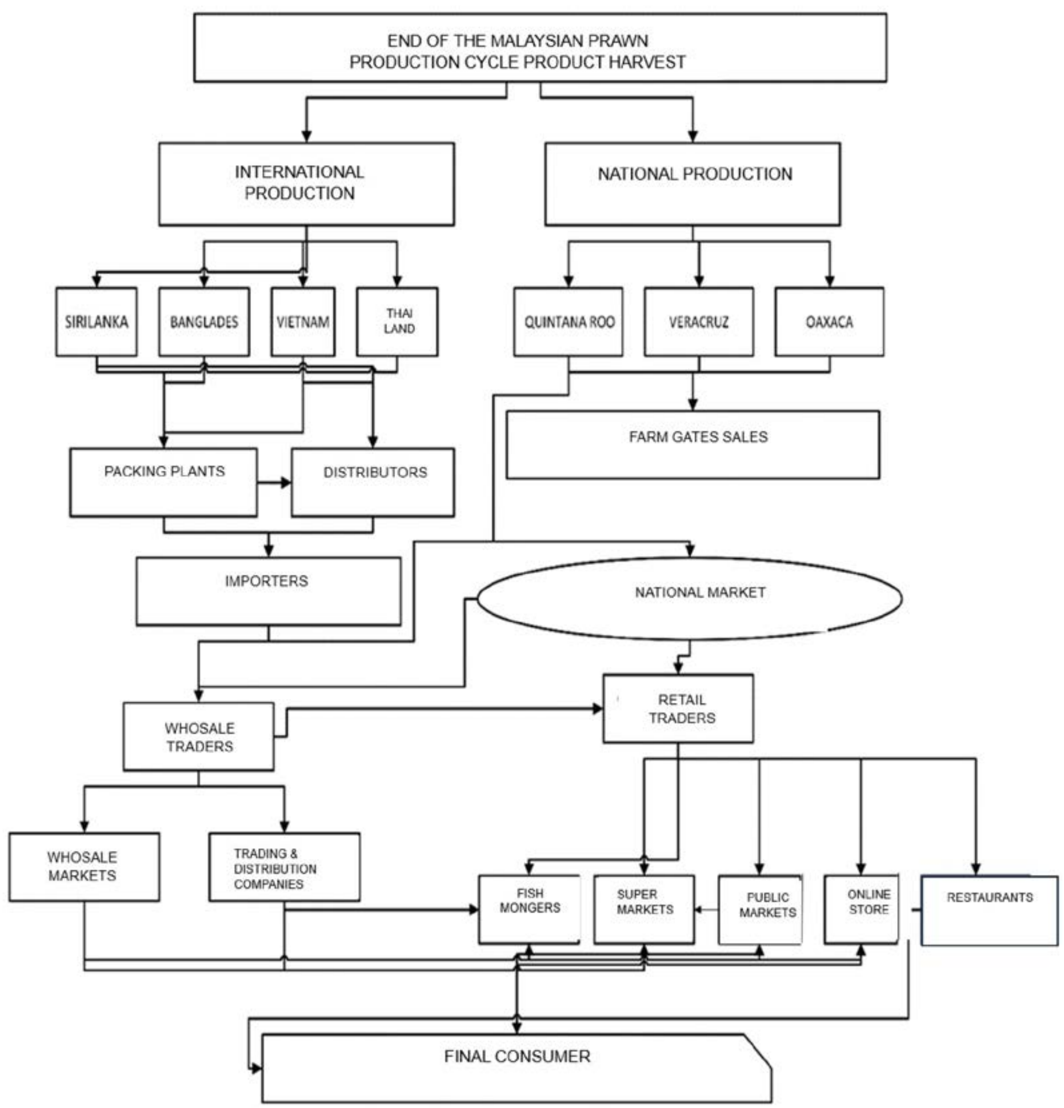

Figure 1. Commercialization structure of Malaysian prawn in Mexico.

Table 2. General motivations to purchase Malaysian prawn (*Macrobrachium rosenbergii*) in Mexico.

Feature	Description
Flavor	The intense and delicate nature of the prawn is its main motivator.
Status	It is a symbol of social status and good taste.
Health	It is perceived as eating healthy and nutritious food. They care about their diet and look for natural and nutritious products.
Culinary experience	It seeks to enjoy a complete gastronomic experience, from the choice of the product to its preparation.
Gourmet experience	Enjoy good food and are constantly looking for new culinary experiences. Appreciates high-quality products and good design.
Quality	Looks for the highest quality, fresh, and sustainably sourced produce.
Exclusivity	Attracted to unique and hard-to-find products.

Source: Prepared by the authors based on information from field research.

Malaysian prawn has proven to be a species with great potential in global aquaculture, especially in Asia. However, in Mexico, its production still faces challenges that limit its development, such as the lack of access to advanced technologies and scarcity of reproducers. For the country to take advantage of the opportunities that the international market offers, it is necessary to invest in technological innovation and to improve local production systems, which would allow Mexico to reduce its dependence on imports and become a relevant competitor in the global market of Malaysian prawn.

CONCLUSIONS

The market structure and commercialization channels of Malaysian prawn vary significantly depending on the region and the level of development of aquaculture. Globally, oligopoly markets predominate where a few actors control most of the production and commercialization, while in local markets like Mexico, the commercialization chain includes several intermediaries that add value in each stage. The added value of Malaysian prawn is influenced by factors like the technology used in production, distribution systems, and consumer perception. These aspects are key to understanding the dynamics of the market and the opportunities to improve competitiveness of the product in global and national markets. Finally, it was found that there are two origins of the product, the national which is incipient and the imported from Asian countries, particularly Vietnam. There are trading companies that stockpile the product and introduce it to the market in the American continent, and Mexico is one of the importers. These imports reach the large supply centers and then are distributed to retail markets and supermarkets. This commercialization chain is motivating to induce national production of the *M. rosenbergii* prawn.

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Colegio de Postgraduados

Financial Feasibility Assessment of the Smart Eco-Friendly Backpacks Project in Mexico

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ABSTRACT

Objective: To determine the financial feasibility of the smart eco-friendly backpack project by calculating the discount rate and the internal rate of return, in order to support the decision to accept or reject the investment proposal.

Design/methodology/approach: Financial project evaluation based on a five-step methodology and the weighted average cost of capital.

Results: An initial investment of $564,420.00 with an 8-year analysis horizon; a discount rate of 12.32%; an internal rate of return of 147.12%; a net present value of $5,588,559.15; a benefit-cost ratio of 1.20; a benefit-to-initial-investment ratio of 10.90; and a payback period of 0.92 years.

Limitations of the study/implications: The results are based on the assumptions made and may not hold true under different conditions.

Findings/conclusions: All indicators meet the acceptance criteria; the financial assessment is positive, and project implementation is recommended, as it represents an attractive investment opportunity. The project not only demonstrates financial profitability but also contributes to environmental sustainability by promoting the use of biodegradable materials.

Keywords: investment, evaluation, net present value, profitability, eco-friendly backpack.

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INTRODUCTION

According to the most recent data from the National Institute of Statistics and Geography (2025), during the second half of 2024, 8.7% of households in urban areas of Mexico reported that at least one of their members had been a victim of robbery or assault either on the street or in public transportation. This type of crime commonly involves the theft of personal belongings such as cell phones, laptops, and backpacks.

The theft of personal belongings represents a serious urban security crisis in Mexico, as thousands of people experience this situation daily, often without reliable records. Therefore, the design of this backpack will contribute to improving localization and

safety. However, conventional backpacks, typically made from materials such as synthetic blends, have a considerable environmental impact due to their slow decomposition process and the resources used in their production (Fundación Bosque Sagrado Dominicana, 2023). Furthermore, the time it takes for a conventional backpack to break down in the environment depends on its specific composition.

Backpacks made from synthetic blends (such as polyester or nylon) can take between 30 and 100 years to fully decompose, as they are not biodegradable and their decomposition process is very slow. Moreover, during decomposition, synthetic fibers break down into small particles that can be ingested by marine and terrestrial organisms, affecting wildlife and entering the human food chain. Additionally, their production requires non-renewable resources and generates higher greenhouse gas emissions (Fábrica de Bolsas Plásticas, 2023).

According to Vogo (2024), conventional backpacks, mostly made from synthetic materials such as polyester, PVC, and synthetic leather, have a significant environmental impact when discarded. These petroleum-derived materials are highly resistant to degradation, contributing to the accumulation of waste in landfills and natural ecosystems. Moreover, during their degradation, backpacks can release microplastics and toxic chemicals, such as persistent organic pollutants, which affect soil and water quality and pose a threat to wildlife and human health (IPEN, 2021).

For this reason, to mitigate these impacts, Gómez, Rodríguez, and Ochaga (2023) recommend choosing backpacks made from recycled or biodegradable materials, reusing and repairing backpacks instead of discarding them, and participating in recycling programs and proper waste management. By adopting these practices, individuals contribute to reducing environmental pollution and promoting more responsible consumption. Therefore, the smart eco-friendly backpack project was developed with the aim of providing security and confidence to all individuals seeking stability in a social environment and protection from theft that could compromise personal safety. The backpack features a global positioning system (GPS), designed to facilitate the easy location of people or belongings, thereby enhancing personal protection. Additionally, the backpack offers high-quality fabric that is eco-friendly, resulting in a 85.72% neutral environmental impact and a 14.28% moderately negative impact. It does not harm the environment, as the product uses sustainable leather made from cactus fiber. According to Rey (2021), cactus-based vegetable leather is revolutionizing the fashion industry; discussing vegetable leather no longer implies a material exclusively for vegans, but rather a sustainable alternative to animal and synthetic leather suitable for anyone seeking an environmentally friendly option.

Nowadays, it is well known that the production of animal-derived leather is highly polluting, in addition to involving animal cruelty. The tanning and finishing processes use chemicals that are often discharged into water, damaging ecosystems and everything within them. Rey (2021) states that bio-based materials, particularly regenerative ones, are part of the sustainable trends of 2021 and represent the best alternative to animal-derived or plastic-based materials. Cactus-based vegetable leather is among the most sought-after by brands and designers aiming to steer their products toward sustainability.

The project involves the production, commercialization, and distribution of backpacks made from cactus-based leather, featuring an internal GPS device, with the aim of providing a high-quality product. The project belongs to the manufacturing industry (secondary sector of the national economy) under the classification Transformation 111108.323003: Manufacture of leather, hide, and substitute products (Secretaría de Economía, 2024). The project also falls within the tertiary sector through the commercialization of its product, classified as 72: Retail trade of leather, hide, and substitute products 623026 (Secretaría de Economía, 2024).

The problem consists of a series of factors that influence the feasibility and sustainability of the project. Concerns begin with the need to conduct a comprehensive financial analysis capable of determining whether the project's business model can generate sufficient revenue to cover its costs at the start of operations. This also involves the creation of a solid financial structure that ensures the feasibility and sustainability of the project in the short, medium, and long term. Its background lies in the initial investment required for the project to commence operations, as there is uncertainty regarding the project's profitability. The generated revenue must be sufficient to cover operating costs and yield an attractive profit margin. If proper financial planning is not carried out, the long-term economic feasibility of the project is put at risk. Moreover, the project depends on securing external sources of financing to cover the initial investment and operating costs. While the project may have some internal resources, it is advisable to rely on loans, investors, grants, or credits that can ensure financial leverage and contribute to increased profitability.

MATERIALS AND METHODS

The research methodology was conducted in three phases: office, field, and office. In the office phase, project planning was carried out through research using confidential primary and secondary sources, in order to define the business idea. In the field phase, the necessary data and information were collected to support the development of the investment project based on the FISH business model (Martínez, Portillo & Del Valle, 2018). In the final office phase, the information was analyzed and processed using the following models and indicators: five-step project financial evaluation, weighted average cost of capital, discount rate (DR), internal rate of return (IRR), net present value (NPV), benefit–cost ratio (BCR), benefit-to-initial-investment ratio (BIR), and payback period (PP).

Step 1. Economic Analysis

Economic analysis aims to determine whether a project contributes positively to the economic well-being of an organization by evaluating benefits and costs from a financial perspective (Sapag & Sapag, 2014). Additionally, Martínez, Portillo & Del Valle (2019) indicate that economic analysis is the process of structuring the operating cost budget and the revenue budget in order to study and understand the project's economic status.

The project's economic status was analyzed, represented by the investment cost budget, the operating cost budget, and the revenue budget. An initial investment of $564,420.00 was estimated, of which 79.53% corresponds to fixed assets ($448,870.00), 10.45% to deferred assets ($59,000.00), and 10.02% to working capital ($56,550.00) (Table 1). An investment program of 6 months was proposed in year 0. Using the straight-line depreciation method, it was determined that the project loses value by $27,369.00 annually, of which $19,994.00 corresponds to the depreciation of fixed assets and $7,375.00 to the amortization of deferred assets. Based on the average useful life method for fixed assets, the analysis horizon was calculated to be 8 years. It was estimated that in year 5, a reinvestment of $42,856.00 will be required for the replacement of fixed assets.

Using the monthly revenue and cost budgets for the first year of operation, the project's cash flow and working capital were determined. The operating cost budget and the project revenue budget were projected over the 8-year analysis horizon. During this period, the established operation schedule was 50% in the first year, 60% in the second year, 70% in the third year, 80% in the fourth year, 90% in the fifth year, and 100% in the sixth, seventh, and eighth years.

Step 2. Financing Analysis

Financing analysis is crucial in project evaluation because it determines how costs will be covered and what impact the cost of capital will have on the project's profitability. A proper financial analysis allows for the identification of the most efficient capital structure, minimizing risks and ensuring that the project is financially viable. It also enables the projection of the project's capacity to generate sufficient cash flows to cover debts and other financial obligations (Cano, 2017).

Table 1. Investment Cost Budget.

No.	CONCEPT	UNIT	AMOUNT	UNIT PRICE ($)	TOTAL INVESTMENT ($)
A. FIXED ASSETS					**448,870.00**
1	Land	m^2	120	2,000.00	240,000.00
2	Building	Unit	1	125,000.00	125,000.00
3	Computer equipment	Part	5	10,000.00	50,000.00
4	Desk	Part	5	1,900.00	9,500.00
5	Printer	Part	1	3,570.00	3,570.00
6	Straight sewing machine	Part	2	8,000.00	16,000.00
7	Desk	Part	1	1,000.00	1,000.00
8	Chairs	Part	6	400.00	2,400.00
9	Shelf	Part	1	1,400.00	1,400.00
B. DEFERRED ASSETS					**59,000.00**
1	Company constitution	Service	1	15,000.00	15,000.00
2	Operating license	Service	1	3,500.00	3,500.00
3	Patents	Service	1	5,000.00	5,000.00
4	Office 365 package	Service	1	4,000.00	4,000.00
5	Training	Service	1	1,500.00	1,500.00
6	Plant and equipment insurance	Service	1	30,000.00	30,000.00
C. WORKING CAPITAL					**56,550.00**
D. TOTAL INVESTMENT					**564,420.00**

Source: Prepared with project data (2025).

Based on the financing needs of the investment project, the capital financing structure was defined by integrating resources from three financial sources: $296,550.00 of equity, representing 52.54% participation, with an annual return of 15.68%; $142,870.00 of subsidies, representing 25.31% participation, with an opportunity cost of 10.00% per year; and $125,000.00 of loans, representing 22.15% participation, with an annual interest rate of 7%. Using the weighted average cost of capital method, the project's discount rate was determined to be 12.32% per year (Table 2).

Using the constant total amortization method, a loan repayment schedule was proposed with a term of 5 years and an interest rate of 7% per year on the outstanding balance, resulting in a constant annual payment of $30,486.34.

Step 3. Analysis of Pro Forma Financial Statements

Pro forma financial statements are projected financial statements that show the financial effects of hypothetical events or transactions, helping management plan and make decisions based on future financial estimates (Weygandt, Kimmel & Kieso, 2019). Step 3 of the five-step financial evaluation involves defining the income statement, the balance sheet, the cash flow statement, and the sources and uses of funds over the project's analysis horizon.

This step encompasses the income statement, the cash flow statement, the statement of sources and uses of funds, the statement of financial position, the calculation of complementary financial ratios for investment projects, the debt service capacity or coverage ratio, the payback period, and the break-even point.

The structuring and analysis of three pro forma financial statements were carried out: the income statement, the cash flow statement, and the balance sheet. The income statement showed net profits in all years of the analysis horizon. The cash flow statement showed positive results in all years of the horizon. In the balance sheet, the two previous financial statements were consolidated in report form (Table 3). It was estimated that, at the end of the analysis horizon, the project's salvage value is $433,463.83, of which $331,774.00 corresponds to the recovery of fixed assets and $101,689.83 to the recovery of working capital.

Step 4. Financial Analysis

Martínez, Portillo & Del Valle (2019) define financial analysis as the process of calculating the Net Present Value, Internal Rate of Return, Benefit/Cost Ratio, and Benefit/Initial Investment Ratio, which determine the project's profitability over the analysis horizon.

Table 2. Financing Structure and Weighted Average Cost of Capital.

No.	CONCEPT	TOTAL	SHARE (%)	FINANCIAL COST (%)	WEIGHTED AVERAGE (%)
1	Partner contribution	296,550.00	52.54	15.68	8.24
2	Subsidy	142,870.00	25.31	10.00	2.53
3	Long term credit	125,000.00	22.15	7.00	1.55
A	Total investment	564,420.00	100.00		12.32

Source: Prepared with project data (2025).

Table 3. Pro forma Balance Sheet.

No.		CONCEPT	0 %	50 %	60 %	70 %	80 %	90 %	100 %
			Year 0	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6 al 8
	ASSETS		($)	($)	($)	($)	($)	($)	($)
A	FIXED ASSETS		448,870.00	428,876.00	408,882.00	388,888.00	368,894.00	391,756.00	371,762.00
	1	SUBTOTAL	448,870.00	448,870.00	448,870.00	448,870.00	448,870.00	491,726.00	491,726.00
	2	Depreciation	0.00	19,994.00	39,988.00	59,982.00	79,976.00	99,970.00	119,964.00
B	DEFERRED ASSETS		59,000.00	51,625.00	44,250.00	36,875.00	29,500.00	22,125.00	14,750.00
	1	SUBTOTAL	59,000.00	59,000.00	59,000.00	59,000.00	59,000.00	59,000.00	59,000.00
	2	Amortization		7,375.00	14,750.00	22,125.00	29,500.00	36,875.00	44,250.00
C	WORKING CAPITAL		56,550.00	716,660.73	1,592,824.75	2,684,999.43	3,993,139.20	5,480,767.69	7,251,174.23
	1	Working capital	56,550.00	56,550.00	65,577.97	74,605.93	83,633.90	92,661.87	101,689.83
	2	Cash flow	0.00	660,110.73	1,527,246.78	2,610,393.50	3,909,505.30	5,388,105.82	7,149,484.40
D	TOTAL ASSETS LIABILITIES		564,420.00	1,197,161.73	2,045,956.75	3,110,762.43	4,391,533.20	5,894,648.69	7,637,686.23
E	SHORT-TERM LIABILITIES								
F	LONG-TERM LIABILITIES		125,000.00	103,263.66	80,005.78	55,119.85	28,491.90	0.00	
	1	Final unpaid balance of credit	125,000.00	103,263.66	80,005.78	55,119.85	28,491.90	0.00	
G	TOTAL LIABILITIES		125,000.00	103,263.66	80,005.78	55,119.85	28,491.90	0.00	
	SHAREHOLDERS' EQUITY								
	1	Share capital	296,550.00	296,550.00	296,550.00	296,550.00	296,550.00	296,550.00	296,550.00
	2	Other capital accounts	142,870.00	142,870.00	142,870.00	142,870.00	142,870.00	142,870.00	142,870.00
	3	Results from previous years	0.00	0.00	654,478.07	1,526,530.96	2,616,222.58	3,923,621.30	5,455,228.69
	4	Exercise result	0.00	654,478.07	872,052.89	1,089,691.62	1,307,398.72	1,531,607.39	1,743,037.54
H	TOTAL SHAREHOLDERS' EQUITY		439,420.00	1,093,898.07	1,965,950.96	3,055,642.58	4,363,041.30	5,894,648.69	7,637,686.23

Source: Prepared with project data (2025).

The project's cash flow was structured over an 8-year horizon, on which the profitability indicators were calculated.

To test the proposal, the calculation of profitability indicators was carried out using the project's financial information, evaluating an investment of $564,420.00 at a discount rate of 12.32%, with cash flows occurring annually over an 8-year analysis horizon.

Step 5. Sensitivity Analysis

According to Gutiérrez & Serrano (2016), sensitivity analysis is used to evaluate the stability of a project's results in response to changes in the main variables, providing valuable information for risk management and decision-making. The project's sensitivity analysis was conducted considering the product's selling price as the risk variable, given that it is the most unstable throughout the year. Three scenarios of price reduction were evaluated: 5%, 10%, and 15%. Within this range, the project does not show sensitivity to these decreases, as it still generates profits; even with a price reduction of up to 15%, the company remains profitable. The project can withstand a price decrease of up to 27.61% while remaining profitable; below this threshold, the project ceases to be viable.

Table 4. Cash Flow Discounted at 12.32 %.

YEAR	TB ($)	TC ($)	UTB ($)	UTC ($)	UCF ($)	CUCF ($)
0	0.00	564,420.00	0.00	564,420.00	−564,420.00	−564,420.00
1	4,490,129.16	3,799,532.09	3,997,625.29	3,382,776.99	614,848.31	50,428.31
2	5,388,154.99	4,490,532.61	4,270,970.59	3,559,461.96	711,508.63	761,936.94
3	6,286,180.82	5,172,547.77	4,436,256.22	3,650,347.94	785,908.28	1,547,845.22
4	7,184,206.66	5,854,608.51	4,513,898.80	3,678,500.86	835,397.94	2,383,243.17
5	8,092,946.49	6,583,859.63	4,527,129.54	3,682,958.42	844,171.12	3,227,414.28
6	8,980,258.32	7,218,879.74	4,472,479.23	3,595,251.78	877,227.45	4,104,641.73
7	8,980,258.32	7,209,851.78	3,981,911.31	3,196,900.28	785,011.03	4,889,652.76
8	8,980,258.32	7,209,851.78	3,545,151.78	2,846,245.39	698,906.39	5,588,559.15
TOTAL			33,745,422.76	28,156,863.61	5,588,559.15	

Source: Prepared with project data (2025).
TB=Total Benefits. TC=Total Costs. UTB=Updated Total Benefits. UTC=Updated Total Costs. UCF=Updated Cash Flow. CUCF=Cumulative Updated Cash Flow.

Table 5. Project Profitability Indicators.

No.	INDICATOR	ACRONYM	VALUE	OBSERVATION
1	Discount Rate	DR	12.32 %	Annually
2	Internal Rate of Return	IRR	147.12 %	Annually
3	Net Present Value	NPV	$ 5,588,559.15	Horizon
4	Benefit–Cost Ratio	BCR	1.20	Horizon
5	Benefit-to-Initial-Investment Ratio	BIR	10.90	Horizon
6	Payback Period	PP	0.92	Years

Source: Prepared with project data (2025).

Table 6. Sensitivity Analysis Based on Product Price.

What happens if the price decreases by:	PRICE ($)	IIR	NPV ($)	BCR	BIR	PP
0 %	2,867.26	147.12 %	5,588,559.15	1.20	10.90	0.92
5 %	2,723.90	123.80 %	4,576,376.27	1.17	9.11	1.12
10 %	2,580.53	100.51 %	3,564,193.38	1.13	7.31	1.42
15 %	2,437.17	77.13 %	2,552,010.50	1.10	5.52	1.95
−27.61 %	2,075.71	12.32 %	0.00	1.00	1.00	8.00

Source: Project data elaboration (2025).

RESULTS AND DISCUSSION

According to the five-step financial project evaluation, conducted with a discount rate of 12.32 % over an 8-year horizon, the results obtained were: 147.12 % IRR, $5,588,559.15 NPV, 1.20 B/C ratio, 10.90 B/II ratio, and 0.92 years payback period.

The ecological backpack project is unique due to its innovative design, which includes a GPS that allows easy tracking of the item in case of theft or loss. Other backpacks already on the market are also made from biodegradable nopal leather; however, instead of a GPS, their distinguishing feature is that they are solar-powered (Fernández, 2021).

Discount Rate

DR=12.32 %. For every $100.00 of annual investment in the project, a financial cost of $12.32 is estimated per year, with cash flows occurring annually over the 8-year analysis horizon. Since the DR is lower than the 147.12 % IRR, which is its critical point, the project is accepted and the evaluation is positive. It is recommended to proceed with the project, as it will generate net profits after covering the initial investment, operating costs, and the financial cost of capital.

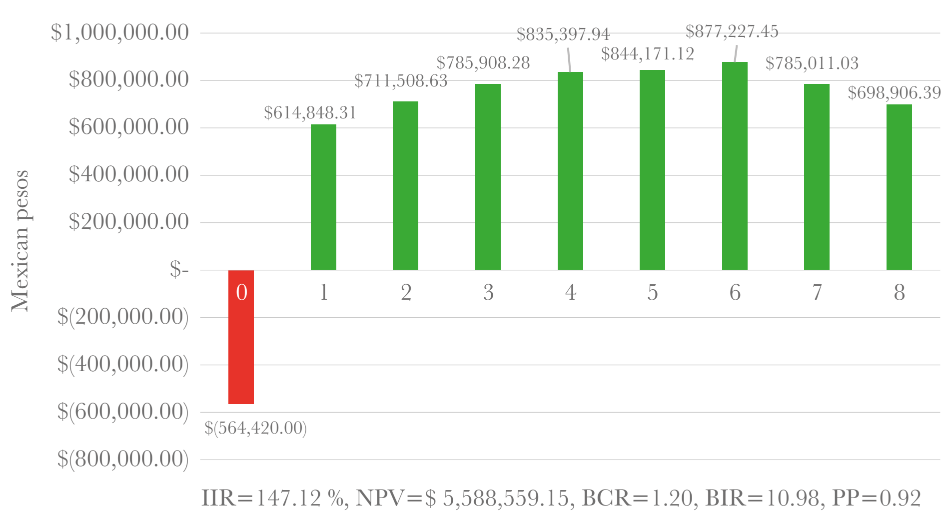

Figure 1. Cash flow discounted at 12.32 %. Source: Own elaboration with project data (2025).

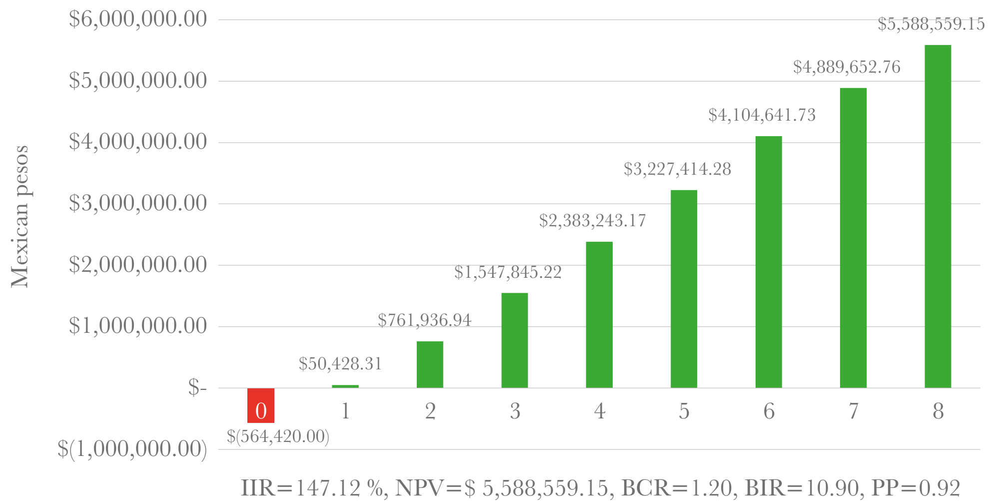

Figure 2. Cumulative discounted cash flow at 12.32 %. Source: Own elaboration with project data (2025).

Internal Rate of Return

IRR=147.12%. For every $100.00 of annual initial capital investment in the project, $147.12 of net profit is generated annually, with cash flows occurring each year over the 8-year analysis horizon. Since the IRR is higher than the 12.32% discount rate (DR), which is its critical point, it is accepted and the evaluation is positive. It is recommended to proceed with the project because it will generate net profits after covering the initial investment, operating costs, and the financial cost of capital.

Net Present Value

NPV=$5,588,559.15. The initial capital investment of $564,420.00 in the project will generate a net profit of $5,588,559.15 at a 12.32% discount rate, with cash flows occurring annually over the 8-year analysis horizon. Since the NPV is greater than 0, with 0 as its critical point, it is accepted and the evaluation is positive. It is recommended to proceed with the project because it will generate net profits after covering the initial investment, operating costs, and the financial cost of capital.

Benefit/Cost Ratio

BCR=1.20. For every $1.00 invested in the project's total costs, $1.20 of total benefits will be generated at a 12.32% discount rate, with cash flows occurring annually over the 8-year analysis horizon. Since the BCR is greater than 1, with 1 as its critical point, it is accepted and the evaluation is positive. It is recommended to proceed with the project because it will generate net profits after covering the initial investment, operating costs, and the financial cost of capital.

Benefit/Initial Investment Ratio

BIR = 10.90. For every $1.00 of initial investment in the project, $10.90 of total benefits will be generated at a 12.32% discount rate, with cash flows occurring annually over the

8-year analysis horizon. Since the BIR is greater than 1, with 1 as its critical point, it is accepted and the evaluation is positive. It is recommended to proceed with the project because it will generate net profits after covering the initial investment, operating costs, and the financial cost of capital.

Payback Period (PP)

PP=0.92 years. The initial investment of $564,420.00 in the project is estimated to be recovered by December 2 of the first year of operation, with cash flows occurring annually, discounted at 12.32% over the 8-year analysis horizon. Since the PP is less than 8 years, with 8 years as its critical point, it is accepted and the evaluation is positive. It is recommended to proceed with the project because it will generate net profits after covering the initial investment, operating costs, and the financial cost of capital.

CONCLUSIONS

It is concluded that the project is financially feasible, as all profitability indicators meet the acceptance criteria; therefore, the project is profitable, accepted, and the evaluation is positive. The IRR of 147.12% is higher than the 12.32% discount rate (DR), the NPV of $5,588,559.15 is greater than $0.00, the B/C ratio is 1.20 (greater than 1), the B/IC ratio is 10.90 (greater than 1), and the payback period (PBP) is 0.92 years (less than the 8-year analysis horizon).

It is recommended to carry out the project, as it is profitable, generating net profits after covering operating costs each year of the analysis horizon, recovering the initial investment, and paying the financial cost of capital.

The project generates a positive environmental impact, as the backpacks are eco-friendly and made from biodegradable materials that are environmentally friendly, contributing to the reduction of the ecological footprint. Furthermore, it provides a sustainable option for young people and the target population in meeting their needs.

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Colegio de Postgraduados

Production of Bioaugmented Composts with *Trichoderma harzianum*

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ABSTRACT
Objective: To develop a bioaugmented compost in a pile system through the addition of submerged fermentation products derived from *Trichoderma harzianum*.
Methodology: Biodegradation kinetics of newspaper were monitored over a 30-day period in a cylindrical air-lift bioreactor inoculated with *Trichoderma harzianum* (IrV6S1C7), aiming to generate fungal biomass and cellulolytic enzymes. Subsequently, the fermentation products were incorporated into a 60 kg pile of organic waste comprising banana, red mango, leaf litter, and lettuce to assess waste degradation time in comparison to an uninoculated control.
Results: By day 30 of fermentation, biomass production of *T. harzianum* reached 67 g/L, while the activities of cellulolytic enzymes carboxymethyl cellulase (CMCase) and filter paperase (FPase) were 2095.51 U/L and 1471.75 U/L, respectively. The compost pile inoculated with *T. harzianum* suspension exhibited a more consistent stabilization of the carbon-to-nitrogen (C/N) ratio over time relative to the control.
Conclusions: The bioaugmented compost enriched with *T. harzianum* and its fermentation derivatives facilitated the production of a stable pre-compost with enhanced nitrogen availability, benefiting both plant growth and the proliferation of beneficial microbial communities.

Keywords: organic waste, bioreactor, submerged culture, cellulases.

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INTRODUCTION
In Mexico, it is estimated that approximately 120,128 tons of municipal solid waste are generated daily (SEMARNAT, 2022), posing significant environmental and economic challenges due to the lack of comprehensive waste management systems. This deficit contributes to the emission of greenhouse gases and the contamination of soils and water bodies caused by leachate discharge (De Anda *et al.*, 2021). Approximately 50% of this waste is composed of organic matter (OM), which can be utilized for compost production. Composting is a low-cost biotechnological process that enables the transformation of organic waste into stable organic matter (mature compost) through the activity of diverse microorganisms (Vargas-Pineda *et al.*, 2019). Despite its advantages, conventional composting techniques are time-consuming. Therefore, the inoculation of organic residues with selected microbial consortia has been proposed to accelerate the composting process and enhance the quality of the final product (Méndez-Matías *et al.*, 2018; Condori Nina, 2022).

Among the microorganisms that can be employed to bioaugment compost and improve its efficacy as a soil fertility enhancer, *Trichoderma* species stand out due to their adaptive capacity and production of a wide range of bioactive metabolites, including enzymes, plant growth-promoting compounds, and volatile organic substances (Hernández-Melchor *et al.*, 2019; Loayza-Dueñas & Gallegos-Jara, 2020). A relevant example was demonstrated by Bellini *et al.* (2023), who evaluated *Trichoderma*-enriched compost for lettuce production and biocontrol of *Fusarium oxysporum* f. sp. *lactucae*. Their findings revealed a 70% reduction in disease severity, increased crop yield, and no adverse impact on the native soil microbial community. Similarly, Méndez-Matías *et al.* (2018) assessed the effect of *Trichoderma harzianum* and *Aspergillus* sp. inoculation during the composting of mezcal agave bagasse and sugarcane bagasse in a pile system in Oaxaca, Mexico. Their results confirmed that the inoculation of lignocellulolytic fungi significantly reduced substrate degradation time and improved the physicochemical properties of the mature compost, compared to a non-inoculated control. Despite the demonstrated benefits of exogenous microbial inoculation in enhancing composting efficiency and product quality, research in Mexico remains at the experimental level. Outcomes are highly dependent on the substrate type and the microbial strains employed, indicating the need for a deeper understanding of the process to facilitate its standardization and large-scale implementation. Based on the above, the aim of the present study was to evaluate the production of pile compost bioaugmented with submerged fermentation products of a native *Trichoderma harzianum* strain, previously isolated from the maize rhizosphere in Irapuato, Guanajuato.

MATERIALS AND METHODS

Microorganisms used

The strain IrV6S1C7, isolated from the rhizospheric soil of maize cultivated in experimental plots in Irapuato, Guanajuato, Mexico (Herrera-Jiménez *et al.*, 2018), was employed in this study. This strain is preserved in glass tubes containing slanted PDA medium at 4 °C and was taxonomically identified as *Trichoderma harzianum* (GenBank accession number MN165442) (Hernández-Melchor *et al.*, 2023). The strain was propagated by point inoculation onto Petri dishes containing PDA medium and incubated at 28 °C for five days. Subsequently, the cultures were exposed to light and ambient temperature for two additional days to promote sporulation.

Newspaper degradation kinetics in bioreactor

A batch submerged culture degradation kinetics experiment was conducted over 30 days using the IrV6S1C7 strain in a cylindrical air-lift bioreactor with a total volume of 6 L and an effective working volume of 5.5 L. The culture medium used was a basal minimal medium (BMM) composed of (g/L): 6.0 Na_2HPO_4, 3.0 KH_2PO_4, 2.64 $(NH_4)_2SO_4$, 0.5 $MgSO_2 \cdot 7H_2O$, 0.015 $CaCl_2$, 3.0 $MnSO_4$, and 3.0 $ZnSO_4$, with the pH adjusted to 4.8 using a 0.05 M citrate buffer (García-Espejo *et al.*, 2016). A total of 27.5 g (0.5% w/v) of untreated newspaper cut into 1 cm×1 cm pieces was used as the sole carbon source. The inoculum consisted of a spore suspension at a concentration of 1×10^6 spores/mL. Newspaper was selected due to its proven efficacy in inducing cellulase production in laboratory-scale submerged cultures using *Trichoderma harzianum* (Hernández-Melchor *et al.*, 2023). The bioreactor was operated under continuous aeration at a flow rate of 3 L/min via a perforated stainless-steel sparger located at the base. Air injection was regulated using a float-type rotameter with an aluminum float (Applikon Biotechnology, Netherlands) and a dual-outlet air pump (802 ELITE). The internal temperature was maintained at 24±1 °C, and pH was stabilized within the range of 4.2-5.0 without the addition of acid or base. Volume loss due to sampling and aeration was compensated weekly by adding sterile distilled water to maintain the working volume (∼70 mL) of the bioreactor.

Analytical determinations

Aliquots (20 mL) were collected every third day to quantify cellulase activity, biomass production, residual cellulose, and reducing sugars. All assays were performed in triplicate.

Quantitative analysis of cellulases

Total cellulase activity was determined using the Filter Paper Assay (FPase), while endoglucanase activity (CMCase) was assessed using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959; Ghose, 1987), employing D-glucose as the standard (calibration curve prepared from a 5 g/L glucose standard solution). The enzymatic activity protocols were conducted as described by Hernández-Melchor *et al.* (2022). Absorbance measurements were taken at 540 nm using a BioTek® Synergy 2 microplate reader. Enzymatic activities were expressed in IU/L (international units per liter).

Determination of biomass and residual cellulose

Fungal dry biomass and cellulose content in the bioreactor were determined according to the method described by Ahamed and Vermette (2009). Dry biomass was calculated by subtracting the residual cellulose weight from the total dry weight of solids, which includes both mycelium and cellulose.

Reducing sugar concentration

Reducing sugars were quantified using the DNS method (Miller, 1959). Optical density (OD) was measured at 540 nm using a BioTek® Synergy 2 spectrophotometer. A calibration curve was constructed from a 5 g/L D-glucose standard solution.

Pile compost production

Organic and garden waste were collected from the Colegio de Postgraduados, Montecillo campus, and the Central de Abastos in Mexico City (CDMX) to construct two composting piles (60 kg each). Each pile was composed of 18 kg of *Musa balbisiana* (plantain), 8 kg of *Mangifera indica* 'Tommy Atkins' (red mango), 23 kg of leaf litter, and 11 kg of *Lactuca sativa* (common lettuce). The piles measured 1×1×0.6 m and were covered with 70% black shade mesh, remaining exposed to outdoor environmental conditions. The waste materials were shredded and cut to an approximate particle size of 10 cm. Proportions of each component were homogenized to ensure uniform composition in the compost piles. One pile served as an uninoculated control, while the second was inoculated with the products derived from the bioreactor-based newspaper degradation process (fungal biomass, enzymes, residual cellulose, and fermentation broth) at the end of the culture kinetics (day 30). One replicate was established for each treatment. The compost piles were aerated through weekly manual turning over a 35-day period to evaluate the effect of microbial inoculation and to obtain a pre-compost undergoing maturation. Moisture levels were maintained within a 70-80% range through the addition of tap water. pH and temperature were monitored in both piles using a multiparameter soil meter. Composite samples (20 g) were collected weekly for 35 days to determine total carbon and nitrogen content, as well as to quantify cellulase activity (CMCase and FPase).

Determination of total carbon and nitrogen

Total carbon content was determined using the Walkley-Black method, while total nitrogen was measured using the Kjeldahl method (Rodríguez & Rodríguez, 2015).

Quantitative analysis of cellulases

From the composite samples collected from the compost piles, 5 g were weighed and mixed with 20 mL of 0.05 M citrate buffer (pH 4.8). The mixtures were incubated at room temperature with agitation at 100 rpm for 1 hour. After incubation, the samples were centrifuged at 400 rpm for 10 minutes. Subsequently, 2 mL aliquots of the supernatant were taken to determine total cellulase activity (FPase and CMCase) as described by Hernández-Melchor et al. (2022).

Statistical analysis

The collected data were statistically analyzed using the SAS software package for Windows 8, version 6.2-9200. All analyses were conducted in triplicate, and the mean ± standard deviation was used to represent the statistical significance of each data set. A 95% confidence interval was applied, and statistical significance was considered valid at $p<0.05$. One-way ANOVA was employed for the analysis.

RESULTS AND DISCUSSION

Newspaper degradation kinetics

A degradation kinetics study was conducted using newspaper as the sole carbon source for the native *T. harzianum* strain (IrV6S1C7), cultivated in a batch-mode submerged system within a cylindrical air-lift bioreactor. Figure 1 presents the results for biomass concentration, residual cellulose, and reducing sugars obtained throughout the 30-day culture period. Biomass production by *T. harzianum* peaked on day 9, reaching a maximum concentration of 97 g/L. Subsequently, a decline in biomass concentration was observed as the culture entered the stationary phase, yielding a final biomass concentration of 67 g/L by day 30.

Figure 1 also illustrates the concentrations of residual cellulose and reducing sugars, both byproducts of newspaper degradation (Centeno Rumbos *et al.*, 2015). The residual cellulose content exhibited a trend similar to that of biomass, increasing proportionally and reaching a maximum of 95 g/L on day 9, followed by a reduction to 43 g/L by day 30.

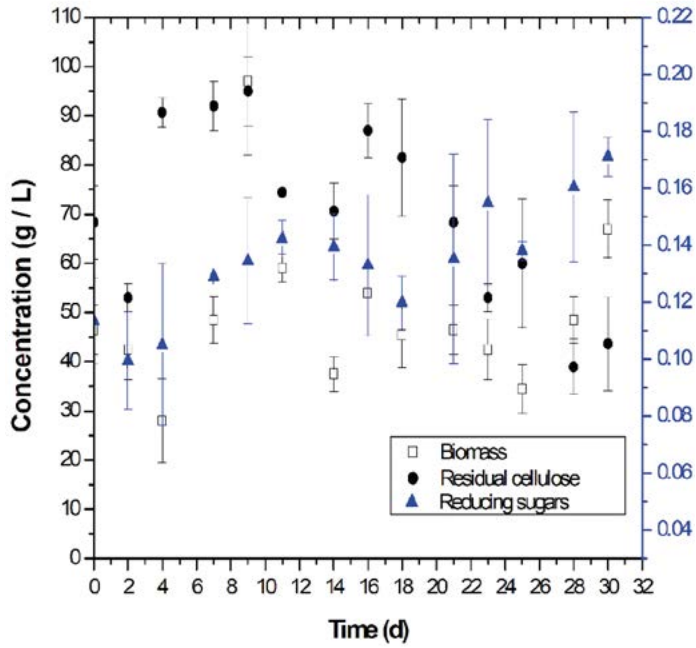

Figure 1. Biomass, residual cellulose, and reducing sugar concentrations over time during newspaper degradation in a cylindrical air-lift bioreactor. Mean values ± standard errors, n=3, ($p \leq 0.05$).

This trend indicates that the degradation of the carbon source (newspaper) was directly linked to biomass production over the course of the kinetics. Similarly, the concentration of reducing sugars steadily increased throughout the cultivation period, reaching a peak of 0.17 g/L on day 30 (Figure 1). This concentration is approximately 50 times higher than the value reported by Centeno Rumbos *et al.* (2015), who evaluated biomass production by *T. reesei* using paper sludge as a carbon source in submerged culture. Newspaper is primarily composed of cellulose, which can be enzymatically hydrolyzed into reducing sugars such as glucose and cellobiose (Hernández-Melchor *et al.*, 2019). Accordingly, the results of this study demonstrate a sequential degradation pattern of the substrate (newspaper), wherein *T. harzianum* biomass enzymatically breaks down the carbon source into its simplest form glucose. Figure 2 presents the enzymatic activity values for cellulases (FPase and CMCase) measured throughout the batch culture kinetics in the bioreactor during newspaper degradation by *T. harzianum*. As shown in Figure 2, CMCase activity increased over time, reaching a maximum of 2219 IU/L on day 7, after which it declined until the end of the cultivation period. In contrast, FPase activity continued to increase steadily over time, reaching its peak value of 1471 IU/L on day 30.

The presence of these enzymes during the biodegradation kinetics of newspaper indicates the breakdown of complex substrates into simpler forms that can be utilized by *T. harzianum* as a carbon source. The maximum cellulase activities observed in this study were 3.2 times lower for CMCase and 2.3 times higher for FPase compared to the values reported by Li *et al.* (2019), who assessed the cellulolytic enzyme production capability of *T. harzianum* LZ117 using cellulose as the carbon source. The differences between the present results and those reported in the literature can be attributed to the nature and

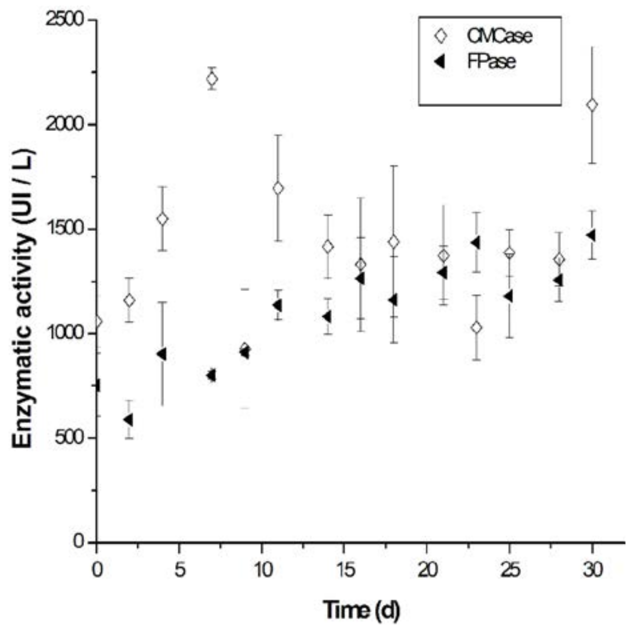

Figure 2. Cellulase activity (FPase and CMCase) over time during newspaper degradation in a cylindrical air-lift bioreactor. Mean values ± standard errors, n=3, ($p \leq 0.05$).

complexity of the substrate, as well as the operational parameters such as temperature, agitation, pH, and the bioreactor configuration all of which significantly influence the induction of enzymatic production in various fungal species (Adnan *et al.*, 2019; Bohacz-Kowalska, 2020). Given the enzymatic capacity of *Trichoderma* to degrade various carbon sources (Hernández-Melchor *et al.*, 2019), its incorporation as a bioaugmentation agent was evaluated for the degradation of organic matter (OM) during composting.

Pile compost production

The composting process was carried out over a period of 35 days to evaluate the effect of adding *T. harzianum* and the products derived from newspaper degradation in the bioreactor to the inoculated (bioaugmented) pile, in comparison to a non-inoculated control pile. Figure 3 presents the recorded values of temperature and pH throughout the composting process.

As shown in Figure 3A, the first five days correspond to the mesophilic phase in both piles. This was followed by the onset of the thermophilic phase, which lasted approximately 14 days and reached a maximum temperature of 44 °C. On day 23, a decrease in temperature was observed, marking the beginning of the cooling phase, which continued until day 35, when final temperatures of 22 °C and 21 °C were recorded in the inoculated and control piles, respectively. This behavior is consistent with that reported by Vargas-Pineda *et al.* (2019), who used waste from a local wholesale market to evaluate its degradation through composting. They described the microbial succession occurring at each stage of the process, highlighting the role of these communities in the degradation of organic matter (OM). In the present study, the early presence of *T. harzianum* in the inoculated pile had a significant effect on the timing of the different composting phases. The pH values in the inoculated

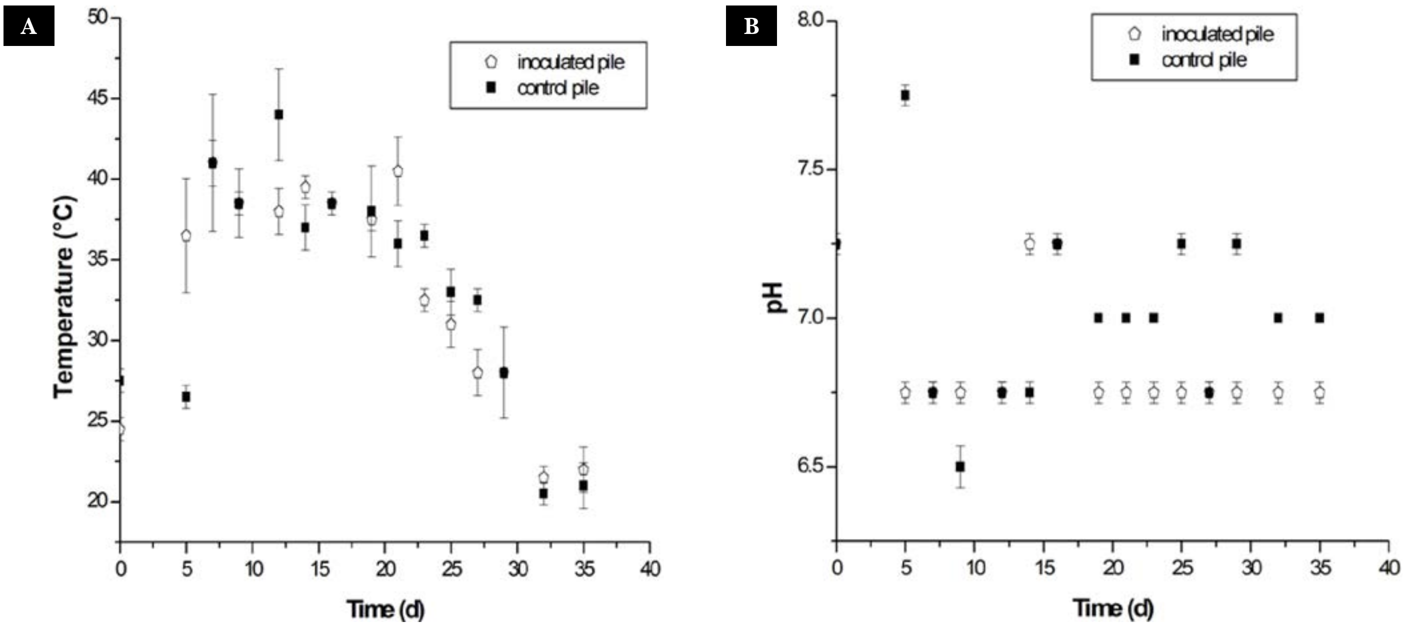

Figure 3. Temperature (A) and pH (B) profiles recorded during the composting process in the inoculated pile and the control pile. Mean values ± standard errors, n=3, ($p \leq 0.05$).

pile remained within a range of 6.7-7.2, while in the control pile they ranged from 6.5-7.7 (Figure 3B). pH plays a critical role in shaping microbial diversity during composting and affects nutrient availability. Generally, fungi tolerate pH levels between 5 and 8, whereas bacteria tend to thrive in slightly alkaline conditions, with optimal growth occurring in a pH range of 6-7.5 (Guano Andrade, 2017).

Carbon/Nitrogen (C/N) Ratio

Table 1 presents the C/N ratio values obtained from both the inoculated and control compost piles over the 35-day period. The C/N ratio in a composting process reflects the availability of carbon and nitrogen necessary for optimal microbial growth during the different composting stages (Bohórquez Santana, 2019). According to Hernández (2003), when the C/N ratio exceeds 33, the decomposition of plant residues tends to be slower; conversely, a ratio below 17 facilitates faster degradation. In the present study, the control pile initially (t_0) exhibited a C/N ratio of 34.3, while the inoculated pile started at 14.3. Over time, the ratios in both treatments fluctuated and eventually reached final values of 10.8 (control) and 11.6 (inoculated) by day 35 (Table 1). Iñiguez *et al.* (2011) indicate that a C/N ratio between 10 and 25 serves as a benchmark for compost maturity and stability. By day 35, both treatments produced a pre-compost undergoing maturation, as inferred from the final C/N ratios. However, additional analyses including heavy metal content, basal respiration, germination tests, and phytotoxicity assessment are required to fully evaluate the quality and maturity of the resulting product.

Based on these results, the pile inoculated with *T. harzianum* maintained a low C/N ratio from the beginning of the composting process through day 35, indicating greater nitrogen availability to support the growth of the fungus and other microorganisms.

Table 1. C/N ratio recorded during the composting process in a pile inoculated with *Trichoderma harzianum* (IrV6S1C7) and a control pile.

Time (d)	C/N ratio	
	Inoculated pile	Control pile
0	14.3	34.3
7	14.7	16.5
14	24.3	9.2
21	9.1	15.4
28	17.1	8.9
35	11.6	10.8

Méndez-Matías *et al.* (2018) conducted a similar study in which they evaluated the C/N ratio during the composting of mezcal agave bagasse and sugarcane bagasse inoculated with *T. harzianum*. At the start of the process, they reported an initial C/N ratio of 55.4, which decreased to a final value of 24.3 by day 133. Their findings demonstrated that the incorporation of lignocellulolytic microorganisms accelerated substrate degradation and led to the production of mature compost. According to the temperature, pH, and C/N ratio data presented, no significant differences could be established between treatments by day 35 as a result of the addition of submerged fermentation products of *T. harzianum*. Therefore, it is recommended to extend the composting period until maturity is achieved in order to properly assess the agronomic value of each treatment.

Quantitative Analysis of Cellulases

Due to the nature of the organic matter (OM) involved in the composting process primarily lignocellulosic materials a complex set of enzymatic mechanisms is required for its degradation (Gómez-García *et al.*, 2019). Among the key enzymes involved are cellulases, which are responsible for breaking down cellulose into simpler molecules. These enzymes are considered high-value bioproducts in various industrial sectors. Consequently, several research groups have explored cellulase production using low-cost lignocellulosic waste as raw material (Zhang *et al.*, 2017; Li *et al.*, 2019; Wang *et al.*, 2020). In light of this, the present study monitored cellulase enzymatic activity throughout the composting process. Furthermore, the presence of these enzymes may be associated with the specific microbial communities present at each stage and the nature of the decomposing materials (Guano Andrade, 2017). Figure 4 shows the enzymatic activity profiles of two cellulases (FPase and CMCase) quantified during composting in a pile inoculated with *T. harzianum* and in a non-inoculated control pile.

Figure 4 shows that the maximum cellulase activities (CMCase and FPase) occurred at the beginning of the composting process, decreasing progressively until day 35. Final values reached 28,123 and 14,098 IU/L for CMCase, and 12,402 and 1,829 IU/L for FPase in the control and *T. harzianum*-inoculated piles, respectively. The enzymatic activity trends over time are consistent with those reported by Guano Andrade (2017), who analyzed cellulase and amylase activity during the composting of sewage sludge and plant residues.

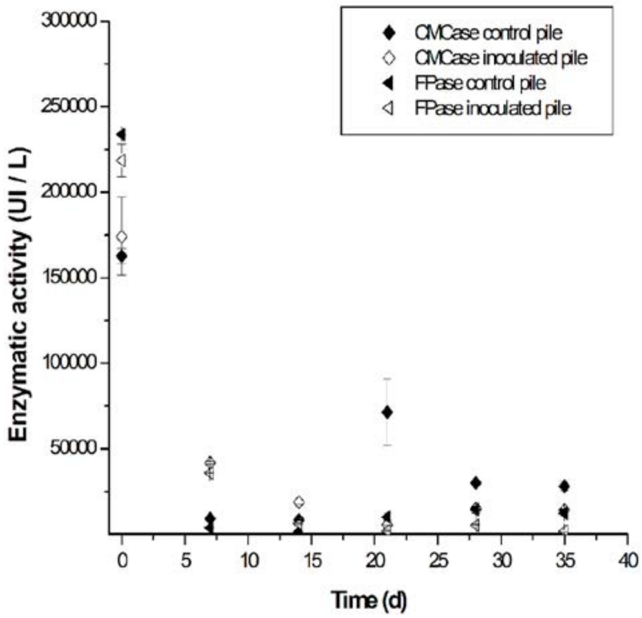

Figure 4. Cellulase activity (FPase and CMCase) over time during 35 days of composting organic matter in a pile inoculated with *T. harzianum* (IrV6S1C7) and a control pile. Mean values ± standard errors, n=3, ($p \leq 0.05$).

Similarly, Li *et al*. (2017) noted that the decrease in enzymatic activity during composting suggests a reduction in available substrates for microbial communities. This decline is also influenced by variables such as temperature and the type of raw material, which affect the dynamics of cellulolytic bacteria and fungi present in the composting material. These factors may help explain the lower enzymatic activity observed in the inoculated pile. Although both treatments exhibited comparable C/N ratios, the complexity of the carbon sources available may differ. Additionally, it is important to consider that the fermentation supernatant added to the inoculated pile contained active cellulases, which may have contributed significantly to the early degradation of substrates. This early activity could lead to the accumulation of simpler molecules by day 35, thereby reducing the need for continued high enzymatic activity. This hypothesis warrants further confirmation through molecular analysis of the compounds present in each pile. Despite the absence of statistically significant differences between treatments, it is worth highlighting *T. harzianum's* ability to degrade complex substrates and produce enzymes of industrial relevance. These findings support the potential for implementing a circular economy approach, whereby fungal biomass and enzymes can be generated from waste degradation and subsequently applied independently across different production scales.

CONCLUSIONS

The *T. harzianum* strain (IrV6S1C7) demonstrated the capacity to degrade newspaper under submerged culture conditions in a cylindrical air-lift bioreactor, effectively producing industrially relevant enzymes such as cellulases, along with fungal biomass. Although the initial addition of *T. harzianum* (IrV6S1C7) as a bioaugmentation agent during the composting process did not yield statistically significant differences compared to the uninoculated control, a pre-compost exhibiting early signs of maturation was successfully obtained by day 35. Therefore, future studies should consider extending the composting period to assess compost maturity, field applicability, economic feasibility, and agronomic validation.

ACKNOWLEDGMENTS

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Reproduction of *Procambarus llamasi* in a recirculating aquaculture system (RAS)

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ABSTRACT

Objective: The reproductive potential of *Procambarus llamasi* was evaluated in an aquaculture recirculation system (ARS).

Design/methodology/approach: The organisms were collected in the community of Xmaben, Hopelchén, Campeche. The experiment was carried out from August to September 2023 in the livestock area of the Colegio de Postgraduados, Campus Campeche. An experimental design with three treatments and one control was used, each with three replicates, randomly distributed in an ARS as follows: T_1: 1Male:2Females, T_2: 1M:3F, and T_3: 1M:4F, and the control. Water physicochemical parameters were recorded daily, and biometric measurements were taken biweekly along with the count of ovigerous females.

Results: The culture of *P. llamasi* in ARS allows for development and reproduction. Furthermore, there was no significant difference in the behavior of the water's physicochemical parameters, despite ammonium levels fluctuating around 0.2 mg/L. The optimal ratio with the highest number of ovigerous females was treatment two (1M:3F), making this the recommended ratio for *Procambarus* culture in Campeche.

Study limitations/implications: It is necessary to test this type of culture system with other *Procambarus* species found in the state, analyzing molting production and calcium concentration in the aquaculture recirculation system.

Findings/conclusions: The acocil (freshwater crayfish) is a viable alternative for obtaining animal protein and can be cultivated in family backyard systems in rural areas of Campeche.

Keywords: reproductive potential, aquaculture recirculation systems, ovigerous females, freshwater crayfish.

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INTRODUCTION

The term *acocil* derives from the Nahuatl word *acocilli* or *acuitzilli*, originating from *atl* (water) and *cuitzilli* or *coitzilli* (that which twists) (Hernández-Pérez & Correa-Benítez, 2022). In Mexico, approximately 44 species have been identified, most of which belong to the genus *Procambarus*, with one species assigned to the genus *Cambarellus* and one to *Orconectes* (Franco-Sustaita, 2014). Among the species of commercial importance,

Procambarus clarkii stands out, with aquaculture and fisheries production valued at 150 million USD (McAlain, 2009). *Acocil* is distinguished by its remarkable adaptability to adverse environments, which enables it to occupy a broad distribution range worldwide. In Mexico, its distribution extends from north to south, with the presence of species such as *Procambarus regiomontanus*, *P. clarkii*, *P. llamasi*, and *P. philosimanus* (Rodríguez-Serna, 2002; Bautista-Ortega *et al.*, 2024). In Campeche, *P. llamasi* is locally known as "camarón de pozo," "camarón de tierra," "camarón de popal," or, incorrectly, as "pigua" (Bautista-Ortega *et al.*, 2024). At present, limited information is available regarding the cultivation of *P. llamasi* and its adaptation to aquaculture recirculation systems (ARS). These systems function as water reuse technologies, employing a series of filters that maintain optimal conditions for the development and growth of aquatic species (García-Pulido *et al.*, 2011). Based on this context, the objective of the present study was to evaluate the reproductive performance of *Procambarus llamasi* in terms of the optimal male-to-female ratio within an ARS, with the purpose of promoting acocil culture and supporting the conservation of this underutilized local resource in rural areas of Campeche.

MATERIALS AND METHODS

The experiment was conducted in the livestock area of the experimental field at the Colegio de Postgraduados (COLPOS), Campeche Campus, located at coordinates 19° 29' 54.1" N, 90° 32' 45.7" W. The organisms were collected from temporary water channels in the community of Xmaben, Hopelchén, Campeche. Subsequently, they were transferred to the aquaculture recirculation system (ARS) and subjected to an acclimation period of 15 minutes. A total of 42 crayfish were used, comprising 30 females and 12 males, with an average initial length of 57.60±7.6 mm and 51.76±9.7 mm, respectively. The number of individuals stocked in each system varied according to treatment: T* (Control): 2 organisms, T_1: 3 organisms, T_2: 4 organisms, and T_3: 5 organisms, each with replicates. The experiment lasted 45 days, and the ARS was placed under natural light conditions with a sheet-metal roof, without photoperiod control. Selected individuals were in phase I, immediately after molting. Water physicochemical parameters were monitored, including nitrites (NO_2), nitrates (NO_3), ammonium $\left(NH_4^+\right)$, and pH, using an API test kit. Temperature (°C) and dissolved oxygen (mg/L) were measured with a portable analyzer (model D09100). Biometric measurements were performed biweekly, recording weight (g) and total length (mm) with a Goldenwall "Precision Instrument" electronic balance and an Esteren digital caliper. Ovigerous females were visually counted for each treatment. Feeding was provided ad libitum using VIMIFOS BUMPER CROP STARTER 40 pellets, formulated with 40% crude protein, 8% crude fat/lipids, 3% crude fiber, 12% moisture, 11% ash, 26% nitrogen-free extract (NFE), and a particle size of 1.8 mm.

The aquaculture recirculation system consisted of 12 fiberglass tanks (161×63.5×23.5 cm) with a capacity of 250 L each. The pumping system included a 1½ hp pump (model 12603 BOS-1SP) and a filtration system composed of plastic caps, volcanic rock (tezontle), mesh scraps, shade mesh, charcoal, and seashells. Each tank was equipped with 1" PVC tubes cut to 11.5 cm in length, serving as shelters for crayfish in each treatment. Water recirculation was continuous, ensuring organic matter removal and homogeneous water movement throughout the system. It is important to note that the ARS operated under balanced conditions, with a water renewal rate of 147.7 L/min. A completely randomized design (CRD) was employed, consisting of three treatments and one control, each with three replicates. Treatments were defined by different female-to-male ratios. Data were analyzed using one-way analysis of variance (ANOVA) with a confidence level of 0.05, followed by Tukey's test to determine significant differences, employing Statistica software version 0.7.0.

RESULTS AND DISCUSSION

Tables 2 and 3 show a significant difference in the number of ovigerous females across treatments, with treatment two (1M:3F) representing the optimal ratio for reproduction. This treatment yielded the highest proportion of ovigerous females and ensured 100% offspring survival. These findings align with Carmona-Osalde *et al.* (2005), who reported that gregarious behavior promotes maturation in both females and males, thereby increasing the likelihood of encounters and successful reproduction. A similar pattern was observed by Bustillos-Garza (2012), who documented that in *Procambarus clarkii*, copulation events occur mainly from May to June, while spawning peaks from August to October, with occasional spawning between November and December. The size of organisms stocked in the ARS also corroborates the observations of Rodríguez-Serna *et al.* (1999),

Table 1. Random distribution of treatments in the SRA.

Treatment	Container number	Description/relationship	Density of organisms (m^2)
* Control	1, 4,11	T*: 1 male and 1 female	2
T_1: 1M/2H	3, 7,10	T1: 1 male and 2 females	3
T_2: 1M/3H	5, 8,12	T2: 1 male and 3 females	4
T_3: 1M/4H	2, 6, 9	T3: 1 male and 4 females	5

Table 2. Number of ovulated females per treatment.

Treatment	Ovate Females
T*	0.00^{b}
T_1	1.00^{ab}
T_2	1.66^{a}
T_3	1.33^{a}

[a,b] Different letters in the same column indicate differences in Tukey statistics ($p<0.05$).

Table 3. Record in ovulated females during the months of August-September 2023.

Effect	Sum of squares	Degrees of Freedom	Analysis of Variance	F. Calculated	Confidence Level
Intercept	12.00000	1	12.00000	72.00000	0.000029
Treatment	4.66667	2	1.55556	9.33333	0.005441
Error	1.33333	8	0.16667		

who indicated that individuals reach sexual maturity at approximately 40 mm in length. Moreover, the female-to-male ratio is a critical factor influencing fertilization success and male competitiveness. These results are consistent with the studies conducted by Yeh and Rouse (1995), who demonstrated that an optimal 1:5 ratio in *Cherax quadricarinatus* cultures promoted successful reproduction, as observed in the present study. It is also important to note that small PVC tubes were used as shelters to prevent cannibalism and ensure organism survival throughout the experimental period.

The physicochemical parameters did not show significant differences throughout the 45-day experimental period. However, a similar behavior was observed in nitrate (NO_3), nitrite (NO_2), and pH levels, while ammonium exhibited minimal values, likely due to the accumulation of sediments and feed residues in the aquaculture system. According to Villalobos (1955), Rojas-Paredes & R. (1998), and Carmona-Osalde *et al.* (2002), species of the genus *Procambarus* exhibit high resistance to unfavorable environmental conditions in their natural habitats, enabling them to adapt and occupy diverse environments, including agricultural canals and drainage systems. Nitrite (NO_2) and nitrate (NO_3) concentrations in the recirculation system remained at 0.0 mg/L, indicating that both the ARS and the biofiltration system based on recycled materials operated effectively without the need for water exchange. This contrasts with the findings of Grajeda-Zabaleta *et al.* (2024), who reported nitrate concentrations of 2.64 mg/L in clear water and 0.75 mg/L in Biofloc systems. It is important to highlight that elevated nitrate levels can induce stress in aquatic organisms and may become toxic at concentrations exceeding 5 mg/L (Bautista & Ruíz, 2011). The pH remained stable at 7.60 across all treatments. This parameter plays a critical role in shrimp growth, as the optimal range has been established between pH 7.0 and 9.0 (Boyd *et al.*, 2001). It should be noted that at values above 8.5 (alkaline conditions), ammonium is converted into toxic ammonia, which may cause organism mortality, whereas values below 5.0 (acidic conditions) can also be detrimental. Water temperature in the ARS fluctuated around 26.73±0.78 °C, while dissolved oxygen averaged 4.17±2.76 mg/L. Within these ranges, crayfish carried out their biological activities (Figure 1). These results are consistent with those of Carmona-Osalde *et al.* (2004), who reported that *P. llamasi* inhabits environments with average temperatures between 20 °C and 29 °C, although it can acclimate to ranges from 5 °C to 35 °C. Furthermore, physiological and reproductive processes occur optimally at 26.8 °C, as also reflected in the present study, where reproduction and ovarian development were observed at 26.73±0.78 °C. Under this context, temperature directly influences molting frequency, as well as feed consumption and assimilation. Dissolved oxygen levels recorded in this study were higher compared to those reported by Grajeda-Zabaleta *et al.* (2024), who observed low oxygen concentrations in Biofloc systems, ranging from 2.6±0.5 mg/L in treatment T1BFT to 3.8±0.12 mg/L in T_2. This difference was attributed to oxygen consumption and competition by bacterial flocs in experimental Biofloc systems. These findings suggest that *Procambarus llamasi* can withstand low dissolved oxygen concentrations, reinforcing its classification as a resilient species with significant potential for aquaculture.

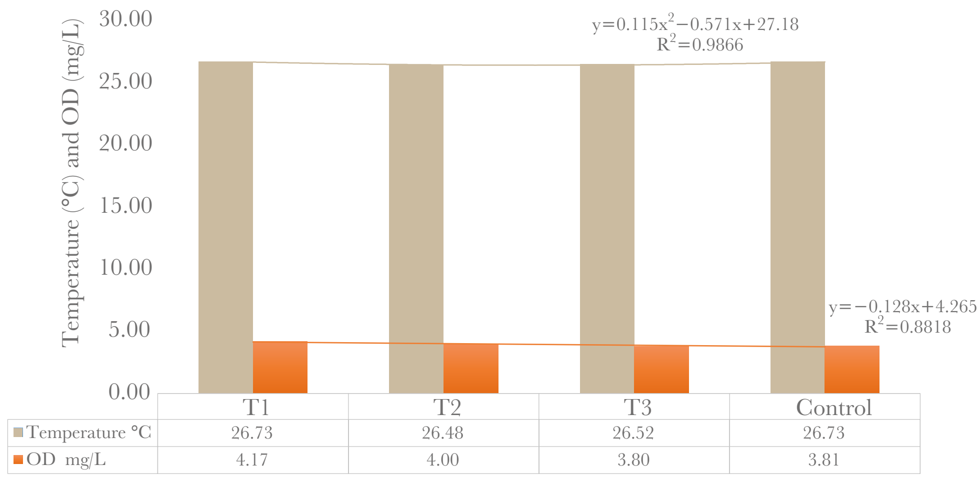

Figure 1. Behavior of temperature and dissolved oxygen in the RAS.

In Figures 2 and 3, a significant difference was observed in the length of the organisms; however, weight did not show significant variation between sexes.

During the initial days of the experiment (day 2), a reduction in the number of organisms was recorded, attributed to the cannibalistic behavior of *P. llamasi*. This outcome was associated with the acclimation process, stocking, and handling of the species, in accordance with the Guidelines for Good Aquaculture Practices (Boyd *et al.*, 2001). It is noteworthy that both length and weight increased by the second biometric evaluation, indicating effective feed utilization. According to the growth rate, results from the third and fourth biometric evaluations revealed that males exhibited greater growth compared to females. This pattern is explained by the fact that females allocate a significant portion of their energy to incubation and juvenile care stages, which is consistent with the findings of Hazlett & Rittschof (1985) (Figures 2 and 3).

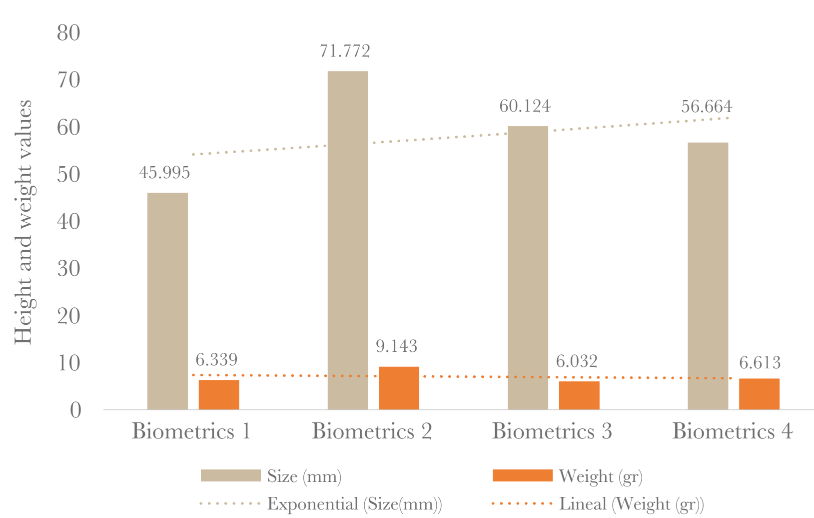

Figure 2. Relationship between length (cm) and weight (g) of female organisms

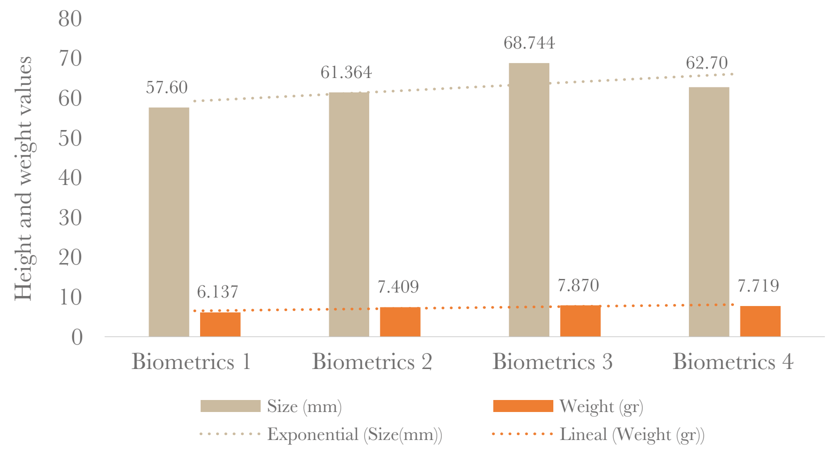

Figure 3. Relationship between length (cm) and weight (g) of male organisms.

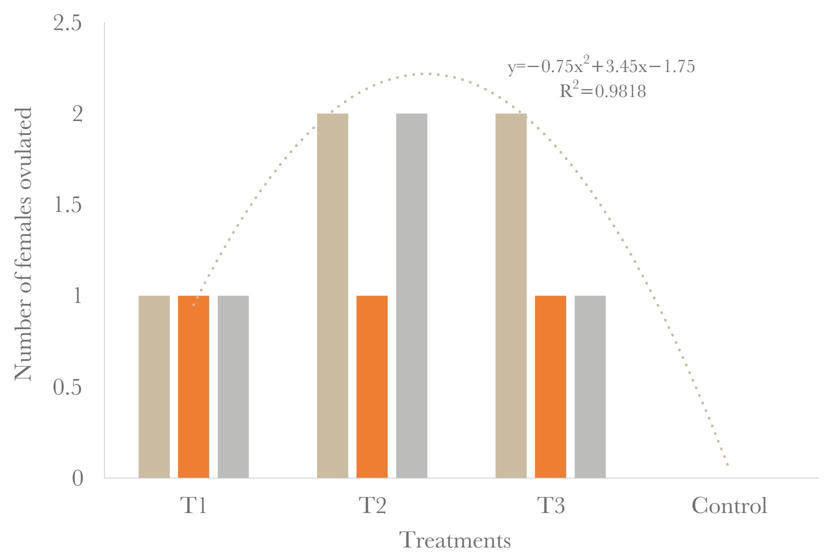

Figure 4. Behavior of ovigerous females in the Aquaculture Recirculation System (ARS).

The results indicated that treatment T2 (1M:3F) yielded superior outcomes compared with T3 and T4, as all females became ovigerous in contrast to the control group. These findings suggest that a single male can successfully mate with two to three females within a 45-day period under ARS conditions (Figure 4). In contrast, organisms from T1 (1M:2F) and the control group remained mostly within shelters, exhibiting minimal feeding activity.

CONCLUSIONS

The culture of *Procambarus llamasi* in an aquaculture recirculation system (ARS) proved acceptable in terms of reproduction and species development, despite the short duration of the experiment and the limited culture space. The physicochemical and water quality parameters in the ARS remained balanced, with water replenishment occurring only through evaporation and minor leaks in the piping system. Treatment two, consisting of one male and three females (1M:3F), yielded the highest number of ovigerous females of *Procambarus llamasi*. Nevertheless, these preliminary results provide a foundation for future validation studies, where maximum stocking density should be considered a key variable in decision-making regarding species reproduction under longer experimental periods. The Campeche acocil represents a promising alternative for obtaining animal protein and, simultaneously, a species suitable for cultivation in AREL (aquaculture under resource-limited conditions) backyard systems in rural areas of Campeche.

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Colegio de Postgraduados

Agriculture and Poverty in Mexico: The Role of Agriculture in Poverty, 2010-2022

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ABSTRACT

Objective: To estimate a model incorporating the variables poverty, extreme poverty, and agricultural income using generalized least squares, in order to demonstrate the effect of agricultural income on poverty and extreme poverty. This analysis aims to inform the design of agricultural public policies that effectively contribute to poverty reduction in the sector.

Methodology: The generalized least squares (GLS) method was employed. The primary variables analyzed were poverty and extreme poverty, while agricultural income, planted area, number of workdays, and daily wages were included as control variables.

Results: Between 2018 and 2022, agricultural income was found to significantly reduce extreme poverty. In prior years, no statistically significant relationship was identified. The number of workdays and daily wages were positively associated with poverty suggesting that increases in planted area, workdays, and wages may, paradoxically, be linked to higher poverty levels.

Limitations: The limited availability of multidimensional poverty and extreme poverty data at the state level poses a constraint on conducting robust time-series analyses.

Conclusions: The findings underscore the need to reassess public policies directed at the agricultural sector, with the objective of enhancing their effectiveness in poverty alleviation.

Keywords: Rural poverty, agricultural income, agricultural production.

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INTRODUCTION

Between 2010 and 2022, agricultural production in Mexico increased from 508 million to 708 million tons (Agri-Food and Fisheries Information System, SIAP, 2024). According to the National Institute of Statistics and Geography (INEGI, 2023), primary sector activities contributed 3.9% to the national GDP, with agricultural activities accounting for 90.9% of that total. These figures highlight both the significant growth in agricultural production over a 12-year period and the dominant role of agriculture within the primary sector. Data from the Agri-Food Outlook (2022) indicated that Mexico ranked 11[th] in global food production and was the leading exporter of several agricultural products: avocado (49% of the global market), tomato (30.7%), asparagus (31.4%), mango (24.7%),

and berries (16%). Additionally, Mexico is the top exporter of agro-industrial products such as beer and tequila. Consequently, the country maintained a positive agri-food trade balance (Secretariat of Agriculture and Rural Development, SADER, 2022). In contrast to the success of certain agricultural products in international markets, Mexico continues to face serious challenges in rural areas, including poverty and extreme poverty. In 2022, 36.6% of the population was living in poverty, and 7.1% in extreme poverty. While these figures represent a decrease from 2010 —when 44.4% of the population was in poverty and 11% in extreme poverty— they remain concerning (National Council for the Evaluation of Social Development Policy, CONEVAL, 2023). Poverty in Mexico is measured multidimensionally, based on income and six social rights: education, health services, social security, housing quality and space, adequate housing, and food access (CONEVAL, 2022). This methodology aims to identify the most vulnerable populations and guide the implementation of appropriate welfare policies. Eradicating poverty is one of the United Nations Sustainable Development Goals (SDGs). Several authors have examined the relationship between agriculture and poverty, suggesting that agriculture can play a key role in poverty reduction (Hammed *et al.*, 2023; Omodero, 2021). Johnston and Mellor (1961) were among the first to assert a direct causal relationship between agriculture and poverty, identifying agricultural income as the primary transmission mechanism —higher agricultural income reduces household poverty levels. Similarly, Sen (1981), through his capabilities approach, emphasized that agriculture contributes to poverty reduction by enhancing access to food, thereby reducing vulnerability among the poor. Ellis and Biggs (2005) also argue for a causal link between agriculture and poverty reduction, particularly in low-income countries, where most of the population resides in rural areas and depends on agriculture for survival. In the same vein, Mellor and Malik (2017) state that growth in agricultural production and smallholder income is the most effective pathway for rural poverty reduction. This occurs through increased spending by commercial farmers in the rural non-agricultural sector, which raises non-farm rural incomes and lowers poverty levels. Schneider and Gugerty (2011) suggest that the link between increased agricultural production and poverty reduction is mediated by job creation in both agricultural and non-agricultural sectors, through backward and forward linkages. These mechanisms help reduce both urban and rural poverty and contribute to lowering food prices. De Janvry and Sadoulet (2010), in a study conducted in Vietnam, found that reductions in rural poverty were associated with increased agricultural productivity. They argue that the agricultural sector is not only crucial for poverty alleviation but also has the capacity to stimulate overall economic growth through its linkages with other sectors. This perspective is reinforced by Ogundipe *et al.* (2017), who highlight that job creation, increased demand for goods and services, and rising agricultural income are essential components in reducing poverty. In Indonesia, Mariyah and Nugroho (2022) found that agricultural production had a statistically significant impact on poverty reduction between 2011 and 2020. They concluded that this effect is primarily achieved through labor absorption, improved agricultural productivity, and a focus on high-value agricultural products. In Burkina Faso, agriculture plays a vital role in the national economy. Despite widespread poverty —particularly in rural areas— increased agricultural productivity has had positive impacts on education, and agricultural value-added has contributed to improvements in life expectancy (Traore *et al*., 2022). A similar situation has occurred in China, where poverty eradication efforts since 2020 have focused heavily on agricultural subsidies. However, a recent study by Wang *et al*. (2024) found mixed results: while agriculture has been effective in reducing poverty in the northern region, its efficiency score for poverty reduction in the southern region remains low. In Mexico, there is limited research on the relationship between agriculture and poverty reduction. This study aims to estimate an econometric model that incorporates poverty, extreme poverty, and agricultural income using generalized least squares, to evaluate the impact of agricultural income on poverty and extreme poverty. The findings are intended to inform the design of public policies that support the agricultural sector in poverty alleviation. The central hypothesis is that increased agricultural income has contributed to poverty reduction over the study period.

MATERIALS AND METHODS

The study was conducted using state-level data. Multidimensional poverty and extreme poverty figures by federal entity were analyzed for the period 2010-2022. This timeframe was selected due to the consistency of poverty data available biennially and the observed increase in agricultural income alongside a reduction in poverty levels. The years included in the analysis were 2010, 2012, 2014, 2016, 2018, 2020, and 2022. The sample consists of 31 Mexican states and Mexico City.

Selected variables

The poverty and extreme poverty variables refer to multidimensional poverty as defined by CONEVAL, which considers a person to be in poverty if they lack sufficient income to meet basic needs and experience at least one of six social deprivations: educational lag, lack of access to health services, social security, housing quality and space, access to basic housing services, and access to adequate nutrition. Extreme poverty refers to individuals who, even when allocating all their income to cover basic needs, fall short and experience three or more social deprivations (CONEVAL, 2022). Both variables were measured by the number of people living in poverty and extreme poverty, with data obtained from the National Council for the Evaluation of Social Development Policy (CONEVAL, 2023).

Agricultural variables

The agricultural income variable corresponds to the value of agricultural production and is calculated as the product of total agricultural output and the rural average price, expressed in monetary units. Planted area refers to the total number of hectares allocated to agricultural cultivation. Data for these variables were sourced from the Agri-Food and Fisheries Information System (SIAP) for the years 2010, 2012, 2014, 2016, 2018, 2020, and 2022. Due to the availability of agricultural census data for 2022, two additional variables were included: agricultural laborers and daily wages. The former refers to individuals employed in agricultural activities receiving a daily wage; this was measured by the number of laborers per state. The latter represents the amount, in monetary units, received by workers for agricultural labor. These variables were only included in the econometric models for the year 2022. The selection of variables was based on theoretical linkages. As Mellor and Malik (2017) suggest, agricultural income is theoretically tied to poverty reduction particularly in rural áreas since higher agricultural income correlates with lower poverty levels. Income growth may result from increased productivity or higher prices. Planted area is associated with this relationship because its expansion can lead to increased food production, job creation, higher income, and ultimately, poverty reduction (Li *et al.*, 2022). Regarding agricultural laborers, their link to poverty is primarily through labor conditions, informality, and low wages, which according to Yacoub and Restiatun (2024) contribute to the persistence of poverty. It is important to note that for the regression analyses, the explanatory variables were categorized as monetary and non-monetary. The first group includes agricultural income and daily wages, while the second comprises planted area and the number of laborers.

In Mexico, multidimensional poverty data is only available every two years, which limits the feasibility of time-series or panel data analysis. Therefore, a cross-sectional analysis was conducted using the ordinary least squares (OLS) method. OLS is a statistical technique used to establish a functional relationship between two variables, X and Y, where Y is the dependent variable and X the independent variable (Mballa & Sauceda, 2018).

To define the model, the Ordinary Least Squares (OLS) method was employed, which aims to minimize the variance of the errors. According to Madala (2004), the model specification is presented in Equation 1.

$$Y_i = \beta_1 + \beta_2 X_i + u_i \tag{1}$$

Where: Y_i=is the variable to be explained. β_1=is the intercept. β_2=is the parameter associated with X_i. X_i=explanatory variable. u_i=is the error term.

The OLS method seeks to minimize errors, therefore, it starts from:

$$Y_i = \beta_1 + \beta_2 X_i + u_1$$

$$Y_i = \hat{\beta}_1 + \hat{\beta}_2 X_i + u_i \tag{1}$$

$$\hat{u}_i = Y_i - \hat{\beta}_1 - \hat{\beta}_2 X_i \tag{2}$$

Equation (2) shows that the residuals are the difference between the observed and estimated values of Y.

$$\sum \hat{u}_i^2 = \sum \left(Y_i - \hat{Y}_i\right)^2$$

$$\sum \hat{u}_i^2 = \sum \left(Y_i - \hat{\beta}_1 - \hat{\beta}_2 X_i\right)^2 \tag{3}$$

and how $\sum \hat{u}_i^2 = f\left(\hat{\beta}_1 + \hat{\beta}_2\right)$ then the differentiation process is obtained:

$$\sum Y_i = n\hat{\beta}_1 + \hat{\beta}_2 \sum X_i \tag{4}$$

$$\sum Y_i X_i = \hat{\beta}_1 \sum X_i + \hat{\beta}_2 \sum X_i^{\;2} \tag{5}$$

n is the sample size and these equations are known as normal equations, by algebraic manipulation the β are obtained.

$$\hat{\beta}_2 = \frac{n\sum X_i Y_i - \sum X_i \sum Y_i}{n\sum X_i^2 - \left(\sum X_i\right)^2}$$

$$\hat{\beta}_2 = \frac{\sum\left(X_i - \bar{X}\right)\left(Y_i - \bar{Y}\right)}{\sum\left(X_i - \bar{X}\right)^2} \tag{6}$$

$$\hat{\beta}_2 = \frac{\sum x_i y_i}{\sum x_i^2}$$

Where $\bar{X}$ and $\bar{Y}$ are the sample means of X and Y

$$\hat{\beta}_1 = \frac{\sum x_i^2 \sum Y_i - \sum X_i \sum X_i Y_i}{n\sum x_i^2 - \left(\sum x_i\right)^2}$$

$$\hat{\beta}_1 = \bar{Y} - \hat{\beta}_2 \bar{X} \tag{7}$$

Since the estimates presented heteroscedasticity problems, the correction was applied using generalized least squares.

It is required that $E(u) = 0$, now the variance of the errors will be: $Var(u) = \Omega$.

$$Q(\beta) = \left(y - X\beta\right)' \Omega^{-1}\left(y = X\beta\right) \tag{8}$$

Developing, deriving, and equating to 0

$$\frac{\partial Q}{\partial \beta} = -2X'\Omega^{-1}Y + 2X'\Omega^{-1}X\beta$$

$$-2X'\Omega^{-1}Y + 2X'\Omega^{-1}X\hat{\beta}_{MCG} = 0 \tag{9}$$

$$\hat{\beta}_{MCG} = \left(X'\Omega^{-1}X\right)^{-1} X'\Omega^{-1}y$$

After the explanation of the estimation of the coefficients, the model was formulated, whose specification is as follows:

$$P = \beta_0 + \beta_1 Inga + \beta_2 Sup\_S + u \quad (10)$$

$$PE = \beta_0 + \beta_1 Inga + \beta_2 Sup\_S + u \quad (11)$$

Where *P* is poverty, *PE* refers to extreme poverty, *Inga* is agricultural income, measured as the value of agricultural production, and *Sup_S* is the planted agricultural area.

Regarding 2022, the availability of data from the 2022 Agricultural Census made it possible to integrate variables to strengthen the analysis. Thus, in the 2022 linear regression analysis, four regressions were performed (Equations 12 to 15), differentiating between monetary and non-monetary variables. The model specification is as follows:

Monetary variables:

$$P = \beta_0 + \beta_1 Inga + \beta_2 Pag\ J + u \quad (12)$$

$$PE = \beta_0 + \beta_1 Inga + \beta_2 Pag\ J + u \quad (13)$$

Where *Inga* corresponds to agricultural income and *Pag J* refers to the payment for day labor, representing the wages received by individuals employed in agricultural activities.

Non-monetary variables:

$$P = \beta_0 + \beta_1 Jor + \beta_2 Sup\_S + u \quad (14)$$

$$PE = \beta_0 + \beta_1 Jor + \beta_2 Sup\_S + u \quad (15)$$

The variable *Jor* refers to the number of day laborers employed in agricultural activities per state, and *Sup_S* corresponds to the planted agricultural area.

The analysis consisted of 16 regressions following the aforementioned general framework. The EViews 12 software was used for model estimation. All models were subjected to the White test for heteroscedasticity in the error terms, which is considered one of the most robust diagnostic tests (Toro *et al.*, n.d.). Additionally, following Hair *et al.* (2021), the models were tested for multicollinearity using the Variance Inflation Factor (VIF), where a value greater than five indicates the presence of collinearity. After conducting the diagnostic tests, heteroscedasticity was detected in some models. These models were subsequently adjusted and corrected using the Generalized Least Squares (GLS) method (Gujarati, 2010). Hypothesis testing was then performed to verify the predictive validity of the models.

RESULTS AND DISCUSSION

According to the results of the hypothesis tests based on the Prob(F) statistic, it was determined that the independent variables jointly have explanatory power over the dependent variable in each of the models. The R^2 statistic measures goodness-of-fit, which "indicates how well the sample regression line fits the data" (Gujarati, 2010, p. 73), and reflects the percentage of variation in the dependent variable explained by the model. A positive coefficient indicates an increase in poverty, while a negative coefficient suggests a reduction in poverty. The regression results, presented in Table 1, show that agricultural income had no effect on poverty levels during the years analyzed.

Table 1. Regression Results: Poverty

Dependent variable: POVERTY						
Model	**Year**		**Coefficients**	**Standard error**	**T-Student**	**P-value**
1	2010	Constant	15.09378	245.8	2.11	0.0436
		Inga	0.00001	0.00003	0.41	0.6829
		Sup_S	0.001493	0.00051	2.90	0.007***
		R^2=0.36	Prob(F)=	0.002		
2	2012	Constant	546.7498	242.017	2.26	0.0316
		Inga	0.000003	0.00002	0.12	0.9080
		Sup_S	0.001638	0.00048	3.43	0.0018***
		R^2=0.31	Prob(F)=	0.004		
3	2014	Constant	561.6029	260.772	2.15	0.0397
		Inga	0.0000	0.00	0.06	0.9518
		Sup_S	0.00172	0.00051	3.38	0.0021***
		R^2=0.27	Prob(F)=	0.009		
4	2016	Constant	0.000	0.000	−0.30	0.7673
		Inga	0.000	0.000	−0.30	0.7673
		Sup_S	0.002	0.0005	3.56	0.0013***
		R^2=0.27	Prob(F)=	0.009		
5	2018	Constant	−0.00001	0.00001	−1.30	0.2026
		Inga	−0.00001	0.00001	−1.30	0.2026
		Sup_S	0.00214	0.00054	3.94	0.0005***
		R^2=0.29	Prob(F)=	0.007		
6	2020	Constant	747.867	316.322	2.36	0.0250
		Inga	−0.00001	0.00001	−0.73	0.4737
		Sup_S	0.00169	0.0006	2.83	0.0084***
		R^2=0.19	Prob(F)=	0.04		

Source: Own elaboration based on data from CONEVAL and SIAP.
Note: * Significant at the 90% confidence level, ** Significant at 95%, *** Significant at 99%.
Hypothesis test (Prob(F)) for the model at α=0.05:
H_0: The coefficients of the explanatory variables are less than or equal to zero; they do not explain the dependent variable.
H_1: At least one coefficient is different from zero; the model explains the dependent variable.
Decision rule: Reject H_0 if Prob(F)<0.05.

In Model 1, an R^2=0.34 was obtained, indicating that in 2010, 34% of the variation in multidimensional poverty in Mexico was explained by the model. The coefficient for Sup_S (planted area) suggests that for each additional hectare, poverty increases by 0.0014 persons, with 99% statistical significance. In Model 2, an R^2=0.31 was obtained. In that year, a one-hectare increase in planted area was associated with an increase in poverty of 0.0016 persons, also with 99% significance. Model 3 yielded an R^2=0.28, and agricultural income (Inga) was not statistically significant. However, planted area was, indicating that each additional hectare increased poverty by 0.0017 persons with 99% probability. In Model 4, the R^2=0.27 and the coefficient for Sup_S remained statistically significant at the 99% level, indicating that a one-hectare increase in cultivated area leads to a 0.0018-person increase in poverty. Model 5 produced similar results, with an R^2=0.29. Agricultural income was again not a significant factor, while planted area was highly significant (99%) and had a positive effect on poverty: each additional hectare increased poverty by 0.0021 persons. In Model 6, the results followed the same trend. The model had an R^2=0.19, and planted area continued to be statistically significant. An increase of one hectare in agricultural area was associated with an increase of 0.0084 persons in poverty, at a 99% confidence level.

The positive association between planted area and poverty contradicts the theory of economies of scale. According to Roest *et al.* (2017), economies of scale imply that the average cost per unit of output decreases as production increases. This theory suggests that an expansion in cultivated land should reduce poverty through increased employment and income. However, in practice, the relationship is more complex, as it is influenced by factors such as land quality, land management, the formation of agricultural cooperatives, and production efficiency, among others (Li *et al.*, 2021). Table 2 summarizes the regression results from Models 7 through 12. The findings indicate that agricultural income had no statistically significant effect on extreme poverty between 2010 and 2016. However, in 2018, a positive relationship between agricultural income and the reduction of extreme poverty was observed.

In 2010, the coefficient of determination was R^2=0.36, and agricultural income was not statistically significant. However, the Sup_S (planted area) variable showed a significant effect at the 95% confidence level, indicating that an increase of one cultivated hectare leads to a 0.000633-person rise in extreme poverty. In 2012, R^2=0.38, and again, agricultural income was not significant. However, planted area remained relevant; with 95% confidence, it was found that an additional hectare increased extreme poverty by 0.00058 persons. Similar findings were observed in 2014 and 2016: in both years, agricultural income showed no statistical relationship with extreme poverty, while planted area was significant, indicating that land expansion contributes to an increase in extreme poverty. Model 11, corresponding to 2018, yielded an R^2=0.39 and demonstrated that a one-unit increase in agricultural income reduces extreme poverty by 0.000008 persons, with 99% confidence (see bold coefficients in Table 2). In 2020, R^2=0.29, revealing that a $1.00 increase in agricultural income results in a 0.000007-person reduction in extreme poverty. Although the coefficient was negative in this year, the confidence level fell to 88%. The results of Models 13 through 16 correspond to the year 2022. In this section,

Table 2. Regression Results: Extreme Poverty.

Dependent variable: Extreme poverty						
Model	**Year**		**Coefficients**	**Standard error**	**T-Student**	**P-value**
7	2010	Constant	15.09378	67.94115	0.22	0.8257
		Inga	−0.000004	0.000013	−0.32	0.7504
		Sup_S	0.000633	0.000277	2.29	0.0296**
		R^2=0.36	Prob (F)=	0.001539		
8	2012	Constant	27.268	54.62579	0.50	0.6214
		Inga	−0.000005	0.000008	−0.62	0.5383
		Sup_S	0.000581	0.000222	2.62	0.0139**
		R^2=0.31	Prob(F)=	0.001092		
9	2014	Constant	6.504879	61.99892	0.10	0.9172
		Inga	−0.00001	0.00001	−0.89	0.3825
		Sup_S	0.000656	0.000253	2.59	0.0148
		R^2=36	Prob(F)=	0.001		
10	2016	Constant	−8.80283	62.86152	−0.14	0.8896
		Inga	−0.00001	0.00001	−1.29	0.2073
		Sup_S	0.00062	0.000234	2.65	0.0128**
		R^2=35	Prob(F)=	0.002		
11	2018	Constant	−27.72477	66.107740	−0.42	0.678
		Inga	−0.000008	0.000004	−1.82	0.0792**
		Sup_S	0.000724	0.00025	2.89	0.0072***
		R^2=39	Prob(F)=	0.001		
12	2020	Constant	70.05538	67.15596	1.04	0.3055
		Inga	−0.000007	0.000004	−1.64	0.1112
		Sup_S	0.000607	0.00023	2.64	0.0133**
		R^2=29	Prob(F)=	0.006		

Source: Own elaboration based on data from CONEVAL and SIAP.
Note: * Significant at the 90% confidence level, ** Significant at 95%, *** Significant at 99%.
Hypothesis test (Prob(F)) for the model at α=0.05:
H_0: The coefficients of the explanatory variables are less than or equal to zero; they do not explain the dependent variable.
H_1: At least one coefficient is different from zero; the model explains the dependent variable.
Decision rule: Reject H_0 if Prob(F)<0.05.

the independent variables were categorized as monetary and non-monetary to assess their specific effects on the dependent variable. This classification led to a significant improvement in model fit: Model 14 had an R^2=0.55, Model 15 reached R^2=0.56, and Model 16 yielded R^2=0.69. Model 13 produced an R^2=0.33. In this case, agricultural income was statistically significant, indicating that a one-unit increase in Inga results in a 0.00001-person reduction in poverty, with 99% confidence. Similarly, the Pag J (payment for day labor) variable was also statistically significant at the 99% level, showing a positive effect: an increase of one monetary unit in daily wage is associated with an increase of 0.66 persons in poverty (see Table 3).

Regarding the non-monetary variables (Model 14), the Jor variable (number of day laborers) was statistically significant at the 99% confidence level and showed a positive relationship with poverty. The result suggests that for each additional person employed in agricultural labor, poverty increases by 0.00188 persons.

The results of Models 15 and 16 refer to extreme poverty. In this context, Model 15, which includes monetary variables, yielded an $R^2=0.56$ and showed that Inga (agricultural income) is negatively associated with extreme poverty. An increase of one unit in income results in a 0.000007-person reduction in extreme poverty, with 99% confidence. Similarly, the Pag_J (payment to day laborers) variable was statistically significant at the 99% level and indicated that an increase in wages is associated with an increase of 0.23 persons in extreme poverty (see Table 4).

Regarding the non-monetary variables, the Jor (agricultural laborers) variable was statistically significant at the 99% confidence level and showed a positive relationship with extreme poverty. Specifically, an increase of one unit in the number of agricultural laborers is associated with an increase of 0.0005 persons in extreme poverty. In contrast to previous years, the Sup_S (planted area) variable did not show statistical significance.

Table 5 summarizes the models in which evidence was found that agricultural income reduced extreme poverty between 2018 and 2022, as well as general poverty in 2022. These findings support the hypothesis proposed at the beginning of this study and are consistent with the results of Warr and Suphannachart (2021), who found that increased agricultural productivity reduces rural poverty in Thailand. Similarly, Chandrarekha *et al.* (2021) observed in India that as agricultural production and *per capita* income rise, poverty decreases particularly in agrarian economies where the majority of the population depends

Table 3. 2022 regression results, poverty.

Dependent variable: Poverty						
Monetary variables						
Model	**Year**		**Coefficients**	**Standard error**	**T-Student**	**P-value**
13	2022	Constant	713.6949	203.4292	3.50832	0.0015
		Inga	−0.00001	0.00001	−1.87134	0.0714*
		PagJ	0.660297	0.134536	4.90795	0.000***
		$R^2=0.33$		Prob (F)	0.003	
Non-monetary variables						
14	2022	Constant	514.9577	210.945	2.44119	0.021
		Jor	0.001748	0.000629	2.77859	0.0095***
		Sup_S	0.000127	0.00045	0.28251	0.7796
		$R^2=0.55$		Prob(F)	0.0000	

Source: Own elaboration based on data from CONEVAL, SIAP, and the 2022 Agricultural Census.
Note: * Significant at the 90% confidence level, ** Significant at 95%, *** Significant at 99%.
Hypothesis test (Prob(F)) for the model at $\alpha=0.05$:
H_0: The coefficients of the explanatory variables are less than or equal to zero; they do not explain the dependent variable.
H_1: At least one coefficient is different from zero; the model explains the dependent variable.
Decision rule: Reject H_0 if Prob(F)<0.05.

Table 4. Regression results 2022. Extreme poverty.

Dependent variable: Extreme poverty						
Monetary variables						
Model	**Year**		**Coefficients**	**Standard error**	**T-Student**	**P-value**
15	2022	Constant	91.46759	37.60456	2.43235	0.0214
		Inga	−0.000007	0.000002	−3.41312	0.0019***
		PagJ	0.232437	0.042901	5.41798	0.000***
		R^2=0.56	Prob(F)	0.000		
Non-monetary variables						
16	2022	Constant	−5.536	43.560	−0.12708	0.8998
		Jor	0.0005	0.0001	5.80702	0.000***
		Sup_S	0.0001	0.0001	0.6539	0.5183
		R^2=0.69	Prob(F)=	0.00		

Source: Own elaboration based on data from CONEVAL, SIAP, and the 2022 Agricultural Census.
Note: * Significant at the 90% confidence level, ** Significant at 95%, *** Significant at 99%.
Hypothesis test (Prob(F)) for the model at α=0.05:
H_0: The coefficients of the explanatory variables are less than or equal to zero; they do not explain the dependent variable.
H_1: At least one coefficient is different from zero; the model explains the dependent variable.
Decision rule: Reject H_0 if Prob(F)<0.05.

Table 5. Key regressions.

Dependent variable: Extreme poverty							
Model	**Year**		**Coefficients**	**Standard error**	**T-Student**	**P-value**	**R^2**
11	2018	Inga	−0.000008	0.000004	−1.82	0.0792**	0.39
12	2020	Inga	−0.000007	0.000004	−1.64	0.1112	0.29
15	2022	Inga	−0.000007	0.000002	−3.41	0.0019***	0.56
Dependent variable: Poverty							
13	2022	Inga	−0.00001	0.00001	−1.87134	0.0714*	0.33

Source: Own elaboration based on data from CONEVAL, SIAP, and the 2022 Agricultural Census. Note: * Significant at the 90% confidence level, ** Significant at 95%, *** Significant at 99%.

on the agricultural sector. The results of this study also align with Umar *et al.* (2023), who reported that increases in agricultural productivity and *per capita* income contributed to poverty reduction in Nigeria. The findings of this research indicate that the effect of agricultural income on reducing extreme poverty was greater than its effect on general poverty. These results contrast with those of Herrero and Bustamante (2025), who, in their analysis of the impact of minimum wage in Ecuador, concluded that wage increases had a stronger effect on general poverty than on extreme poverty. This discrepancy may be due to differences in study periods and variables used. Between 2010 and 2016, no statistical relationship was found between agricultural income and poverty likely due to income levels during that period. These findings are consistent with those of López *et al.* (2016), who, using municipal-level data from Mexico, were unable to establish a clear link between agricultural production and poverty reduction. One possible explanation for the limited impact of agricultural income on poverty reduction relates to the geographic concentration of poverty in southern Mexican states. While regional disparities between the north and south can be attributed to multiple factors, agriculture plays a significant role. According to CONEVAL (2022), Chiapas, Oaxaca, and Guerrero are the states with the highest poverty levels. In these states, agricultural production focuses on grasses, sugarcane, and maize largely for subsistence markets with limited commercialization. In contrast, the states that have experienced agricultural growth are located in the central and northern regions, producing crops such as tomatoes, berries, and avocados, which are part of a more developed agricultural market. For example, states like Guanajuato, Michoacán, Sinaloa, Nayarit, and Baja California have reduced poverty during the study period. With regard to the relationship between planted area and poverty/extreme poverty, it was found that an increase in cultivated land correlates with higher poverty. This contradicts the results of Quin and Zhang (2022), who found in West Java that increasing cultivated area reduced poverty but only through the implementation of regional agricultural clusters and specialization. However, when analyzing farmers within horizontal integration models, their results were consistent with those found in this study, concluding that increased planted area does not reduce poverty. These findings also contrast with Li *et al.* (2020), who reported that plantation land is associated with higher farmer income, though they emphasized the importance of crop diversification. Similarly, Heger *et al.* (2020) identified a link between planted area and poverty reduction in Sub-Saharan Africa, but noted that the effect depends largely on improvements in land quality. The results of this study suggest that expanding cultivated land may increase poverty potentially due to stagnant agricultural productivity. Rural farmers often face capital constraints that limit investment in productivity improvements. Low productivity restricts income, which in turn limits reinvestment in soil quality, perpetuating poverty. Another key factor is land distribution: if agricultural land is concentrated among a small group of farmers, the positive effects of land expansion on poverty reduction are diminished. On the other hand, the relationship between the number of agricultural laborers and both poverty and extreme poverty was found to be positive. This relationship is complex and may be linked to so-called poverty traps situations where poverty persists due to self-reinforcing mechanisms (Millán, 2018). As Nevárez and Castro (2025, p. 4) describe, it is "a condition in which the income of poor individuals does not rise above a certain threshold, causing them to remain in poverty." In rural settings, these traps are often multidimensional, involving economic, biophysical, and social processes that interact to create reinforcing dynamics that maintain the trap (Radosavljevic *et al.*, 2021). In this study, the estimates show that when a person enters the agricultural labor force, poverty increases. These results are plausible: agricultural jobs typically require lower skills and qualifications than those in other economic sectors. For individuals who lack education or training and are not qualified for work outside of agriculture, remaining in that sector may be their only option —even if it means staying in poverty. It is worth noting that since 2021, agricultural laborers have been included in the list of minimum wage earners. According to the National Minimum Wage Commission (CONASAMI, 2024), in 2021, the minimum wage for agricultural workers was $213.30 MXN/day in the northern zone and $160.19 MXN/day in the rest of the country. In

2022, those wages rose to $260.34 and $195.93, respectively a 22% increase. These figures imply a monthly income (assuming 30 days of work) of $7,810.20 in the north and $5,879.90 elsewhere. However, the extreme poverty line for income the monetary value of the food basket in December 2022 for a household of four was $6,520.84 in rural areas (CONEVAL, 2023). This means that the minimum required expenditure on food exceeds the household income of an average agricultural worker. Despite increases in the minimum wage for agricultural laborers, poverty persists. This is largely because most agricultural workers are employed on a temporary or casual basis, without access to social benefits (Flores, 2021). Employment agreements are often verbal, and in some cases, there is no fixed wage agreement (Posadas, 2018). Additionally, wage increases are not uniformly applied across the country. According to Barrón *et al*. (2019), in the third quarter of 2021, 44% of agricultural workers earned less than the value of the basic food basket. Furthermore, while agricultural income has grown at an average annual rate of 18% between 2010 and 2022 (based on SIAP data, 2023), its poverty-reducing effects were only observed during the 2018–2022 period. Ceddia (2019) attributes this to income inequality and land concentration. Vaquiro (2021) also emphasizes that in southern Mexican states where poverty is more persistent agricultural households must diversify income sources to escape poverty. Lastly, it is important to note that agricultural laborers typically migrate two or three times a year, depending on harvest seasons, in order to stay employed year-round (Barrón *et al*., 2019). For example, in San Quintín (northwestern Mexico), a strong agricultural labor market has emerged, relying heavily on low-cost indigenous labor from the country's poorest southern states (Villegas & Camarena, 2024). In short, low wages, job insecurity, and high labor turnover all reinforce the poverty trap that perpetuates the precarious conditions faced by agricultural workers. Wage increases are necessary but alone, they are insufficient to significantly reduce poverty.

CONCLUSIONS

Although agricultural production increased during the study period, agricultural income only began to have an effect on the reduction of multidimensional extreme poverty starting in 2018, and its effect on general poverty was observed only in 2022. This suggests that, while agriculture is a potentially powerful tool for poverty reduction, in the case of Mexico, other factors must be considered such as land tenure, market conditions, climate change, and public policy. The marginal impact of agriculture on poverty reduction found in this study highlights the need for deeper analysis in future research to evaluate the behavior of these variables as more data becomes available. It is recommended to reinstate social support programs targeting rural agricultural producers, aimed at reducing poverty through subsidies, financing mechanisms, or direct cash transfers.

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Colegio de Postgraduados

Sustainability of nature-based enterprises in Mexico

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ABSTRACT

Objective: To evaluate the performance of the economic, social, and environmental dimensions of Nature-Based Enterprises (NBE) in Mexico, with the aim of identifying positive deviants and the key factors that explain such differentiation.

Design/Methodology/Approach: An exploratory mixed-methods study was conducted using the enterprise as the unit of analysis. A total of 23 NBEs across 13 Mexican states were evaluated through purposive sampling. Between May and September 2023, 23 surveys were administered to enterprise representatives, and 9 interviews were conducted with key informants. The analysis included a descriptive phase and the calculation of economic, social, and environmental performance indices using an adapted methodological framework. Subsequently, hierarchical cluster analysis identified four categories of enterprises consolidated, developing, adapting, and incipient which were compared through ANOVA, mean difference tests, and chi-square tests.

Results: The study revealed that NBEs in Mexico achieved an average sustainable performance index of 0.85, with over 60% of cases scoring above the mean. Economic performance showed the greatest variability, social performance was moderate, and environmental performance remained relatively consistent across enterprises.

Limitations/Implications: The use of a purposive, non-probabilistic sample limits the generalizability of the findings to the broader NBE population.

Findings/Conclusions: The consolidated enterprises represented the closest cases to positive deviants. The findings suggest that NBE performance is enhanced by participatory governance, a clear organizational mission, economic diversification, certifications, and investment in capacity building and marketing. Conversely, key obstacles include reliance on initial subsidies, seasonal activity patterns, regulatory gaps, and weak organizational cohesion.

Keywords: economic diversification, governance, nature-based enterprises, sustainable performance.

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INTRODUCTION

The accelerated loss of biodiversity and the degradation of ecosystems represent global challenges with direct impacts on human well-being, food security, and economic stability (Meza & Rodríguez, 2022). International organizations such as the Organisation for Economic Co-operation and Development (OECD), the International Union for Conservation of Nature (IUCN), and the United Nations Development Programme

(UNDP) have highlighted a financing gap that hampers both the fulfillment of international commitments and the continuity of conservation policies (Deutz *et al.*, 2020). In Mexico a megadiverse country and an emerging economy these challenges are intensified by the need to simultaneously fund development priorities, biodiversity conservation, and climate policies, all within a context of increasing pressure on natural resources and declining budgets for the environmental sector. In response, alternative mechanisms are needed to complement public and international financing (Peña-Azcona *et al.*, 2022; Sosa & Ivanova, 2025). At the international level, Nature-Based Solutions (NbS) have been recognized as strategies that seek to align development goals with ecosystem protection (Meza & Rodríguez, 2022). The OECD (2020) defines them as "actions that protect, sustainably manage, or restore natural ecosystems, with the goal of maintaining or enhancing ecosystem services to address a range of social, environmental, and economic challenges." Within this framework, enterprises that contribute to the development and implementation of NbS are known as Nature-Based Enterprises (NBE) (Collier *et al.*, 2023).

Kooijman *et al.* (2021) and McQuaid *et al.* (2021) define NBEs as businesses engaged in economic activities that use nature sustainably as a central component of their product or service offering. Their interaction with nature may be direct through cultivation, harvesting, sustainable use, or ecosystem restoration or indirect, via the planning, implementation, or management of NbS. These enterprises contribute positively to biodiversity and ecosystem services.

NBEs may adopt various organizational forms, including conventional businesses, social enterprises, community associations, or non-governmental organizations (McQuaid *et al.*, 2021). In the literature, institutions such as the World Bank, FAO, and UNDP have referred to these entities as Community Forest Enterprises (CFEs), Sustainable Forest Enterprises, or Community-Based Tourism Enterprises (World Resources Institute *et al.*, 2008). In Mexico, the General Law on Forest Development recognizes similar entities under the term Social Forestry Enterprises (DOF, 2024). Studies conducted in Mexico have documented both the characteristics and limitations of these enterprises. Maldonado-Alcudia and Maldonado-Alcudia (2019) point out that nature tourism cooperatives need to strengthen their organizational and certification capacities. Bañuelos-Frías *et al.* (2020) note that game hunting tourism can be profitable and contribute to conservation, although ejido-level organizations face challenges. Likewise, Engbring and Hajjar (2021), Galicia *et al.* (2022), and Hernández *et al.* (2022) report that CFEs face trade-offs between productivity and conservation, and struggle with training, infrastructure, governance, and certification. Other relevant cases include tourism enterprises in Oaxaca (Palomino & López, 2019), in Oaxaca and Chiapas (Medina & Sánchez, 2023), and in Yucatán (de Yta-Castillo *et al.*, 2025). These studies converge in recognizing NBEs as key actors in addressing conservation, development, and social well-being challenges. However, the study of how organizational models, governance structures, and business culture influence their performance remains limited. Additionally, there is a growing need to integrate economic, social, and environmental indicators in performance measurement frameworks (Bota-Avram, 2023; Jonäll *et al.*, 2025; Stroe *et al.*, 2022). In this context, it is relevant to analyze NBEs that, despite facing similar constraints and conditions as their peers, achieve significantly better outcomes referred to as positive deviants (Pascale *et al.*, 2010). Understanding their strategies and key success factors can offer valuable evidence on the viability of NBEs. This study is guided by the research question: What factors explain why some NBEs become positive deviants? Accordingly, the objective was to evaluate the economic, social, and environmental performance of NBEs in Mexico through an exploratory study aimed at identifying positive deviants and the key factors that explain such differentiation. The main contribution of this work is to provide integrated empirical evidence on the performance of NBEs in Mexico, establishing a comparative baseline to inform public policy design, funding programs, and business strategies oriented toward sustainability.

MATERIALS AND METHODS

The study followed a mixed-methods approach, combining qualitative and quantitative techniques through surveys, interviews, and result triangulation.

Unit of analysis

The unit of analysis was the enterprise. Selection was carried out through purposive sampling (Rofiaty *et al.*, 2024), considering only those enterprises whose economic activity uses nature as the central element of their product or service offering in Mexico, regardless of their legal structure, in accordance with the definition of a Nature-Based Enterprise (NBE).

Identification and selection of enterprises

The selection process began with a literature review and consultation of the Wildlife Directorate's database (CONANP, 2022), complemented by searches on websites and social media, as well as guidance from subject matter experts who facilitated contact with technical staff and representatives of potential enterprises. Only enterprises that met the following criteria were included:

- Nature is the central axis of their economic activity;
- The enterprise was operational during the interview period;
- The legal representative agreed to participate and provide the required information.

Data collection

The survey was developed based on a literature review and validated through a pilot test in April 2023. It included 50 questions covering general characteristics, decision-making, organizational structure, legal compliance, natural resource use, training, products and services, profitability, partnerships, and satisfaction. Most questions were closed-ended, while open-ended questions explored the motivations for creating the enterprise, its activities, offered products/services, and alliances. Additionally, nine key informant interviews were conducted with researchers from academic institutions and technical experts in biology, forestry, and natural resource engineering. These interviews focused on resource management, enterprise adaptation, and NBE challenges. Updated information was also requested on Wildlife Management Units (UMA) (SEMARNAT, 2023), which helped validate the data and enrich the analysis. A formal invitation was then sent to the selected enterprises, of which 23 agreed to participate. Their representatives owners, general directors, or members of the governing council were interviewed either in person or through virtual meetings between May and September 2023. The collected data were systematized using Excel, and descriptive profiles were created for each enterprise. Table 1 presents the main characteristics, based on the size classification defined by DOF (2023).

Performance index

The performance of NBEs was assessed using the Nature-Based Enterprise Sustainable Performance Framework, which was adapted from the FAO's Sustainability Assessment of Food and Agriculture Systems (SAFA) Framework (FAO, 2014) and the Sustainability Evaluation Methodological Framework developed by Pineda-Vázquez *et al.* (2019). This framework incorporated a hierarchical structure comprising dimensions, general criteria, specific criteria, and indicators (see Table 2).

Table 1. Sample of nature-based enterprises.

ID	State	Municipality	Legal and organizational status	Surface (ha)	Size	Company's line of business
E1	Hidalgo	Huasca de Ocampo	Natural Person	1051.6	micro	Adventure Tourism
E2	Mexico	Amecameca	Cooperative Society	96.7	small	Wildlife Observation and Forestry
E3	Guanajuato	San Miguel de Allende	Civil Association	67	medium	Environmental Education
E4	Sonora	Álamos	Civil Association	114	micro	Wildlife Observation
E5	Yucatán	Tizimín	Civil Association	2300	micro	Environmental Education
E6	Veracruz	Coatzacoalcos	Owned by a Public Company	1048	small	Environmental Education
E7	Veracruz	Catemaco	Variable Capital Corporation	13.5	medium	Wildlife Observation
E8	Tlaxcala	Nanacamilpa de Mariano Arista	Rural Production Society	74	micro	Wildlife Observation
E9	Mexico	Tepetlaoxtoc	Ejido	5	micro	Wildlife Observation and Forestry
E10	Veracruz	Córdoba	Natural Person	4	micro	Environmental Education and Agroforestry
E11	Puebla	Cuetzalan del Progreso	Cooperative Society	2	small	Adventure Tourism
E12	Veracruz	San Andrés Tuxtla	Social Solidarity Society	983.5	small	Adventure Tourism
E13	Veracruz	San Andrés Tuxtla	Natural Person	15	small	Environmental Education and Agroforestry
E14	Chiapas	Tzimol	Cooperative Society	1200	micro	Adventure Tourism
E15	Puebla	Tetela de Ocampo	Rural Production Society	4	micro	Adventure Tourism
E16	Aguascalientes	San José de Gracia	Natural Person	200	micro	Sport Hunting
E17	Jalisco	Tamazula de Gordiano	Natural Person	180	micro	Environmental Education
E18	San Luis Potosí	Alaquines	Natural Person	20204	micro	Sport Hunting
E19	Sinaloa	El Fuerte	Natural Person	4502	micro	Sport Hunting
E20	Sonora	La Colorada	Natural Person	2116	small	Sport Hunting
E21	Sinaloa	El Fuerte	Natural Person	3568	micro	Wildlife Observation
E22	Querétaro	Pinal de Amoles	Natural Person	7	micro	Wildlife Observation
E23	Mexico	Texcoco	Natural Person	1	small	Adventure Tourism

ID=Nature-Based Enterprise identifier.

Performance framework

The performance framework encompassed indices for three dimensions. Economic performance was assessed based on financial resilience, considering factors such as stability, fulfillment of obligations, cash flow generation, and responsiveness to external changes. Environmental performance focused on habitat and species conservation, as well as compliance with the legal framework. Meanwhile, social performance included aspects of stakeholder engagement, satisfaction, and corporate ethics (FAO, 2014; Pineda-Vázquez *et al.*, 2019).

Index processing and calculation

The collected data were systematized into a data matrix through the coding of variables and indicators, enabling the transformation of qualitative and categorical responses into comparable numerical values, as established by Pineda-Vázquez *et al.* (2019). Subsequently, the coded data were processed in Excel®, assigning equal weights to each specific criterion within its respective general criterion, and to each general criterion within its corresponding dimension. This procedure resulted in the Dimension Performance Index (DPI) for the

Table 2. Sustainable performance framework of NBEs.

Dimension	General criteria	Specific criteria	Indicators
Environmental	Conservation	Presence of natural vegetation	1
		Integrated habitat management	5
		Presence of water bodies	2
		Soil status	1
		Distribution and status of managed species	3
	Compliance with legal and operational guidelines	Annual reports to the appropriate authority	1
		Species inventory	1
		Legal accreditation of use	1
		Awareness of legislation	1
		Management plan	1
		Consistent with objectives	2
Economic	Investment Vulnerability	Promoción	3
		Capacitación y desarrollo del personal	1
		Certificaciones que demuestran el compromiso de sostenibilidad	2
		Rentabilidad	2
	Engagement Overall Satisfaction	Diversificación de ingresos	1
		Liquidez	2
		Estacionalidad	3
Social	Regulatory Compliance and Corporate Ethics Investment	Actividades de participación social	1
		Actividades de vinculación interinstitucional	1
		Satisfacción de los implicados	3
	Vulnerability	Legalidad	4
		Misión explicita	1
		Reglamento interno	1

Adapted from FAO (2014) and Pineda-Vázquez *et al.* (2019).

environmental, economic, and social dimensions, each considered of equal importance in the overall performance assessment. At each hierarchical level, scores were estimated using the arithmetic mean of the corresponding values.

Statistical analysis

Spearman's rank correlation coefficients (rho) were calculated among the three DPIs to evaluate potential associations. Since only the economic DPI exhibited a normal distribution, results were interpreted in accordance with Hinkle *et al.* (2003). To classify the legally constituted Nature-based enterprises (NBEs) according to their performance levels, a hierarchical cluster analysis was conducted using three variables. A total of 22 observations were included, while one excluded observation (E23) was subsequently assigned to the most similar group. The Ward method, recommended for hierarchical groups involving multiple variables, was employed for case grouping, while squared Euclidean distance was used to determine the similarity between examined enterprises (Aguilar-Gallegos *et al.*, 2015). Visual analysis of the dendrogram identified four distinct clusters.

Group comparison

Differences among groups were evaluated using analysis of variance (ANOVA) and mean comparison tests for quantitative variables, and contingency tables with the chi-square test (χ^2) for categorical variables. Of the 77 variables analyzed, only those with statistically significant differences were reported. Subsequently, a second analysis was conducted with the 15 enterprises from the two highest-performing groups, applying the same statistical methods. All analyses were performed using SPSS® software, version 27.0.

RESULTS AND DISCUSSION

General characteristics

The analyzed Nature-based enterprises (NBEs) have been in operation for periods ranging from 3 to 50 years. The number of permanent employees varies between 1 and 75, with an average of 15, and 60.8% of the NBEs also employ temporary staff. These enterprises operate under various land tenure regimes: communal (ejidal), private, and state-owned. Their direct representatives such as legal holders, general directors, or presidents of the communal board have an average age of 56 and exhibit diverse educational backgrounds: three with basic education, nine with upper-secondary, seven with tertiary education, and four holding postgraduate degrees. Regarding their origin, 39% of the NBEs were established to utilize natural resources from their territories, 30% were driven by environmental conservation interests, 22% aimed to diversify income sources, and 4% were created to access public subsidies. Additionally, 70% received financial support during their early operational stages, primarily through programs offered by the National Forestry Commission.

Enterprise performance

Sustainable performance index

The Sustainable Performance Index (SPI) ranged from 0.57 to 0.97, with a mean value of 0.85; 65% of the enterprises exceeded this average. Spearman correlation coefficients revealed strong positive correlations between the SPI and both the economic and social Dimension Performance Indices (DPI), while the correlation with the environmental DPI was moderate (Table 3).

Economic performance

The Economic Dimension Performance Index (DPI) exhibited high variability. Enterprise E16 recorded the lowest value at 0.64, while E1 achieved the highest at 0.92. Overall, 61% of the enterprises scored above the mean (Figure 1).

Social Performance

Enterprise E23 recorded the lowest social DPI at 0.39, while E3, E4, and E15 achieved the highest possible scores. Sixty percent of the enterprises performed above the general mean (0.90). Notably, 100% of these enterprises contribute to job creation and the strengthening of labor capacities.

Environmental Performance

The lowest environmental DPI was observed in E23 with a score of 0.68, while E1, E3, and E4 reached the maximum value. The main environmental activities carried out by the NBEs include habitat conservation, waste collection, fire prevention, reforestation, and soil conservation. It was estimated that 60% of the enterprises are engaged in the restoration of

Table 3. Correlations between the SPI and the performance dimensions by dimension.

	SPI	DPI economic	DPI social	DPI environmental
SPI				
DPI económico	0.843**			
DPI social	0.726**	0.375		
DPI ambiental	0.579**	0.230	0.441*	

SPI: Sustainable Performance Index. DPI: Dimension Performance Index.
*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

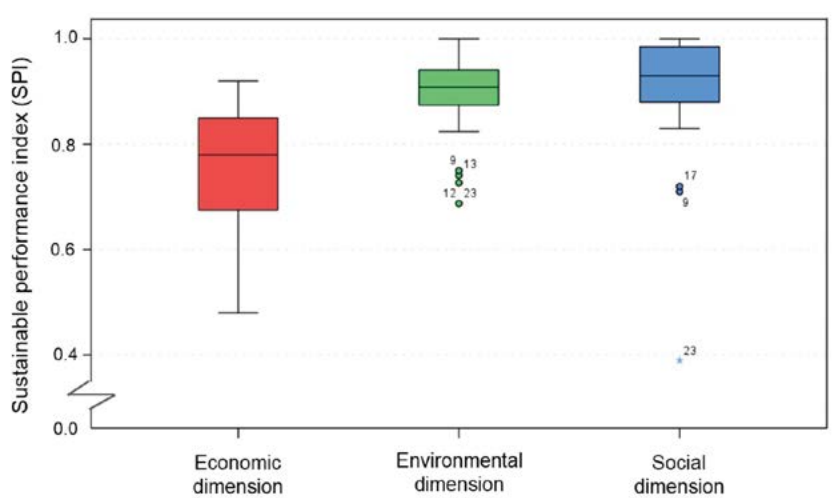

Figure 1. Variability of Sustainable Performance Across Enterprise Dimensions.

degraded areas; 52% exclusively manage native species, and 60% reported the presence of species classified under risk categories according to NOM-059-SEMARNAT-2010 within their territories.

Enterprise typology

A qualitative visual analysis of the dendrogram allowed the identification of four groups (Figure 1). Based on the results of the Dimension Performance Indices (DPI), the groups were named as follows: Group 1 (G1): Consolidated. Group 2 (G2): In Development. Group 3 (G3): In Adaptation. Group 4 (G4): In Incipient Development.

Table 4 illustrates the differences between groups with respect to the indices of the three evaluated dimensions and the general categories. Figure 3 displays the Sustainable Performance Index (SPI) for the identified groups.

Table 4. Comparison of means of variables within groups.

Indicator	G1	G2	G3	G4
Economic DPI	0.87 ± 0.03^{c}	0.80 ± 0.04^{c}	0.55 ± 0.06^{a}	0.68 ± 0.06^{b}
Investment Capacity#	0.98 ± 0.03^{a}	0.91 ± 0.12^{ac}	0.55 ± 0.12^{b}	0.79 ± 0.05^{cb}
Vulnerability Capacity&*	0.75 ± 0.075^{a}	0.69 ± 0.140^{ab}	0.54 ± 0.022^{b}	0.57 ± 0.07^{b}
Social DPI	0.97 ± 0.031^{a}	0.93 ± 0.04^{a}	0.92 ± 0.07^{a}	0.66 ± 0.19^{a}
Engagement Capacity**	0.71 ± 0.49^{a}	0.75 ± 0.46^{a}	0.25 ± 0.50^{a}	0.75 ± 0.5^{a}
Satisfaction Capacity**	0.88 ± 0.13^{a}	0.68 ± 0.22^{a}	0.66 ± 0.30^{a}	0.37 ± 0.32^{a}
Control Capacity**	0.97 ± 0.03^{a}	0.94 ± 0.04^{a}	0.92 ± 0.08^{a}	0.66 ± 0.19^{a}
Environmental DPI#	0.96 ± 0.03^{a}	0.87 ± 0.06^{b}	0.94 ± 0.03^{a}	0.77 ± 0.09^{b}
Conservation Capacity	0.94 ± 0.07^{a}	0.86 ± 0.07^{a}	0.93 ± 0.04^{a}	0.89 ± 0.09^{a}
Regulatory Compliance Capacity#**	0.99 ± 0.03^{a}	0.88 ± 0.13^{bc}	0.96 ± 0.05^{ab}	0.65 ± 0.20^{c}

DPI: Dimension Performance Index. C: General Criterion. a, b, c: Different letters indicate significantly different means ($p<0.1$) within each row. ± Values following the sign represent the standard deviation. Tests used: Scheffé, # Mann-Whitney U, and Games–Howell, ** ANOVA.

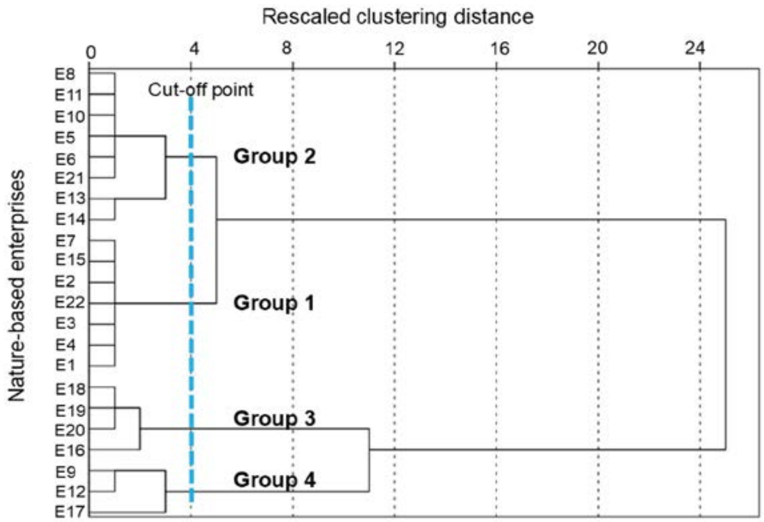

Figure 2. Groups Resulting from the Hierarchical Cluster Analysis.

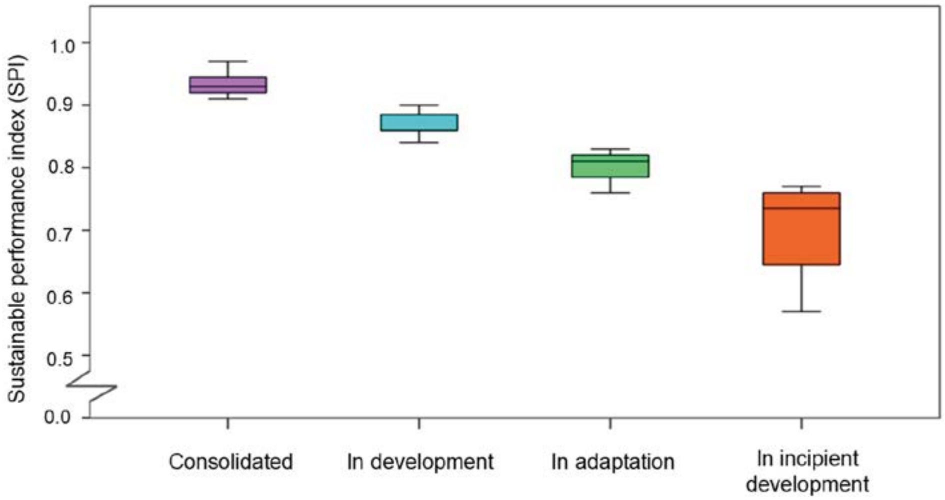

Figure 3. Sustainable Performance Index by Group.

Distinct attributes were identified among the groups, including investment capacity, income diversification, and environmental certifications. These contrasts are presented in Table 5.

Comparison of highest-performing groups

The comparison between the groups with the highest SPI scores (G1* and G2*) revealed significant differences in both economic and environmental indices, consistently favoring the consolidated group (Table 6).

The analysis of attributes associated with sustainable performance revealed that G1* presents greater advantages in conservation and income diversification, while G2* relies more on targeted strategies. Details are shown in Table 7.

This study showed that Nature-based enterprises (NBEs) in Mexico reach, on average, a high Sustainable Performance Index (SPI=0.85), with over 60% of the cases scoring above the mean. This finding directly answers the research question by demonstrating that, despite common limitations, some enterprises achieve superior performance, establishing themselves as positive outliers.

The strong correlation between the economic and social Dimension Performance Indices (DPIs), along with the moderate correlation between the environmental DPI and the SPI, confirms the relevance of these dimensions to overall performance (Table 3). However, the weak correlation between the social and environmental DPIs indicates that strong environmental performance does not always translate into social benefits. This suggests the influence of other organizational or contextual factors a pattern consistent with findings in Puebla (Hernández *et al.*, 2022), but differing from cases in Oaxaca, where participatory governance and indigenous cultural components generate synergies between both dimensions (Engbring & Hajjar, 2021; Palomino & López, 2019). The economic DPI was the most variable dimension (Figure 1), associated with disparities in market access, financing, and management capacities (Galicia *et al.*, 2022; Hernández

Table 5. Differentiating Attributes of NBEs by Group.

Attribute	G1 (%)	G2 (%)	G3 (%)	G4 (%)	(x^2)	p<
Economic dimension						
Investment in digital marketing	85.7	62.5	0	0	12.05	0.007
Investment in social media marketing	85.7	87.5	25	25	8.75	0.033
Investment in training	57.1	75	0	0	9.92	0.019
Sufficient income to cover expenses and generate profits	85.7	62.5	0	25	9.04	0.029
Income has increased in the last 2 years	100	62.5	0	75	11.43	0.01
Income has decreased in the last 2 years	0	0	75	0	16.39	0.001
Income from wildlife watching	57.1	25	0	75	6.36	0.096
Income from adventure tourism	57.1	12.5	0	0	7.78	0.051
Income from agricultural activities	14.3	37.5	100	50	7.81	0.05
Income from sport hunting	0	12.5	100	25	14.57	0.002
Income from gastronomy	85.7	62.5	0	75	8.38	0.039
Seasonality in the use of natural resources	0	12.5	100	25	14.57	0.002
Social dimension						
Committee of decision-making representatives	57.1	25	0	0	6.33	0.097
Reference marketing	14.3	25	100	100	13.55	0.004
International visitors received	100	75	25	50	7.65	0.054
Mission: conservation, research, and environmental education	71.4	25	0	25	6.78	0.079
Environmental dimension						
Natural vegetation >90% of the property	85.7	12.5	75	50	9.05	0.029
Pest and disease control	71.4	12.5	25	0	8.58	0.035
Reports to the appropriate institution	71.4	37.5	100	25	6.50	0.089
Species inventory	100	50	100	25	10.01	0.018
Knowledge of applicable legislation	100	75	100	25	9.77	0.021
Management strategy considers 3 dimensions	85.7	37.5	50	0	8.04	0.045
Management strategy considers ≥2 dimensions	14.3	62.5	50	100	8.04	0.045
ADVC Certification	57.1	25	0	0	6.33	0.097
UMA Certification (free-living)	42.9	12.5	100	25	8.98	0.03

ADVC: Voluntarily Designated Conservation Area. UMA: Wildlife Conservation Management Unit.

et al., 2022). The social DPI ranked in the middle, while the environmental DPI showed more homogeneous patterns, consistent with literature reporting widespread compliance with regulatory frameworks and basic conservation practices (Engbring & Hajjar, 2021). Nevertheless, the cluster analysis (Table 4) revealed that such environmental homogeneity is relative and that performance is shaped by factors such as the quality of natural resources, access to certifications, and organizational capacity aligned with

Table 6. Relationship Between the Two Highest-Scoring SPI Groups and Variables Associated with Sustainable Performance.

Indicador	G1*	G2*
Economic DPI	0.87 ± 0.03^{a}	0.80 ± 0.04^{b}
Social DPI	0.97 ± 0.03^{a}	0.93 ± 0.04^{a}
Environmental DPI#	0.96 ± 0.03^{a}	0.87 ± 0.06^{b}
SPI	0.94 ± 0.02^{a}	0.87 ± 0.02^{b}

SPI=Index of Sustainable Performance. DPI=Dimension Performance Index. [a, b]: Different letters indicate means with significant differences ($p<0.01$) within each row. ± Values following the sign represent the standard deviation, according to independent samples t-test. Mann–Whitney U test ($p<0.05$).

Table 7. Comparison between G1* and G2* enterprises.

Attributes	G1* (%)	G2* (%)	(x^2)	p≤
Environmental dimension				
Natural vegetation >90% of the property	85.71	12.5	8.04	0.009
Natural vegetation ≥50% of the property	14.29	62.5	3.62	0.084
Pest and disease control	71.43	12.5	5.40	0.035
Species inventory	100.0	50	4.77	0.051
Management strategy considers 3 dimensions	85.71	37.5	3.62	0.084
Management strategy considers ≥2 dimensions	14.29	62.5	3.62	0.084
Mission: conservation, research, and environmental education	71.43	25	3.23	0.100
Income from adventure tourism	57.14	12.5	3.35	0.100

findings by Galicia *et al.* (2022) and Pineda-Vázquez *et al.* (2019). This suggests that apparent environmental uniformity is not absolute but rather conditioned by governance arrangements and productive contexts. Groups G3 and G4 are characterized by low investment in training and marketing, concentrated governance structures, and limited regulatory knowledge (Table 5). In particular, although G4 has increased income from wildlife observation and gastronomy, it exhibits the weakest performance in conservation actions and compliance with legal-operational guidelines, linked to a lack of regulatory awareness. These patterns reflect trade-offs between income generation and conservation, similar to those described in community forestry enterprises (Engbring & Hajjar, 2021; Galicia *et al.*, 2022), ecotourism ventures (Hernández *et al.*, 2022), and communities with limited technical-regulatory knowledge, which intensify negative impacts (Pineda-Vázquez *et al.*, 2019). Although similar to G4, G3 differs in achieving greater legal-operational environmental compliance, explained by its focus on sport hunting under the UMA (Wildlife Conservation Management Unit) framework. This strengthens legal and environmental control but limits economic benefits due to quotas and seasonality (Bañuelos-Frías *et al.*, 2020), consistent with the reported decrease in income. Its referral-based marketing responds to the need to attract "trusted" clients who meet legal requirements and to a focus on international visitors. G2 represents an intermediate stage moving toward the G1 profile: it invests more in digital marketing and, most notably, in training, thus enhancing capabilities that complement public support. Although it preserves a smaller proportion of natural vegetation compared to G1, it maintains operational practices aligned with sustainability. The literature describes such enterprises as "in transition": they incorporate training and conservation but still exhibit limited impact (Galicia *et al.*, 2022; Pineda-Vázquez *et al.*, 2019). In contrast, G1 achieved the highest performance, combining financial strength, investment in digital marketing (*e.g.*, websites), income diversification, preservation of natural vegetation, and management strategies that explicitly integrate the three dimensions of sustainability along with participatory structures. These features present across various legal formsposition it as the group most closely aligned with positive outliers. These results align with studies that indicate successful cases of economic, social, and environmental balance typically share factors such as robust governance, long operational trajectories, and land extensions that facilitate sustainable management. Solid governance, environmental certification, diversification, and reinvestment in conservation and social cohesion are common characteristics (Engbring & Hajjar, 2021; Galicia *et al.*, 2022; Prins *et al.*, 2024). The comparative analysis between G1* and G2* revealed that the differences lie in income generation strategies and the integration of environmental approaches (Tables 6 and 7). G1* maintains a high proportion of natural vegetation, consistently complies with legal standards, and prioritizes conservation, research, and environmental education as core strategies. This orientation aligns with a logic of resource dependence, which motivates NBEs to ensure the long-term preservation of natural assets (Wigger & Shepherd, 2020) and supports evidence that implementing strong environmental policies ensures resource conservation and reliable access (Regmi *et al.*, 2023). While G2* has made progress in training, conservation, and organizational innovation, its consolidation may still be constrained by external factors (McQuaid *et al.*, 2021; Solís-Mendoza *et al.*, 2024). Thus, strengthening NBEs also requires a supportive institutional and regulatory environment. The literature highlights that sustainable performance results from the interaction between internal and external factors. Among the former, having a clear vision and mission from the outset translated into regulations, boards, and associative structures is key, as seen in G1* enterprises (Engbring & Hajjar, 2021; Pineda-Vázquez *et al.*, 2019). This organizational foundation supports social cohesion, legitimacy, and intergenerational continuity. However, realizing these elements depends on enabling external conditions such as supportive public policies, access to differentiated markets, and collaboration networks (Galicia *et al.*, 2022). Together, these internal and external conditions determine whether an enterprise remains in an incipient stage or consolidates as a successful model of sustainability. In this context, NBEs represent a complementary mechanism for mobilizing resources toward conservation, partially helping to bridge the biodiversity financing gap. By generating income linked to sustainable activities, these enterprises channel local and private capital toward the protection of natural resources (Álvarez-Peredo & Contreras-Hernández, 2023; Peña-Azcona *et al.*, 2022).

CONCLUSIONS

The study demonstrated that Nature-based enterprises (NBEs) in Mexico achieve, on average, a high level of sustainable performance (SPI=0.85), with over 60% of the cases above the mean, although with marked differences across dimensions and groups. Economic performance was the most variable, social performance ranked at an intermediate level, and environmental performance remained more homogeneous due to widespread compliance with basic conservation practices. The cluster analysis identified four groups consolidated, developing, adapting, and incipient and revealed that sustainability is enhanced by participatory governance and a clear mission, economic diversification, access to certifications, and investment in capabilities and marketing. The main structural obstacles are related to initial support dependency, limited current financing, production seasonality, regulatory constraints, and weak organizational cohesion. Strengthening these enterprises requires robust internal structures, flexible financial instruments, access to differentiated markets, and territorial collaboration networks. Although the exploratory nature of the study and the limited sample size prevent generalization, the findings provide initial evidence of the existence of positive outliers and the ways in which they overcome common limitations. Future research should incorporate longitudinal analyses and mixed methodologies that integrate both quantitative and qualitative indicators to deepen understanding of the factors that explain the sustainability of NBEs in Mexico and Latin America.

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Colegio de Postgraduados

Nonparametric estimation of compensatory variation of recreational service consumers in a biosphere reserve in Mexico

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ABSTRACT

Objective: This study aims to estimate and compare the compensating variation associated with recreational access to the La Michilía Biosphere Reserve, Durango, to provide robust technical evidence for its sustainable management and conservation.

Design/methodology/approach: A contingent valuation method was employed using a dichotomous referendum format (yes/no), based on a survey administered to 148 residents from neighboring communities. Willingness to pay was estimated using both non-parametric approaches (Kaplan-Meier, Kriström, and Turnbull) and a parametric logistic regression model.

Results: The average compensating variation estimated through the non-parametric Turnbull method was $41.33 per visitor, whereas the logistic model yielded an estimate of $52.30. This discrepancy highlights the conservative bias of non-parametric methods, which mitigate the risk of overestimation due to restrictive functional form assumptions.

Limitations/implications: The positive skewness in the distribution of willingness to pay particularly affects the Kriström method especially its Spearman-Karber variant by extrapolating beyond the offered price range, which may constrain its empirical validity.

Findings/conclusions: The findings demonstrate a significant economic valuation of recreational services by the local population. The estimated values support the potential implementation of access fee schemes as a viable financing mechanism, contingent upon comprehensive social and regulatory assessment.

Keywords: environmental recreational services, protected natural areas, parametric functional form, logistic regression.

INTRODUCTION

In Mexico, Protected Natural Areas (PNAs) represent a key policy instrument of the Mexican state for the conservation of ecosystems, plant and animal species, and the environmental services they provide. Currently, according to the General Law of Ecological Balance and Environmental Protection and the National Commission of Natural Protected Areas, there are 226 federally designated PNAs, of which 182 are terrestrial and 44 are marine (SEMARNAT, 1988; CONANP, 2024a). These areas are considered public goods due to their ecological, social, and cultural functions; however, they face increasing challenges related to financing, governance, and community engagement (Bezaury, 2024; Miteva *et al.*, 2019). The La Michilía Biosphere Reserve, located in the state of Durango, stands as a prominent example within the national system of protected areas. Spanning over 35,000 hectares, the reserve hosts endemic species, temperate ecosystems, and rural communities with communal, ejidal, and federal land tenure systems (Halffter, 2011). Its inclusion in international databases such as the World Database of Protected Areas (UNEP-WCMC, 2019) and its designation by UNESCO (2023) underscore its global ecological significance. Nevertheless, it faces specific challenges stemming from institutional fragmentation, pressure from economically incompatible activities, and limited coordination between local and institutional stakeholders (Brenner & Job, 2022). Although a formally published Management Program exists (CONANP, 2010), its implementation and public dissemination have been markedly limited (SEMARNAT, 2022). This situation has significantly hindered participatory planning, access to updated information, and the program's effectiveness as a guiding instrument for territorial management of the biosphere reserve. Recreational access to La Michilía remains scarce, and activities such as ecotourism, wildlife and plant observation, hiking, and interpretive walks are underdeveloped (CONANP, 2024a; México Desconocido, 2023). This limitation has been documented in previous studies on forest recreation planning in protected areas in southern Durango, which highlight the need to strengthen infrastructure and the design of recreational experiences (Pérez *et al.*, 2008). According to the Federal Rights Law, the current entrance fee for protected natural areas is $23.35 MXN per person per day (CONANP, 2024b); however, it remains unclear whether La Michilía currently applies this fee scheme. In this context, estimating the compensating variation associated with recreational access becomes a relevant tool to assess public use value and inform evidence-based management decisions (Morey & Rossmann, 2008). The aim of this study is to estimate and compare the compensating variation derived from recreational access to the La Michilía Biosphere Reserve, using both parametric and non-parametric methods to generate economic evidence that supports the valuation of the area's public use. It is important to clarify that the research does not aim to prescribe normative decisions but rather to produce empirical information that can be considered by decision-makers within their institutional mandates and policy instruments, in order to design and implement programs and projects related to the management and use of natural resources considered public goods.

MATERIALS AND METHODS

To estimate the compensating variation associated with recreational access to La Michilía, the contingent valuation method was employed using a dichotomous referendum format (yes/no). This approach followed the methodological guidelines proposed by Mitchell and Carson (1989), Arrow *et al.* (1993), Carson and Steinberg (1990), and Johnston *et al.* (2017). The latter provided key insights into experimental survey design for discrete choice preferences, thereby enhancing the structural validity of the applied format. A custom-designed questionnaire comprising 20 questions was developed specifically for this study. A pilot test was conducted in the field to refine the wording, sequence, and sensitivity of the questions prior to the final implementation. Redundant items were removed, and the phrasing of ambiguous questions was clarified. The survey was administered in 2021 to a sample of 148 individuals over the age of 18, all residing in four communities adjacent to the reserve. Although convenience sampling was employed, efforts were made to ensure the greatest possible diversity in age, gender, and occupation. Given the exploratory nature of the study and the use of convenience sampling, it is not possible to report a statistical margin of error or confidence level. However, the sample is considered to offer a useful approximation of the potential visitor profile for the biosphere reserve under study.

The variables analyzed included the hypothetical price offered (MX$20-MX$60) within the simulated market scenario presented in the questionnaire. A detailed description of the variables considered in the study is provided in Table 1.

The compensating variation for recreational access to La Michilía was estimated following the methodologies proposed by Kriström (1990), Turnbull (1976), and Haab and McConnell (2002). The estimations were conducted using R software version 4.2, specifically the “DCchoice” package, as documented by Nakatani (2025) and Aizaki *et al.* (2014). The logistic regression equation used to estimate the probability that an environmental service user accepts the offered price (PRC) is presented in Equation 1.

$$\Pr(Si) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 P + \beta_2 X_2 + \ldots + \beta_k X_k)}} \tag{1}$$

Table 1. Variables used in the estimation of compensating variation.

Variable	Description	Scale
PRC	Price offered to the respondent in the willingness-to-pay question	Discrete (MX$20, MX$30, MX$40, MX$50, MX$60)
RSP	Respondent’s answer in the single-bounded referendum format	Dichotomous Nominal (Yes/No)
HHI	Household income	Discrete ($/month)
EDU	Educational attainment	Ordinal Categorical
AGE	Age	Discrete (years)
GEN	Gender	Dichotomous Nominal
FMS	Family size	Discrete

Source: own elaboration.

Where: $\Pr(Si)$ It is the probability that an individual will agree to pay a monetary amount (P) to have access to the environmental services of La Michilía; β_0 is the constant term (intercept); β_1 It is the coefficient that weights the offered price P; $\beta_2,\ldots,\beta_k$ are the coefficients of the additional explanatory variables (income, age, education, among others); $X_2,\ldots, X_k$ correspond to the interviewee's socioeconomic variables and are the basis of the natural logarithm.

Once the logistic regression model has been estimated, it is possible to retrieve the parameter (β_1) which weights the offered price and allows for the calculation of the compensating variation, or the average willingness to pay (WTP), for access to the recreational and biodiversity services of La Michilía. The analytical expression used to obtain the compensating variation or willingness to pay (WTP) indicator is presented in Equation 2.

$$WTP_{media} = \frac{\alpha + \sum_{k=1}^{m} \gamma_k \bar{X}_k}{\beta_1} \qquad (2)$$

Where: α is the estimated intercept and corresponds to the respondent's base utility when all covariates are equal to zero; β_1 is the coefficient that weights the offered price in the single-bound referendum model and corresponds to the marginal sensitivity to changes in price, and its expected sign is negative; γ_k is the coefficient of the covariate k which represents the marginal impact of the covariate on the respondent's utility; $\bar{X}_k$ is the average value of the covariate k; m is the number of covariates with k=1, 2,..., m; and the summation symbol indicates the aggregation of the resulting values of the respective parameter that weights the average value of the covariate k in this case (Haneman, 1984).

The empirical estimation of the logistic regression model was conducted using the VGAM package in R, following the methodological guidelines for discrete response data analysis in contingent valuation studies as described by Hanemann and Kanninen (1999). This approach allows the modeling of acceptance probability as a function of the offered price (PRC) and socioeconomic covariates, ensuring statistical consistency in the estimation of compensating variation. Finally, regarding the methodological framework employed in this study, Bateman *et al.* (2002) provide a systematic overview of stated preference techniques and recommend the use of dichotomous formats to enhance empirical validity and minimize hypothetical bias in environmental valuation studies. This approach enables the simulation of hypothetical markets and the derivation of monetary estimates for stated preferences, including components of existence or non-use value (Carson & Hanemann, 2005).

RESULTS AND DISCUSSION

The descriptive statistics of the variables used in the study are presented in Table 2.

Table 2. Descriptive statistics of the key variables in the study.

Variable	Mean	Standard deviation	Minimum value	Maximum value
PRC	40.0	14.2	20.0	60
RSP	0.64	0.48	0.0	1
HHI	2,447.8	1,482.7	1,002	6,911
EDU	3.1	1.3	1	6
AGE	37.1	14.9	18	77
GEN	0.53	0.50	0.0	1
FMS	4.2	1.9	1.0	15

Note: The definition of the variables is given in Table 1.
Source: Prepared by the authors based on R-DCchoice runs.

Figure 1 shows the survival curve estimated for calculating the average willingness to pay (WTP) for La Michilía, based on field data. The horizontal axis displays the initial price offered to respondents regarding their willingness to pay for access to the reserve's services. The vertical axis presents the empirical survival probability, which represents the proportion of individuals willing to pay each offered amount or more. The survival function essentially represents the demand curve for access to the reserve's services; therefore, the area under this curve corresponds to the average WTP per individual for access to these services.

Table 3 presents the estimated results obtained using the Kriström method. As shown in the table, a survival probability of 1.0 indicates that 100% of respondents would be willing to access the reserve free of charge. Conversely, a survival probability of 0.5667 implies that 56.67% of respondents would be willing to pay $60 for access to the recreational and biodiversity services.

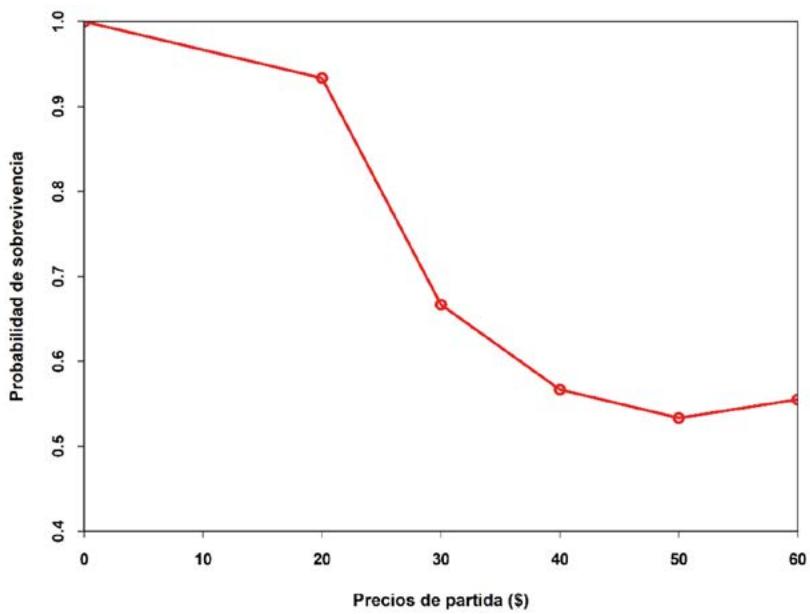

Figure 1. Kriström survival function.

Table 3. Estimated DAP and survival probability of Kristöm.

Starting price ($)	Probability of survival	Average willingness to pay (MX$/visitor)	
0	1.0000	Kaplan-Meier	41.34
20	0.9333	Spearman-Karber	81.66
30	0.6667	Median	60.00
60	0.5667		
50	0.5333		
60	0.5000		
∞	0.0000		

Note: WTP=Willingness to pay. NA=Not applicable.
Source: Prepared based on Nakatani (2023) and Kriström (1990).

Similarly, the results from the Turnbull model adjustment used to estimate the average compensating variation under a single-bounded referendum format, as well as the interpretation of its survival probability, are analogous to those of the Kriström method, as shown in Table 4. In this case, a survival probability of 1.0 indicates that 100% of respondents would be willing to enter the reserve free of charge, while a survival probability of 0.5333 indicates that 53.3% would be willing to pay $50 for access to La Michilía's services.

One way to assess the behavior of the WTP estimates obtained through non-parametric procedures is to compare them with their parametric counterpart, which assumes a specific functional form. This comparison is essential to evaluate the robustness of the estimates. The estimated parameters of the logistic regression model which models the respondent's acceptance probability as a sigmoid function of the offered prices as well as the associated test statistics, are presented in Table 5.

As can be seen, the parameter (γ_1) which weights the price offered to the interviewee (PRC) is statistically significant at 99% confidence. The parameter (γ_2) which weights family income (HHI) is significant at 95% confidence level. Both parameters are well behaved as they present the expected sign; that is, negative for γ_1 y positivo para γ_2. The parameters of the remaining covariates are not statistically significant; however, their

Table 4. Estimated WTP and survival probability of turnbull.

Starting price ($)	Probability of survival	Average willingness to pay (MX$/visitor)	
0	1.0000	Kaplan-Meier	41.33
20	0.9333	Spearman-Karber	44.17
30	0.6667	Median	55.00
60	0.5667		
50	0.5333		
60	0.5000		
∞	0.0000		

Note: WTP=Willingness to pay.
Source: Prepared based on Nakatani (2023).

Table 5. Estimated parameters of the logistic regression model.

Variable	Parameter	Standard error	z value	Pr(>|z|)
Intercept	3.2826	1.2281	2.673	0.0075 ***
PRC	−0.0591	0.0151	−3.914	0.0001 ***
HHI	0.0004	0.0002	2.032	0.0421 **
AGE	−0.0252	0.0142	−1.778	0.0754 *
EDU	0.0064	0.2059	0.031	0.9753
GEN	0.5336	0.3973	1.343	0.1792
FMS	−0.1258	0.1128	−1.115	0.2649

*** significant at the 99% level, ** significant at the 95% level, * significant at the 90% level. Note: Variable names correspond to those defined in Table 1. Source: Prepared using R-VGAM.

inclusion is common in economic valuation studies. The expected sign of these covariates was not specified *a priori*, as no economic theory dictates whether they should be positive or negative. The average willingness to pay (WTP), estimated using the logistic regression model and calculated through the expression in Equation 2 (see Materials and Methods), was $52.30 per potential user of La Michilía's services. Before proceeding with the results discussion, it is important to note that WTP values were estimated using non-parametric estimators —Kaplan-Meier and Spearman-Kärber under the Kriström and Turnbull approaches, as well as a parametric logistic model to incorporate covariates. The Kaplan-Meier estimate ($41.33) was selected for discussion due to its ability to reflect the actual range of offered prices ($0-$60), avoiding invalid extrapolations such as that of the Spearman-Kärber method ($81.66). Its consistency with both the Kriström and Turnbull approaches ($41.33) supports its reliability, while the discrepancy with the logistic regression estimate ($52.30) suggests that parametric assumptions may overestimate the compensating variation (or WTP) in this context. Although the vast majority of reviewed contingent valuation studies apply parametric approaches, Soncco and Armas (2008) compared WTP for water protection services using both parametric and non-parametric methods in the Jequetepeque River basin, Peru. Their study employed a linear logit model for the parametric approach and Turnbull and Kriström techniques for the non-parametric methods. Their results showed no significant differences between methods, and they recommend using multiple approaches in the economic valuation of ecosystem services to obtain more reliable and precise estimates, emphasizing that both approaches are complementary.

Cerda and Vásquez (2005) applied contingent valuation to estimate WTP for water use from the Claro River in Talca, Chile, using both parametric and non-parametric approaches. Their results show a similar pattern to those of the present study, with parametric WTP values approximately 20% higher than their non-parametric counterparts. This corroborates the tendency of parametric methods to yield higher WTP estimates due to the imposition of a smooth, symmetric functional form on the data. Moreover, the inclusion of covariates that increase the probability of payment tends to extrapolate higher values than those strictly observed in the empirical data unlike more conservative non-parametric methods. As illustrated, the use of non-parametric methods responds to the need for prudent estimates, especially when compared to parametric models, and better alignment with observed behavior within the offered price range. Their conservative nature helps avoid overestimations stemming from restrictive functional assumptions, which is particularly relevant in contexts where pricing sensitivity must be evaluated with caution. An important point to underscore is that, like all research on compensating variation, this study faces methodological limitations. Hypothetical bias persists due to the simulated payment scenarios, even though the referendum format recommended by Arrow *et al.* (1993) which tends to reduce such bias was applied. External validity is limited by the sample size and scope (148 respondents from communities near La Michilía). Moreover, while non-parametric methods are conservative, they can be affected by positive skewness in the WTP distribution. For instance, the Spearman-Kärber variant extrapolates beyond the offered range. These limitations do not invalidate the results but do constrain their applicability to public policy design concerning natural resource management.

CONCLUSIONS

This study concludes that local residents in the region surrounding the La Michilía Biosphere Reserve recognize and value the recreational and ecosystem services provided by this protected natural area. This is evidenced by their willingness to pay a monetary fee to enjoy its recreational, environmental, and ecosystem benefits. The application of the Kaplan-Meier method yielded a robust and consistent estimate of the average willingness to pay, set at $41.33 per visitor, a value aligned with both the Kriström and Turnbull approaches. This result supports the hypothesis that there is a positive economic valuation among nearby municipalities for recreational access to the reserve. The evidence suggests that implementing a recreational access fee could be a viable strategy to strengthen the reserve's financing and management, contributing to both natural resource conservation and the well-being of local communities. Ultimately, the findings of this study provide relevant information for decision-making processes aimed at economic diversification and the formulation of public policies that promote the sustainable use and effective governance of protected natural areas like La Michilía. This research is framed within a strictly academic context, with the primary objective of generating technical evidence on the economic valuation of ecosystem services. It does not intend to issue normative or operational recommendations. While the results may inform public decisions, the study does not explicitly propose the implementation of a fee system. In complex contexts such as that of La Michilía, it is up to the competent authorities and local stakeholders to deliberate on the feasibility of any access scheme, considering the results of this study as a technical input.

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Colegio de Postgraduados

Evaluation of microbial consortia for the control of *Rhizoctonia solani* kühn in serrano pepper under greenhouse conditions

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ABSTRACT

Objective: To evaluate the effectiveness of bacterial consortia and endophytic fungi for the control of *Rhizoctonia solani* in serrano pepper.

Design/methodology/approach: Two experiments were established: the first used two-month-old chili plants, and the second used one-month-old plants. The following treatments were inoculated in the root zone: T1=*Bacillus cereus* P5 + *Irpex lacteus* P7B, T2=*Paenibacillus polymyxa* BACAGA + *Bacillus glycinifermentans* CORI2 + *Bacillus subtilis* PI04 + *Xylaria feejeensis* PASI22, T3=*Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE, T4=Negative control (without microorganisms), and T5=Positive control (inoculation of *R. solani*). In the first experiment, disease severity was estimated using a diagrammatic scale. In the second, it was assessed using two methods: a diagrammatic severity scale in the first, and a millimeter ruler in the second. Using the data obtained, the area under the disease progression curve (AUCPE) was estimated for each treatment. The incidence and severity were assessed over 13 days, and the area under the disease progress curve (AUCPE) was estimated. At the end of the experiment, the fresh and dry weights of each treatment were estimated. The data were subjected to analysis of variance and means test (Tukey, p≤0.05) using SAS version 9.0. Results: The best treatment was T3, as it reduced the severity of the disease caused by *R. solani*, registering AUDPC values of 3.17 for severity in the first experiment, AUDPC of 24.90 (first method) and 10.19 (second method) in the second experiment. T5 showed the highest severity values, with an AUDPC of 11.45 in the first experiment and AUDPC of 54.70 (first method) and 23.91 (second method) in the second experiment.

Findings/conclusions: The best treatment to reduce the severity of *R. solani*-induced disease was T3 treatment.

Limitations/implications: Future research is required to evaluate the effectiveness under open-field conditions of the microbial consortia evaluated in the present study.

Keywords: *Capsicum annuum*, disease, beneficial microorganisms, severity, incidence.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is a crop in high global demand (Reddy *et al.*, 2016). Mexico ranks as the second-largest producer worldwide, with an annual output

of 3,681,061 metric tons (FAOSTAT, 2023). This crop is widely consumed, as its fruit is attributed with nutraceutical properties due to the presence of antioxidant compounds such as carotenoids, tocopherols, and capsaicinoids (Imran *et al.*, 2018). However, chili production is significantly impacted by the fungus *Rhizoctonia solani*, which causes root rot and wilting, affecting both seedling emergence and plant development, and can reach an incidence of up to 40% under field conditions (Varma *et al.*, 2020; Tuncer & Eken, 2023). This pathogen is particularly difficult to control due to its ability to persist in the soil for extended periods and its broad host range (Abbas *et al.*, 2022; Sam-On *et al.*, 2024). Moreover, *R. solani* exhibits high virulence and genetic diversity, complicating the development of effective management strategies (Tuncer & Eken, 2023). Traditionally, the control of *R. solani* relies on the application of synthetic fungicides. However, these have raised environmental and human health concerns (Ayaz *et al.*, 2023; Wu *et al.*, 2019). In response to this issue, there has been growing interest in evaluating more sustainable control methods that do not compromise environmental integrity. Within this context, biological control emerges as a promising alternative for disease management (Pawaskar & Kerkar, 2021). Globally, various management strategies have been assessed, including the use of biocontrol agents such as *Bacillus* spp. and *Trichoderma* spp., which have demonstrated effectiveness in reducing disease severity and promoting plant growth (Wu *et al.*, 2019; Abhinav *et al.*, 2023; Huang *et al.*, 2017). In Mexico, *R. solani* is widely distributed across all chili-producing regions, with reported incidence rates exceeding 30% (Montero-Tavera *et al.*, 2013; Vásquez-López *et al.*, 2009). Nevertheless, studies on biological control of *R. solani* under *in vivo* conditions in this crop remain scarce. In light of the above, the aim of this study was to evaluate the effectiveness of microbial consortia composed of bacteria and endophytic fungi in the control of *R. solani* in serrano pepper plants.

MATERIALS AND METHODS

Geographic location of the experimental site

Two greenhouse experiments were conducted at the Faculty of Agricultural and Environmental Sciences of the Autonomous University of Guerrero (FCAA-UAGro), located in Iguala de la Independencia, Guerrero, Mexico, at geographic coordinates 18° 21' 19.70" N and 99° 32' 54.16" W, at an elevation of 750 meters above sea level. The experiment was repeated twice at different times under greenhouse conditions. Two-month-old chili plants were used in the first experiment, and one-month-old plants in the second. In both experiments, relative humidity was recorded using a Datalogger Extech RTH10® at hourly intervals. To increase relative humidity within the experimental area, transparent plastic bags measuring 90×120 cm were placed over each treatment for approximately 72 hours.

Isolation of the phytopathogenic fungus

In San Vicente Palapa, a locality in the municipality of Tepecoacuilco de Trujano, Guerrero, a sampling was conducted in a serrano pepper field where plants showing symptoms of basal rot were collected. The estimated disease incidence was 15%. The plants exhibited wilting, root rot, basal stem rot, and overall collapse. Symptomatic plants were processed at the Laboratory of Plant Physiology and Biotechnology (FCAA-UAGro), where isolate P5SPA was obtained and identified at the cultural and morphological level as *Rhizoctonia solani*.

Obtaining beneficial bacteria and fungi

The bacterial and fungal strains used in this study were provided by the Laboratory of Plant Physiology and Biotechnology at FCAA-UAGro. The microbial strains evaluated included: *Paenibacillus polymyxa* (BACAGA), *Bacillus glycinifermentans* (CORI2), *Bacillus subtilis* (PI04), *Bacillus cereus* (P5), *Bacillus* sp. (SALGUA), *Trichoderma* sp. (TRICOE), *Irpex lacteus* (P7B), and *Xylaria feejeensis* (PASI22).

Planting of plant material

Seeds of serrano chili were used, disinfected with 5% NaOCl for three minutes, and rinsed three times to remove excess NaOCl (Calaña-Janeiro, 2019). Seeds were sown in 200-cell seedling trays filled with Peat Moss® substrate. Nine days after germination, the seedlings were transplanted into plastic pots containing Peat Moss® and placed in a greenhouse.

Experimental design

Both experiments followed a completely randomized design with five treatments and 20 replications, totaling 100 experimental units. Each experimental unit consisted of one serrano chili plant. The treatments applied were as follows:

T1 = *B. cereus* P5 + *Irpex lacteus* P7B; T2 = *P. polymyxa* BACAGA + *B. glycinifermentans* CORI2 + *B. subtilis* PI04 + *X. feejeensis* PASI22; T3 = *Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE; T4 = Negative control (no microorganisms); T5 = Positive control (*R. solani* P5SPA inoculation)

Evaluation of *R. solani* biocontrol

Serrano pepper plants were first inoculated with beneficial microorganisms according to their respective treatments. Four days later, they were inoculated with *R. solani* P5SPA, following the methodology proposed by Villajuan-Abgona (1996). The fungi *R. solani* P5SPA, *Irpex lacteus* P7B, *Xylaria feejeensis* PASI22, and *Trichoderma* sp. TRICOE were cultured on potato dextrose agar (PDA) supplemented with 1 mL/L gentamicin and incubated for 14 days under laboratory conditions. Bacterial strains (*P. polymyxa*, *B. glycinifermentans*, *B. subtilis*, *B. cereus*, and *Bacillus* sp.) were grown on PDA (without antibiotics) and incubated at 28 °C for 48 hours. From the fungal cultures, 19 mm diameter mycelial plugs were extracted using a sterile cork borer and placed at the base of each plant stem using sterile wooden sticks (autoclaved at 121 °C for 20 minutes). Bacterial colonies were suspended in sterile water and adjusted to 10_2 CFU/mL using a hemocytometer. Then, 50 mL of each suspension was applied at the base of each plant stem.

Beneficial microorganisms were inoculated according to their respective treatment, and four days later, *R. solani* P5SPA was introduced, following the aforementioned procedure. The positive control consisted of *R. solani* P5SPA disks, while the negative control consisted of PDA disks without mycelium.

Estimation of incidence and severity

Disease incidence and severity caused by *R. solani* were evaluated every 24 hours for 13 days in both experiments. Incidence was calculated using the following formula:

$$(\textit{number of diseased plants / total number of plants per treatment}) \times 100$$

In the first experiment, disease severity was assessed using the scale proposed by Horsfall and Barratt (1945). In the second experiment, two methods were used:

Method 1: Modified scale from Andrade-Luna *et al.* (2017).

where: 0 = no symptoms, 1 = initial basal stem rot, 2 = moderate basal stem rot, 3 = stem constriction, 4 = collapsed plant with turgid leaves, 5 = collapsed plant with wilted leaves, 6 = advanced stem rot and leaf drop, 7 = severe stem rot, 8 = generalized rot.

Method 2: Severity was measured using a millimeter ruler.

Severity and incidence data over time were used to calculate the Area Under the Disease Progress Curve (AUDPC) as described by Campbell and Madden (1990). Analysis of variance and mean separation (Tukey, $p \leq 0.05$) were performed using SAS software version 9.0.

Fresh and dry biomass weight

Following the final incidence and severity evaluation, both fresh and dry biomass weights were measured for each experimental unit/treatment in both experiments. For fresh weight, plants were removed from the soil, washed with running water to remove substrate, and weighed using a compact OKAYA® scale. For dry weight, plants were placed in kraft paper bags and dried in a NOVATECH® oven at 65 °C for 72 hours. The resulting data were analyzed by ANOVA and Tukey's test ($p \leq 0.05$) using SAS software version 9.0.

RESULTS AND DISCUSSION

Environmental conditions of the experiment

In the first experiment, conducted between October and November, the average temperature and relative humidity inside the greenhouse were 25 °C and 68%, respectively. In the second experiment, carried out from November to December 2024, the average temperature and relative humidity were 21 °C and 55%, respectively.

Disease incidence

Experiment one

In the first experiment, symptoms of the disease caused by *R. solani* P5SPA appeared six days after inoculation. The highest incidence was recorded in the positive control (T5) at 45%, while the lowest incidence was observed in T3 at 5%. By the end of the experiment, T5 reached 50% incidence, whereas T3 remained the lowest at 15%. The negative control remained asymptomatic, with 0% incidence (Figure 1).

Experiment two

In the second experiment, initial symptoms of the disease appeared five days after inoculation with *R. solani*. The positive control exhibited 25% incidence, while T3 showed the lowest incidence at 10%. At the end of the experiment, the positive control (T5) recorded the highest incidence at 90%, while T3 maintained the lowest at 65%. The negative control remained asymptomatic throughout the experiment (0% incidence) (Figure 2).

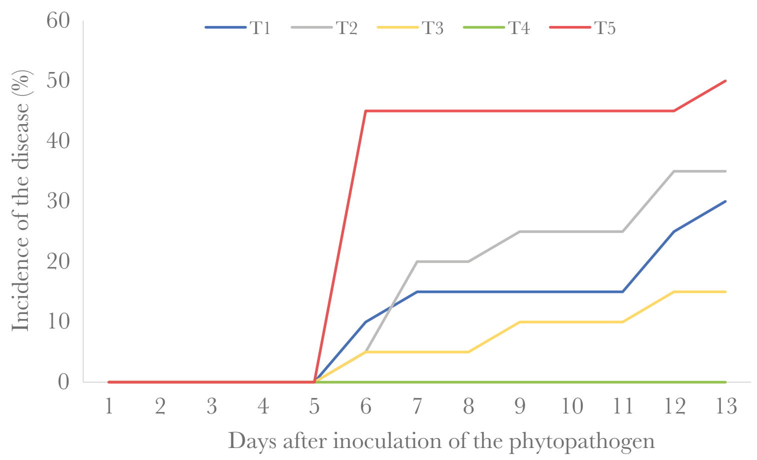

Figure 1. Disease incidence caused by *R. solani* in two-month-old serrano pepper plants (Experiment One).

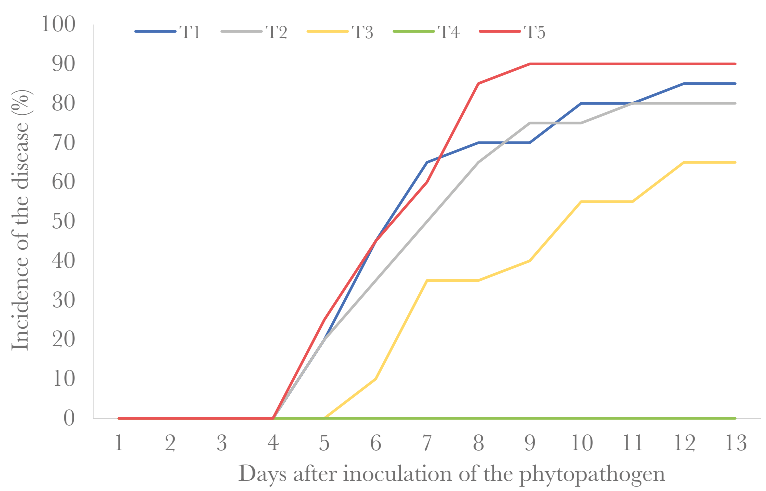

Figure 2. Disease incidence caused by *R. solani* in one-month-old serrano pepper plants (Experiment Two).

Analysis of AUDPC and biomass

Experiment one

The analysis of the Area Under the Disease Progress Curve (AUDPC) for disease incidence revealed that treatment T3 resulted in the lowest disease incidence, with an AUDPC of 3.37. In contrast, the positive control inoculated with *R. solani* P5SPA exhibited an AUDPC of 17.00 (Table 1). Regarding disease severity, treatments T1, T2, and T3 showed no significant differences, with AUDPC values of 3.12, 5.02, and 3.17, respectively. The positive control recorded a severity value of 11.45, while the negative control remained asymptomatic (Table 1). Significant differences were observed in plant biomass. The negative control produced the highest fresh and dry weights, with 5.90 g and 0.62 g, respectively. In contrast, the positive control yielded the lowest biomass, with 2.57 g (fresh weight) and 0.46 g (dry weight). Treatments T1, T2, and T3 produced intermediate values (Table 1).

Experiment Two

In the second experiment, significant differences were also observed in AUDPC values for disease incidence. T3 recorded the lowest incidence with 34.12 AUDPC, while the positive control exhibited the highest with 63.25 AUDPC (Table 1). Regarding severity estimated by Method 1, significant differences were found among treatments. T3 and T2 recorded values of 24.90 and 40.62 AUDPC, respectively, while the positive control reached 54.70 AUDPC (Table 1). Using Method 2, T3 showed the lowest disease severity with 0.19 AUDPC, whereas the positive control had the highest with 23.9 AUDPC. The negative control remained asymptomatic throughout the experiment (Table 1). Biomass data in the second experiment also showed statistical differences. The negative control produced the highest biomass, with 0.66 g (fresh) and 0.10 g (dry). The positive control recorded the lowest values, with 0.02 g and 0.01 g of fresh and dry weight, respectively (Table 1).

In the present study, the most effective treatment in reducing the incidence and severity of *R. solani*-induced disease in chili was T3 (*Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE). In contrast, the positive control exhibited the highest values for both variables (Table 1). Similarly, Abeysinghe (2009) reported that the application of *Bacillus*

Table 1. Mean comparison of AUDPC for disease incidence and severity, and plant biomass.

Treat†	Experiment 1				Experiment 2				
	Inc	Sev	FBW	DBW	Inc	SevM1	SevM2	FBW	DBW
T1	6.25 ab¶	3.12 ab	3.52 b	0.62 ab	57.37 ab	46.70 ab	18.77 ab	0.06 b	0.02 bc
T2	8.62 ab	5.02 ab	3.92 ab	0.66 ab	53.50 ab	40.62 b	15.11 ab	0.10 b	0.03 bc
T3	3.37 b	3.17 ab	4.30 ab	0.75 ab	34.12 b	24.90 b	10.19 bc	0.13 b	0.05 b
T4	0.00 b	0.00 b	5.90 a	0.96 a	0.00 c	0.00 c	0.00 c	0.66 a	0.10 a
T5	17.00 a	11.45 a	2.57 b	0.45 b	63.25 a	54.70 a	23.91 a	0.02 b	0.01 c

†Treat=Treatment; Inc=Incidence; Sev=Severity; FBW=Fresh biomass weight; DBW=Dry biomass weight; SevM1=Severity of method one; SevM2=Severity of method two.
¶Different literals in the same column indicate statistical differences ($p \leq 0.05$).

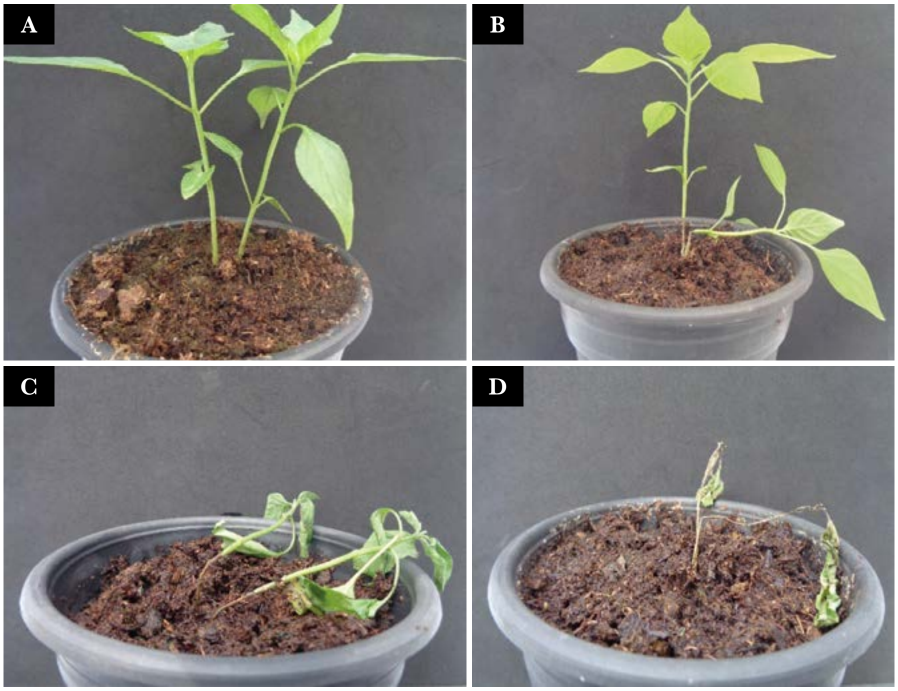

Figure 3. Representation of selected severity levels from the scale used in Experiment 2, Method 1: A) 0=no symptoms, B) 3=stem constriction, C) 5=collapsed plant with wilted leaves, D) 8=generalized rot.

subtilis CA32 (seed-inoculated) and *Trichoderma harzianum* (soil-inoculated) significantly reduced disease severity in chili, recording a severity value of 1.55 compared to 4.75 in the untreated control. Likewise, Espinoza-Ahumada *et al.* (2019) demonstrated that a combination of *Trichoderma* spp. and *Bacillus* spp. reduced disease severity in chili plants to 8.33%, compared to 19.45% in the positive control.

These findings are consistent with the results of the present study and may be attributed to the biocontrol capabilities of *Trichoderma* spp. This fungus employs multiple mechanisms, including mycoparasitism (Wang *et al.*, 2024), which involves direct contact and penetration of *R. solani* hyphae, as well as competition for nutrients and space, and antibiosis through the secretion of antifungal metabolites and enzymes such as chitinases, glucanases, and proteases (Abbas *et al.*, 2022; Kaur *et al.*, 2021; Halifu *et al.*, 2020). Additionally, there is evidence that *Bacillus* spp. can suppress *R. solani* by producing broad-spectrum antimicrobial compounds, including volatile organic compounds (VOCs) that disrupt the pathogen's cellular structure and induce oxidative stress (Ali *et al.*, 2023; Abbas *et al.*, 2019). The combined application of *Bacillus* and *Trichoderma* may also result in synergistic effects against *R. solani*, depending on environmental conditions and the specific strains used (Zhou *et al.*, 2021; Poveda & Eugui, 2022). Treatment T1 (*B. cereus* P5 + *I. lacteus* P7B) showed a moderate reduction in disease severity caused by *R. solani* (Table 1). Notably, *I. lacteus* P7B was recently documented as a mycoparasite capable of antagonizing 100% of 22 phytopathogenic fungi tested (Palemón-Alberto *et al.*, 2024). Therefore, its combination with *B. cereus* may have contributed to the disease suppression observed in this treatment. In Mexico, Pineda-Suazo *et al.* (2021) demonstrated the *in vitro* antagonistic activity of

I. lacteus against *Fusarium* spp., *Colletotrichum* spp., and *Phytophthora* spp., with growth inhibition percentages ranging from 16.7% to 46.3%. Moreover, White and Traquair (2006) reported that *I. lacteus* reduced the germination of *Botrytis cinerea* sclerotia by 100%, while in untreated controls, all sclerotia germinated. Regarding treatment T2 (*P. polymyxa* BACAGA + *B. glycinifermentans* CORI2 + *B. subtilis* PI04 + *X. feejeensis* PASI22), it also showed a moderate reduction in disease severity (Table 1). Chávez-Ramírez *et al*. (2020) reported that *P. polymyxa* exhibited *in vitro* antagonistic activity, inhibiting *R. solani* and *Pythium ultimum* by 70-80%. *B. glycinifermentans* has also been identified as an antagonist of pathogens such as *Fusarium* spp., *F. graminearum*, *Alternaria alternata*, and *F. oxysporum* f. sp. *radicis-lycopersici* (Afordoanyi *et al*., 2023). As for *X. feejeensis*, there are no prior reports of its antagonistic effect against *R. solani*. This study presents the first evidence of its use in combination with other microbes (*P. polymyxa*, *B. glycinifermentans*, and *B. subtilis*), which resulted in a moderate reduction of disease severity (Table 1). Brooks *et al*. (2022) did report *X. feejeensis* as a potent antagonist under *in vitro* and *in vivo* conditions against *F. oxysporum* and *A. alternata*. The highest biomass was recorded in the negative control (T4), while the lowest was observed in the positive control (T5). The remaining treatments showed intermediate biomass values (Table 1). These results clearly indicate the extensive damage caused to the overall plant system by the pathogen, a phenomenon well documented in the literature (Sharma *et al*., 2017; Huang *et al*., 2017).

CONCLUSIONS

The most effective treatment for controlling *R. solani* in serrano pepper was T3 (*Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE). Treatments T1 (*B. cereus* P5 + *Irpex lacteus* P7B) and T2 (*P. polymyxa* BACAGA + *B. glycinifermentans* CORI2 + *B. subtilis* PI04 + *X. feejeensis* PASI22) exhibited moderate effects in disease control. Further studies under open-field conditions are required to evaluate the biocontrol efficacy of these microbial consortia, particularly that of T3, which proved to be the most effective combination for reducing both the incidence and severity of *R. solani*.

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Colegio de Postgraduados

Descriptive Study of the Theoretical and Conceptual Framework of the Intercropped Milpa System with Fruit Trees in Veracruz

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ABSTRACT

Introduction: The Intercropped Milpa System with Fruit Trees (MIAF) constitutes an agroforestry strategy that integrates maize, beans, or other legumes with fruit species, creating a more resilient and sustainable agroecosystem.
Objective: This study aims to analyze, from a soil fertility perspective, the theoretical and conceptual contributions of the MIAF system in the Los Tuxtlas region of Veracruz, in order to support its relevance to agricultural productivity and the improvement of farmers' quality of life.
Methodology: A state-of-the-art analysis was conducted on agroecosystems and the MIAF system through a comprehensive review of the Scopus database. Disciplines, activities, and theories directly related to this system were examined, emphasizing its role in agricultural sustainability.
Results: A total of 13,142 documents related to the term "Agroecosystem" were identified over the past 15 years, spanning 10 countries, four document types, and 11 thematic areas. Regarding "MIAF," 33 scientific articles published between 2015 and 2025 across 10 countries were found. Twelve disciplines and their respective activities were characterized, and five theories closely linked to the system were identified, forming a robust conceptual framework to understand its functioning and potential. Conclusion: MIAF emerges as an innovative alternative to conventional agricultural systems. Its capacity to generate ecological, economic, and social benefits combined with its comprehensive approach positions it as a strategic option for food security, rural development, and environmental conservation.

Keywords: Theory, MIAF, terraces, hillsides.

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INTRODUCTION

The term Agroecosystem (AES) originates from the combination of Agro (land as a source of production) and Ecosystem. Agriculture derives from the Latin ager (field) and culture (to cultivate), and is defined as the activity through which human beings manage

natural resources, energy, and information to produce and reproduce plants and animals for anthropocentric purposes (FAO, 2001). The concept began to be used with the creation of the journal Agro-Ecosystem (Harper, 1974) and subsequently, in Mexico, with the first seminar on Agroecosystems (Hernández X., 1977).

Currently, the agroecosystem is conceived as a conceptual construction that refers to physical spaces modified by humans to generate social satisfiers, both material and immaterial, and, like ecosystems, includes biotic and abiotic components and their interactions (Pérez-Vázquez, 1996). For Hernández X. (1977), it is a transformed ecosystem for the use of resources in agriculture, livestock, forestry, and wildlife. Odum (1984) defined it as a domesticated ecosystem, situated between natural and artificial ones. Conway (1987) described it as an ecological system altered to produce fibers or food, while Marten (1988) defined it as a complex of air, water, soil, plants, animals, and microorganisms in an area modified for agricultural purposes.

The evolution of the concept has not been linear, as it has integrated diverse approaches and disciplines; for example, early agronomic approaches (19^{th}-20^{th} centuries), focused on efficiency and yield (Liebig, 1843; King, 1911); agricultural ecology (mid-20^{th} century), which recognized ecological complexity in agriculture (Odum, 1953; Watt, 1968); agroecology (from the 1970s to the present), consolidated as a holistic and social discipline integrating ecology, economy, and society (Altieri, 1987; Gliessman, 2007); and agroecology for food sovereignty and social justice, with an emphasis on fair and equitable food systems (Holt-Giménez *et al.*, 2013; IPES-Food, 2016). These transformations involve significant methodological challenges, as well as the need for holistic, multidisciplinary, participatory, long-term, adaptive approaches based on agroecology. According to Gliessman (2007), IFOAM (2008), and FAO (2018), holistic, participatory, and systems approaches stand out, along with qualitative and quantitative research methods oriented toward sustainability. On the other hand, the Los Tuxtlas region, Veracruz, is characterized by high biodiversity, abundant natural resources, and complex terrain with steep slopes. However, the expansion of the agricultural frontier and traditional tillage practices have led to soil degradation, water erosion, and decreased fertility, compromising agricultural productivity and the socioeconomic stability of small producers. In this context, since the late 1980s, technologies adapted to local conditions have been developed with the aim of improving agroecosystem sustainability. One of the first innovations was the implementation of Live Wall Terraces (TMV) in 1988, using *Gliricidia sepium* (Jacq.) hedgerows reinforced with runoff filters. This technology allowed for the delimitation of cultivable strips and the gradual formation of terraces, reducing erosion and improving water and nutrient retention. In a period of eight years, TMV reduced soil losses from 146 t ha^{-1} yr^{-1} under traditional tillage to only 2 t ha^{-1} yr^{-1}. Likewise, after five years of observation, the runoff coefficient decreased from 30% to 15%; sediment analyses also revealed a higher concentration of organic matter and nutrients in TMV, although total losses were lower than in the conventional system. The loss of exchangeable bases (K, Ca, and Mg) was also significantly lower in TMV, evidencing its effectiveness in conserving soil fertility. In terms of productivity, the average annual maize yields reached 4.95 t ha^{-1} in live wall terraces, compared to 4.12 t ha^{-1} in traditional tillage. Furthermore, the stability of production was higher in TMV. However, continuous planting over 13 maize cycles showed a slight decrease in fertility, reflected in soil acidification (0.43 pH units), reduction in organic matter (0.56%), and losses of potassium and calcium in the donor section of the terrace. These results showed that, although TMV is effective in mitigating erosion and improving yield stability, it does not fully resolve the challenges of agricultural sustainability. In response to this situation, INIFAP and the Colegio de Postgraduados developed in 2003 the Intercropped Milpa System with Fruit Trees (MIAF) as a technological evolution of TMV. This multi-objective system seeks to rescue the central principles of the traditional milpa, incorporating modern agronomic knowledge. MIAF integrates a fruit tree (overstory), maize (mesocrop), and a legume such as bean (understory), enabling intense crop interaction over time and space, optimizing the use of natural resources. The reported benefits of MIAF encompass biological, technical, and socioeconomic dimensions: increased net income and family employment, water erosion control, improved soil fertility, higher productivity, microclimate regulation, promotion of biodiversity, carbon sequestration, and long-term sustainability (Nájera *et al.*, 2018). In this regard, MIAF is conceived not only as an agricultural technology, but as a comprehensive rural development strategy capable of simultaneously addressing the environmental and socioeconomic problems of small producers in Los Tuxtlas. The need for this study lies in the fact that the Los Tuxtlas region is currently under significant agricultural and environmental pressure, resulting in fertility loss, erosion, and low yield stability. These conditions directly affect producers' quality of life and threaten regional food security. Analyzing the MIAF system from a soil fertility perspective will scientifically support its relevance as a viable alternative to traditional systems, providing a conceptual and practical framework to guide public policies, conservation strategies, and agricultural extension programs. The hypothesis is that the implementation of the MIAF system in the Los Tuxtlas region improves soil fertility and agricultural productivity, while strengthening the socioeconomic resilience of small producers in contrast to conventional systems. Based on this, the aim of the research was to analyze, from the perspective of soil fertility, the theoretical and conceptual contributions of the MIAF system in the Los Tuxtlas region, Veracruz, in order to support its relevance in agricultural productivity and in the improvement of the producers' quality of life.

MATERIALS AND METHODS

Stage I. Information Search and Analysis

In the first stage, a state-of-the-art analysis of the concept of Agroecosystem and the Intercropped Milpa System with Fruit Trees (MIAF) was carried out through scientific articles indexed in the Scopus platform, considering keywords, titles, and abstracts. Based on this information, corresponding metrics were generated for the aforementioned terms, as well as bibliometric maps of these concepts using the VOSviewer software. Additionally, a description was made of the main disciplines and activities that, from the perspective of the authors of this document, should be considered when studying the MIAF system in Mexico, as well as a description of the main theories inherent to the study of the multi-objective MIAF system. The bibliometric and scientific mapping study of the literature on MIAF and related concepts was conducted following PRISMA criteria for record selection/filtering and classical guidelines for "science mapping" (Page *et al.*, 2021; Aria & Cuccurullo, 2017; van Eck & Waltman, 2010). Regarding eligibility criteria, inclusion and exclusion concepts were applied. For inclusion, peer-reviewed articles and reviews, proceedings and chapters with scientific committees, and key institutional technical manuals providing theory, methods, or empirical evidence on MIAF/TMV, soil fertility/erosion, productivity, and socioeconomic aspects were considered. For exclusion, non-peer-reviewed outreach notes, documents lacking complete metadata, duplicates, and out-of-scope works were discarded.

Screening and Extraction Process

Screening was carried out in two phases (title-abstract and full text) by two reviewers, with discrepancies resolved by consensus. A PRISMA 2020 diagram is reported, and Cohen's kappa was calculated for inter-rater agreement (Page *et al.*, 2021). For data extraction, the following were considered: year, document type, country/affiliation, funding, journal, keywords (author/indexed), citations, cited references, methods, soil variables (pH, OM, N, P, K, Ca, Mg), productivity/yield metrics, and erosion/runoff indicators when available.

Bibliometric Indicators

Three indicators were considered. The first was production and impact, which included the annual number of publications, total/average citations, and field index. The second was core sources, focusing on the identification of "core" journals through Bradford's law and dispersion zones (Bradford, 1934; Vickery, 1948; contemporary analyses and cautions). The third indicator was collaboration structure, which examined co-authorship networks (authors, institutions, countries) and cluster density.

Scientific Mapping and Topics

This activity included the co-occurrence of keywords (author/indexed) and co-citation of references to identify research schools and fronts (Callon *et al.*, 1991; Cobo *et al.*, 2011). Core, emerging, and declining topics were analyzed (temporal evolution through moving windows). The expected thematic axes considered were: (i) soil conservation/erosion–runoff; (ii) fertility and Cation Exchange Capacity (CEC); (iii) topological design and densities (overstory/mesocrop/understory); (iv) productivity and income; and (v) socio-environmental dimensions and gender. Following the mapping, an in-depth reading was conducted of clusters related to Los Tuxtlas/TMV (erosion control, fertility, productivity) to contextualize why the region demands this study: steep slopes, historical erosion, and the TMV_2MIAF technological trajectory with evidence of reduced soil loss/runoff and improved yields.

On another note, regarding the maps generated with VOSviewer, it is necessary to mention that this is a free, Java-based software program designed to construct, visualize, and explore bibliometric networks. It was created to clearly and precisely represent complex maps of scientific literature using advanced features such as zooming, panning, and intelligent labeling, particularly useful when handling hundreds or thousands of elements. Furthermore, this software uses a technique known as VOS mapping (Visualization of

Similarities), in which the distance between two elements reflects the strength of their relationship: the closer they are, the stronger the relationship (*e.g.*, co-authorship, co-occurrence, citations).

Stage II. Interpretation of Results

Based on the generated information, a theoretical descriptive analysis was conducted on the main theories developed under a systemic approach within the Intercropped Milpa Agroecosystem with Fruit Trees (MIAF).

RESULTS AND DISCUSSION

State-of-the-Art Metrics on Agroecosystems and the MIAF System

When conducting a joint bibliographic search using the terms Agroecosystem and Intercropped Milpa System with Fruit Trees (MIAF), only two scientific articles were found, published in 2021 and 2023. However, when analyzing the terms separately, significant differences were identified in the coverage of the literature. For the term Agroecosystem, a total of 13,142 documents published over the last 10 years (2015-2025) were retrieved, spanning 10 countries, four main document types, and 11 distinct thematic areas (Figure 1). In this context, the high volume of scientific production reflects a growing global interest

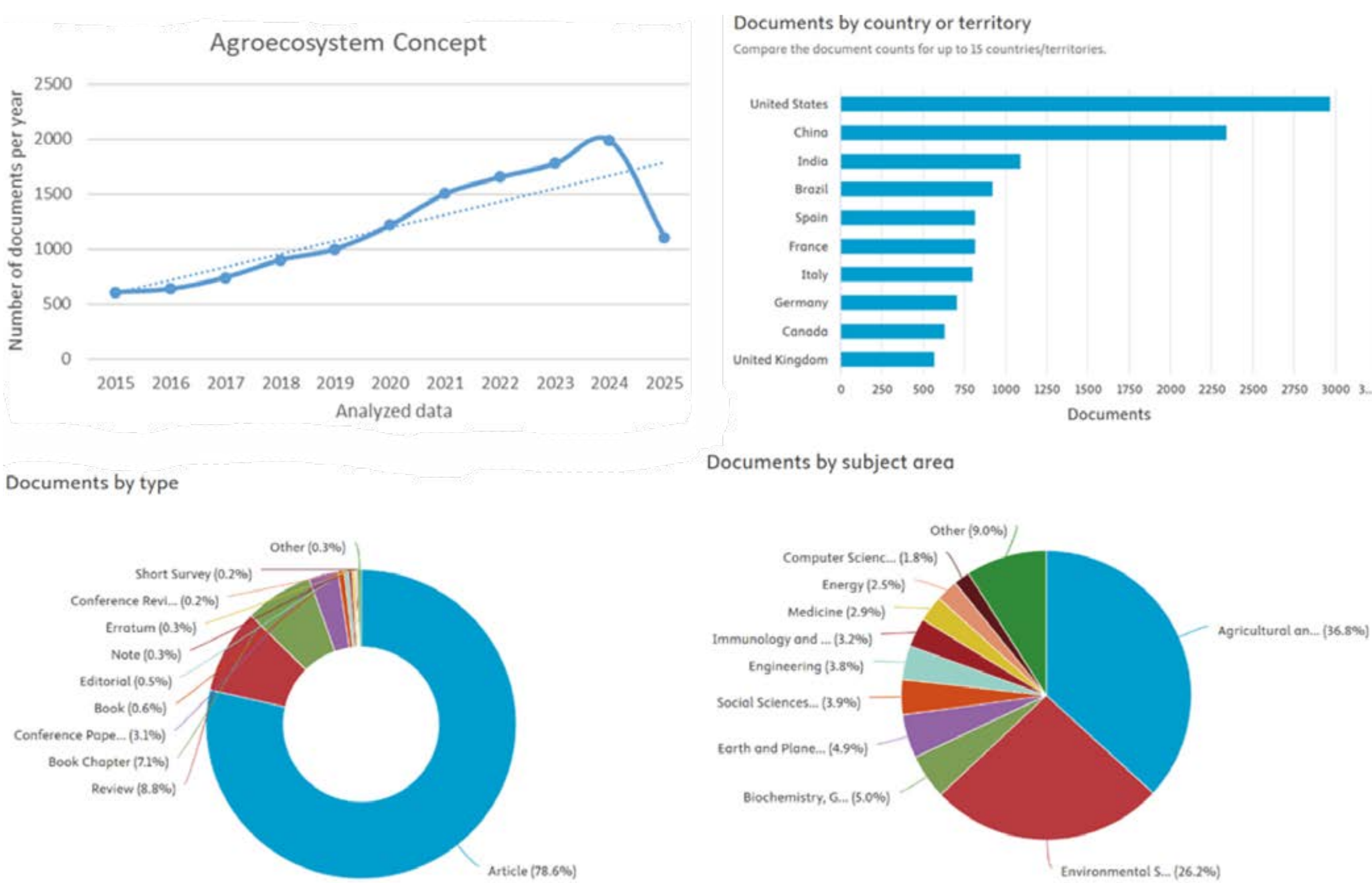

Figure 1. Metrics found on the Scopus platform with the word Agroecosystem.

in agroecosystems, driven by contemporary challenges such as food security, resilience to climate change, and the need for sustainable agricultural systems (Altieri & Nicholls, 2020). The predominance of the United States and China in scientific output aligns with their leadership in agricultural and technological research, as well as with their access to infrastructure, funding, and supportive policies for innovation in the agricultural sector (FAO, 2022).

On the other hand, the concentration of studies within the fields of Agricultural Sciences and Environmental Sciences is consistent with the traditional techno-ecological focus of agroecosystem research. However, a noticeable underrepresentation of disciplines such as Economics, Sociology, and Political Science is observed fields that are essential for addressing the socioeconomic, cultural, and governance dimensions necessary to achieve the comprehensive sustainability of these systems (Gliessman, 2018). This pattern suggests that, although ecological and technical knowledge is well documented, significant gaps remain in understanding the human and social dimensions of agroecosystems. These gaps hinder the development of management strategies that are both effectively sustainable and globally equitable.

Regarding the second keyword, Intercropped Milpa System with Fruit Trees (MIAF), a total of 33 documents published between 2015 and 2025 were identified (Figure 2). It was observed that, since the year 2000, there has been a significant increase in research

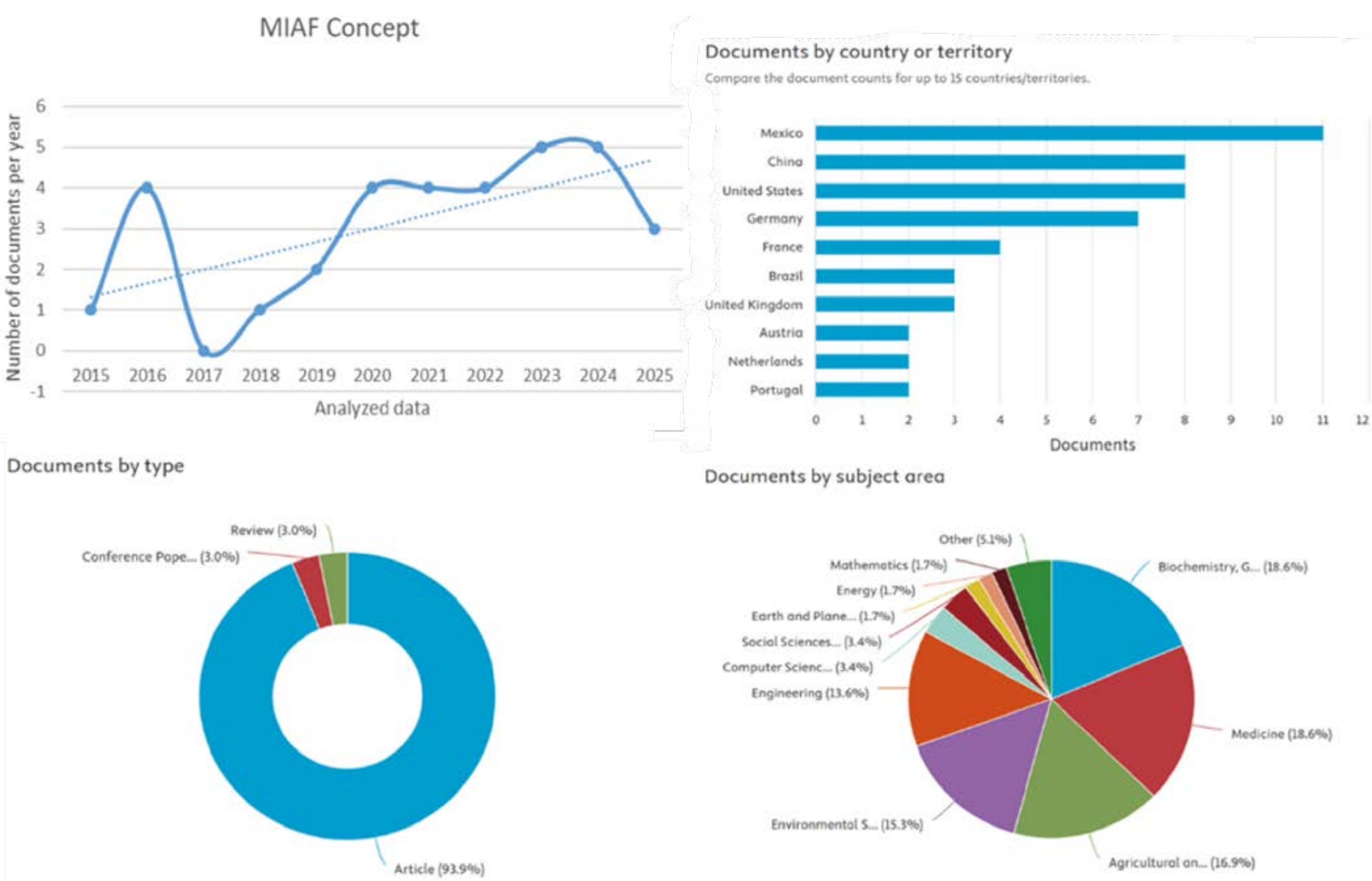

Figure 2. Metrics found on the Scopus platform with the word MIAF.

publications on this topic, with Mexico standing out as the country with the highest scientific output, followed by China, the United States, and Germany. Of the documents reviewed, 93.9% correspond to scientific articles. In terms of thematic distribution, the documents were mainly classified under Agricultural Sciences and Agriculture (16.9%), Biochemistry (18.6%), Engineering (13.6%), and Environmental Sciences (15.3%). Despite the growing recognition of the Intercropped Milpa System with Fruit Trees (MIAF) as a sustainable agricultural strategy in tropical regions, scientific output on the subject remains limited, revealing a research niche that is still underexplored (Hernández *et al.*, 2021). Mexico's predominance in MIAF research aligns with its origin and development as an agricultural technology, primarily implemented and refined within the country (INIFAP, 2017). The thematic distribution of existing studies, which includes areas such as biochemistry and engineering, reflects a multidisciplinary interest in the system. However, a research gap persists in the analysis of socioeconomic dimensionsparticularly concerning system adoption by farmers and its impact on rural development highlighting the need for studies that integrate technical, environmental, and social aspects (Ordoñez-Ovalle *et al.*, 2022).

On the other hand, Figure 3 shows the bibliometric map generated by the VOSviewer software for the concept of Agroecosystems, where the various connections between keywords and the aforementioned concept can be observed. Likewise, Figure 4 presents the bibliometric map based on keywords for the second concept, Intercropped Milpa System with Fruit Trees (MIAF), revealing that most published works are primarily related to the study of fruit trees, soils, and carbon sequestration.

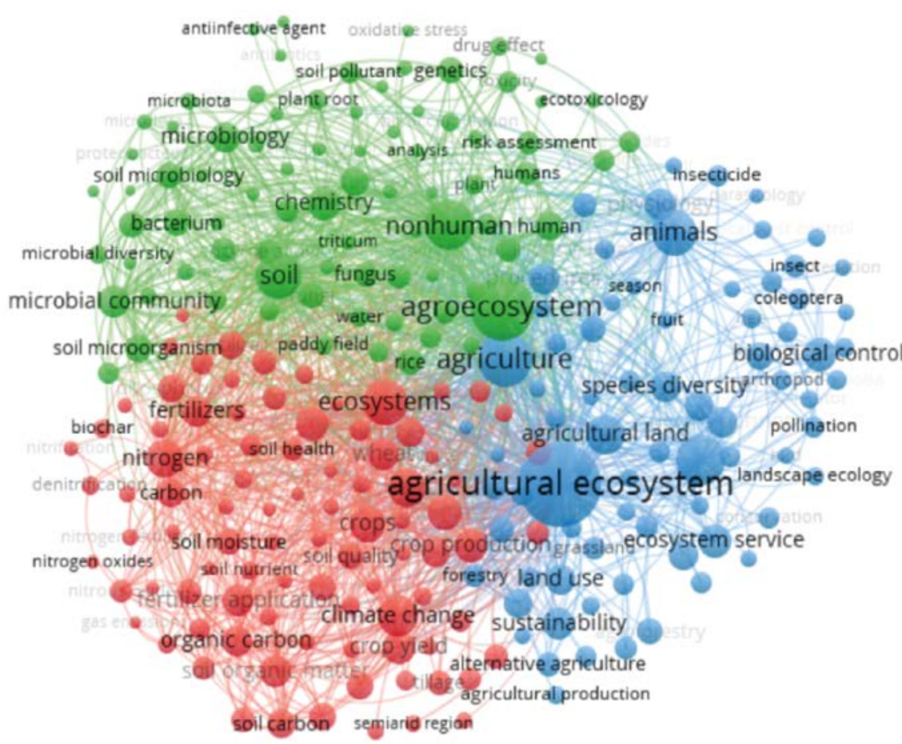

Figure 3. Bibliometric analysis of the Agroecosystem concept generated with the VOSviewer program.

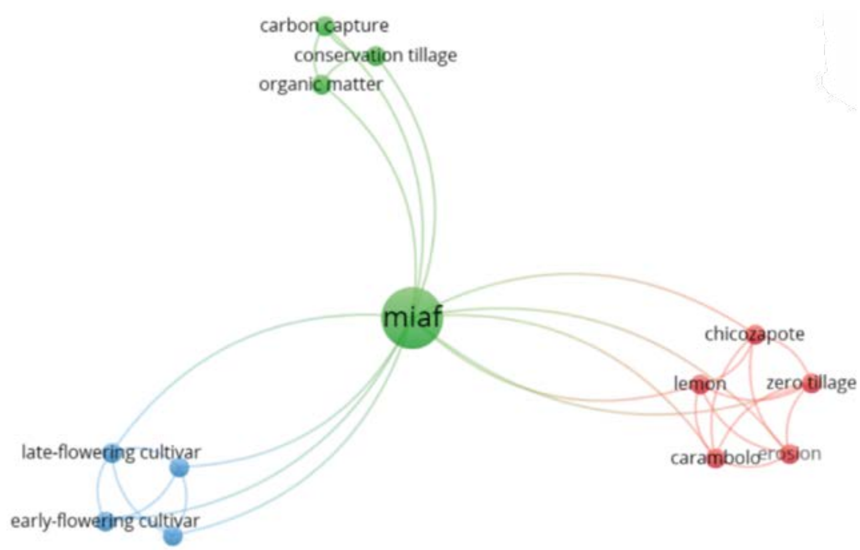

Figure 4. Bibliometric analysis of the concept Milpa Intercalada en Árboles Frutales (MIAF) generated with the VOSviewer program.

In this regard, the bibliometric analysis revealed that, around the concept of agroecosystems, the co-occurrence network is mainly concentrated in ecological dimensions, highlighting a focus on biological productivity, biodiversity, and ecosystem services. However, key terms such as public policies or family farming are notably absent, despite being essential for understanding the practical applicability and social viability of these systems particularly in highly vulnerable rural contexts (Wezel *et al.*, 2020). In the case of the MIAF concept, the predominant association with terms such as carbon sequestration underscores its potential contribution to climate change mitigation and environmental sustainability. Nevertheless, there remains a gap in its connection to socioeconomic categories such as profitability, technological adoption, or gender. The omission of these dimensions limits the construction of a comprehensive vision of the system and reduces the potential to generate public policy proposals that foster its scaling-up (Arriaga-Vázquez *et al.*, 2020). Within this framework, the use of bibliometric tools like VOSviewer constitutes a valuable strategy for identifying trends and patterns in scientific production; however, its scope must be complemented by qualitative and interdisciplinary approaches that allow for contextualizing the findings, interpreting knowledge gaps, and linking academic analysis with the real needs of agricultural systems (Van Eck & Waltman, 2017).

Disciplines and Activities that Must Be Implicit in the MIAF

Given that MIAF is considered a multi-objective system, Table 1 presents the main disciplines and activities that should be taken into account when studying the Intercropped Milpa System with Fruit Trees in Mexico. The integration of agroecological and traditional knowledge within the MIAF system stems from its combination of traditional agricultural practices with agroecological principles, promoting biodiversity, resilience, and sustainability. Its effective implementation depends on the collaboration of various

Table 1. Disciplines and activities to consider when studying the MIAF system in Mexico. Source: Prepared by the authors, August 2025.

No.	Discipline	Activities to be carried out in the MIAF system
1	Edaphology or soil science	• Generate nutritional doses for annual and perennial crops. • Analyze soil flora and fauna. • Conduct field and laboratory studies to analyze the physical, chemical, and biological properties of the soil. • Conduct research on soil formation processes, such as weathering, erosion, and sedimentation. • Evaluate soil fertility and its ability to support plant growth. • Study the impact of human activities on the soil, such as agriculture, deforestation, and pollution. • Teach soil science courses to producers, technicians, officials, and the general public on sustainable soil management practices. • Evaluate soil quality for agriculture, forestry, and construction. • Conduct environmental impact studies for development projects. • Participate in the development of public policies related to soil management, land conservation, and food security. • Promote the adoption of sustainable soil management practices among farmers and other land users. • Communicate the results of their research to the general public through articles, books, conferences, and presentations. • Raise public awareness about the importance of soil for life and the environment. • Promote public understanding of the challenges and opportunities related to sustainable soil management.
2	Agrology or crop science	• Techniques and methodologies for seedbed preparation and cultivation of annual and perennial crops. • Soil fertility management. • Weed control. • Water management. • Soil conservation. • Assessment and monitoring.
3	Agronomy	• Soil preparation (tillage, fertilization, and planting) • Crop management (irrigation, weed control, pest and disease control, pollination) • Harvest and post-harvest • Agricultural extension (technology transfer, training, and technical assistance) • Natural resource management • Plant physiology activities • Soil science activities • Plant breeding activities • Plant genetics activities • Agroecology • Improving annual and perennial cultivation techniques • Ensuring sustainable food production for MIAF families • Environmental protection and the well-being of rural communities
4	Agricultural entomology	• Insect identification and classification • Insect biology and ecology • Assessing damage to annual and perennial crops • Pest management strategies • Protecting MIAF crop yields • Preserving crop quality • Promoting sustainable agriculture
5	Phytopathology	• Disease diagnosis and identification • Pathogen biology and epidemiology • Pathogen-host interactions • Disease management strategies • Protecting crop health • Preserving biodiversity • Promoting sustainable agriculture

Table 1. Continues...

No.	Discipline	Activities to be carried out in the MIAF system
6	Weedology or Weed Science	• Weed biology and ecology • Weed damage • Agroecological weed control methods • Herbicide resistance • Sustainable weed management • Increasing agricultural productivity • Improving crop quality • Environmental protection • Developing new weed control and management strategies
7	Hydrology of water conservation or management	• Rainwater and irrigation monitoring • Development of water management plans • Implementation of strategies to reduce water consumption, improve water use efficiency, and protect water quality. • Educational programs to inform the public about the importance of water, the challenges of water management, and responsible consumption practices. • Awareness campaigns to raise awareness about the value of water, the need to conserve it, and ways to reduce individual and collective consumption. • Promoting the active participation of various stakeholders in water-related decision-making. • Development of efficient technologies to improve water use efficiency in various sectors, such as agriculture, industry, and domestic use. • Implementation of innovative solutions to address water management challenges, such as water reuse and rainwater harvesting.
8	Pomology	• Variety selection and evaluation (Varietal research, varietal trials, and variety recommendations) • Fruit tree propagation (Selection of propagation material, propagation techniques, and rootstock production) • Fruit orchard establishment (Site selection, soil preparation, and tree planting) • Fruit orchard management (Pruning, irrigation, fertilization, weed control, and pest and disease management) • Harvest and postharvest (Ripening, harvesting techniques, and postharvest handling)
9	Agricultural topography or soil conservation engineering	• Land slope analysis • Soil condition assessment • Identification of erosion problems • Design and construction of contour lines • Design and construction of terraces • Design and construction of levees • Monitoring the effectiveness of soil conservation works • Environmental impact assessment • Raising awareness about the importance of soil conservation • Training in soil conservation techniques
10	Post-harvest science and technology	• Maintain the quality of harvested products • Minimize harvest and post-harvest losses • Extend the shelf life of products • Add value to harvested products • Analyze post-harvest physiology • Combat post-harvest diseases and pests • Manage temperature, relative humidity, and packaging of MIAF products • Select the appropriate harvest time • Harvesting techniques • Protect and preserve harvested products • Prevention and treatment • Evaluation and control • Added value

Table 1. Continues...

No.	Discipline	Activities to be carried out in the MIAF system
11	Sociology	• Design and conduct research • Analyze data and interpret results • Teach sociology courses • Develop teaching materials • Supervise graduate students • Provide consulting services to organizations • Conduct social impact studies • Participate in social movements • Develop public policies • Write scholarly articles, as well as books and articles for the general public • Give lectures and presentations
12	Agricultural marketing or agricultural marketing	• Demand analysis • Opportunity identification • Product design • Brand and packaging development • Consumer communication • Demand stimulation • Supply chain management • Distribution channel development • Cost and competition analysis • Pricing strategies • Negotiation with buyers • Customer relationship development • Risk identification and assessment • Implementation of risk management strategies • Sustainable agricultural practices for marketing

disciplines such as agronomy, ecology, anthropology, and economics, among others enabling a comprehensive understanding and optimization of interactions among system components, including crops, fruit trees, and cultural practices. In this regard, the study of MIAF in Mexico represents a paradigmatic example of how interdisciplinary integration can enrich the understanding and improvement of sustainable agricultural systems, as this holistic approach is essential to addressing the contemporary challenges of rural agriculture in Mexico.

Systemic Thinking Theories Focused on the MIAF System

The open systems approach provides a valuable perspective for understanding the functioning of MIAF and its potential for sustainable agriculture, since by recognizing the dynamic interactions between system components and their environment, it becomes possible to design management strategies that promote food production, environmental conservation, and the well-being of rural communities. Moreover, MIAF is also an ancestral agricultural practice that combines the cultivation of maize, beans, and squash with the production of fruit trees, and has been recognized for its capacity to improve soil fertility, increase biodiversity, and provide food security in rural communities (Cortés-Flores *et al.*, 2012).

Table 2 presents the main theories conceptualized from a systems thinking perspective, which allow contextualization of how the Intercropped Milpa System with Fruit Trees (MIAF) can be analyzed as a complex system. This system is composed of various interconnected elements, including:

- Biological components: maize, beans, squash, fruit trees, soil, microorganisms, pollinating insects, and other organisms.
- Abiotic components: water, sunlight, temperature, soil pH, nutrients, and minerals.
- Socioeconomic components: agricultural practices, traditional knowledge, local markets, public policies, and community needs.

Systems thinking provides a theoretical framework for understanding the interaction between these components, evaluating not only the internal functioning of MIAF but also its impact on the environment and rural communities. In this way, these theories enable a comprehensive analysis of MIAF, taking into account its ecological, social, and economic dimensions, and facilitating the design of sustainable and resilient strategies for its management.

Table 2. Theories to consider when studying the Intercropped Milpa System in Fruit Trees (MIAF). Source: Prepared by the authors, August 2025.

Theory	Evidence / Case Studies	Benefits for the MIAF	Concrete examples
Open Systems	Cortés-Flores *et al.*, 2012; Gómez-Méndez *et al.*, 2014; Harvey *et al.*, 2014	It enables the exchange of energy, matter, and information; it promotes sustainability and food security.	Fruit trees fix nitrogen; crops provide organic matter; pollinators facilitate reproduction; interaction with climate and agricultural practices
Self-organization	Altieri, 2009; Gómez-Méndez *et al.*, 2014; Nicholls y Murren, 2010	It generates emerging patterns, resource efficiency, resilience, and adaptation.	Spatial distribution of trees and crops; competition for light and nutrients; feedback between growth and resource availability
Feedback	Capra, 2012; Gunderson y Holling, 2002; Altieri, 2009	It maintains stability, regulates processes, and optimizes productivity and sustainability.	Water and nutrient cycling; biological pest control; soil fertility dynamics; positive and negative loops
Synergy	Harvey *et al.*, 2014; Liniger *et al.*, 2015; Altieri, 2009; Gómez *et al.*, 2014	Maximizes ecological, social, and economic outcomes; improves sustainability, resilience, and multifunctionality	Improved soil fertility; natural pest control; biodiversity conservation; microclimate regulation; increased productivity
Resilience	Altieri y Nicholls, 2000; Jones *et al.*, 2008; Altieri, 2009	Shock absorption capacity, climate change adaptation, and food security	Crop and tree diversity; functional redundancy; feedback and learning; continuous production in the face of drought, flooding, or pests

CONCLUSIONS

This research broadens and contextualizes the concept of Agroecosystem, emphasizing the need to explicitly integrate social, cultural, political, and economic dimensions into its study, as these are essential for understanding productive systems such as MIAF. A total of 12 disciplines and their key activities for its management and sustainability were identified, along with five systemic theories that explain its integrated functioning laying the foundation for the design of public policies, multidisciplinary intervention strategies, and future applied research that prioritizes socio-environmental sustainability and the well-being of rural communities. Despite the methodological limitations inherent in its bibliometric and theoretical-descriptive approach such as dependence on specific databases (Scopus) and the limited volume of direct scientific output on MIAF this study highlights the system's potential as a sustainable alternative to improve soil fertility, productivity, and socio-ecological resilience in regions like Los Tuxtlas. Its practical applications include the design of public policies aimed at soil conservation, agricultural extension, and integrated rural development. However, it is recommended that future research adopt interdisciplinary approaches (with emphasis on socioeconomic and gender dimensions), experimentally evaluate long-term agronomic and environmental impacts, and explore adoption and scaling strategies involving active participation from producers.

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Colegio de Postgraduados

Biostimulants and insecticides for a biorational management of Serrano pepper (*Capsicum annuum* L.) in protected conditions in macrotunnel

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ABSTRACT

Objective: to evaluate the effect of two biostimulants and two insecticides on the biorational management of Serrano pepper cultivation under protected macrotunnel conditions.

Design/Methodology/Approach: three biostimulant treatments were evaluated 1: T22® (*Trichoderma harzianum* strain T22); 2: Mix® (*Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma asperellum*, *Trichoderma koningii*); and 3: control (tap water), applied at doses of 0.5% (w/v). In addition, three insecticide treatments were evaluated 1: Movento® (Spirotetramat) at a dose of 250 mL ha^{-1}; 2: the bioinsecticide *Isaria javanica* strain 304 at a dose 1 L ha^{-1}, and 3: control (tap water). The experimental design was a factorial of treatments in randomized complete blocks. The response variables for the biostimulants were weight, equatorial diameter and polar diameter of the fruit, as well as the total weight of 20 fruits. For insecticides, a single application of each product was made. An aphid sampling was made before insecticides application, then four more afterwards at 3, 5, 7 and 14 days after application (daa).

Results: results demonstrated a greater potential of the biostimulant formulated with *T. harzianum* strain T22 to increase the weight and size of Serrano pepper fruits. Also, the insecticide based on spirotetramat was more effective for aphid control; whereas the fungus *I. javanica* strain 304 was efficient at 7 and 14 daa, with mortality percentages less than 60%.

Findings/Conclusions: the interactions of biostimulants and insecticides had beneficial effects on weight and size of Serrano pepper fruits, as well as on aphid pest control.

Keywords: vegetables, *Trichoderma harzianum*, spirotetramat, *Isaria javanica*, aphids.

INTRODUCTION

In Mexico, vegetables compose approximately 20% of national agriculture production. Among vegetables, green peppers account for the highest production 3 069 865 tons (Megagrams, Mg), of which Serrano pepper contributes with 348 197 Mg. Those

Mexican states with the highest Serrano pepper production are Sinaloa, San Luis Potosí, Michoacán, Veracruz and Guanajuato (SIAP, 2023). The Serrano pepper is an important crop in Mexico as it is consumed fresh; also, it is a source of economic resources through labor in farms. In addition, its cultivation generates foreign exchange in the agrifood exports sector (Ramírez-Seañez *et al.*, 2023). Most of this pepper is grown on lands in the open and very little under protected conditions. This generates a considerable demand for inputs, such as fertilizers and commercial insecticides (Barrón-Contreras *et al.*, 2022; Díaz-José *et al.*, 2023).

Currently, production systems that depend heavily on chemical inputs are characterized by increasing production costs and contributing to the problems of environmental pollution and decreased soil fertility (Silveira-Gramont *et al.*, 2018; Esquivel-Valenzuela *et al.*, 2019) (Galindo *et al.*, 2020; Guerrero & Guevara, 2021). Therefore, production systems nowadays should consist of ecological or bio-rational approaches, which reduce the excessive use of conventional fertilizers and insecticides, while increasing efficiency in plant nutrition and plant protection against pests. One of these strategies is the use of plant biostimulants and biological or new generation insecticides, which are used in the biorational management of crops (Murillo-Cuevas *et al.*, 2020).

The term biorational refers to certain types of components, mostly biological. But, if they are synthetic, then should be structurally similar and functionally identical to those that are biological. These have advantages in terms of risks of contamination or pest resistance, while offering acceptable efficiency in pest control or plant nutrition (Murillo-Cuevas *et al.*, 2023). Biorational products include microorganisms, plant extracts, basic substances, as well as non-pesticide products such as bio-stimulants, biological performance enhancers, plant health promoters and soil conditioners (Matyjaszczyk, 2018). Their use in plant production varies, some act directly against pests, are highly selective and their efficiency is similar to that of chemical products, while others act mainly on plants, increasing their tolerance to various biotic and abiotic factors (Shankar y Tripathi, 2021).

Biostimulants formulated with beneficial microorganisms, such as fungi and bacteria promoting plant growth, act on the solubilization of minerals and nitrogen fixation, as well as through physiological and genetic mechanisms that stimulate the production of phytohormones, enzymes, lipopolysaccharides and organic compounds (Caulier *et al.*, 2019; Lephatsi *et al.*, 2021). In addition, these biostimulants improve tolerance to biotic and abiotic stresses, as they activate genes in plant defense system to produce phenols, enzymes, amino acids, and organic acids (Backer *et al.*, 2018; Vocciante *et al.*, 2022). Fungi of the genus *Trichoderma* increase the height, stem diameter, aboveground biomass, and root volume of habanero pepper plants; as well as yield in fruit number and fruit weight (Cristóbal-Alejo *et al.*, 2021). Likewise, formulations of *Bacillus* bacteria increase weight and size of habanero pepper fruits (Adame-García *et al.*, 2021; Mejía-Bautista *et al.*, 2022).

Bioinsecticides are products developed from entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*, among others, which are used in pest management strategies, since they are environmentally compatible. These products offer economic benefits to farmers, while contributing to the protection of the environment and consumer health (Pacheco *et al.*, 2019). In artisanal productions in rural communities, it is reported that bioinsecticides control pests in lettuce, onion, cabbage, potato, and coriander crops, causing mortalities of more than 80% within 72 hours, compared to control (González-Maldonado and García-Gutierrez, 2012). Compatibility and efficiency of *B. bassiana* and *M. anisopliae* in the control of whiteflies in lettuce is also demonstrated, in addition to the fact that *B. bassiana* and *M. anisopliae* independently control aphids in cabbage leaves (Silva *et al.*, 2020).

Likewise, within the biorational management of pests, state-of-the-art insecticides formulated with active ingredients belonging to new chemical families, such as Spirotetramat and Spiromesifen, are included. These compounds have a low incidence of cross-resistance with conventional insecticides, so they are recommended as part of strategies to prevent the development of resistance in pest populations (Cortez-Mondaca *et al.*, 2018; Díaz *et al.*, 2019).

The objective of this study was to evaluate the effect of two biostimulants and two insecticides on the biorational management of Serrano pepper cultivation under protected macrotunnel conditions.

MATERIALS AND METHODS

This research was established in the vegetable production area of the Instituto Tecnológico de Úrsulo Galván, located in the municipality of Úrsulo Galván, in the central coastal region of the state of Veracruz, Mexico (19° 24’ 43.12” N; 96° 21’ 32.66” W). The experiment was implemented in a 3×30 m macrotunnel greenhouse (90 m^2), covered with an anti-aphid mesh. A drip irrigation system was used and two seedbeds with white and black plastic mulching were established. Plant arrangement consisted of one plant every 25 cm in staggered (triangular) planting, which allowed a total of 120 plants per bed and 240 per macrotunnel. During the crop cycle, an average temperature of 28.4 °C and an average relative humidity of 73.5% were recorded.

Serrano pepper seed (*Capsicum annuum* L.), variety chister-522 was used, which was provided by INIFAP Campo Experimental Cotaxtla. For seedling production, seeds were previously inoculated with mycorrhiza (*Rhizophagus intraradices*) supplied by INIFAP, then sown in polyethylene trays. The seedlings were transplanted to a soil with the following characteristics pH 6.81, electrical conductivity (EC) 81.3 μS, bulk density (DAP) 1.3 g mL^{-1}, sand content 31.64%, silt 31.16%, clay 37.98%, organic matter (M.O.) 3.11%, potassium (K) 0.18 Cmol kg^{-1}, calcium (Ca) 12.04 Cmol kg^{-1}, magnesium (mg) 3.83 Cmol kg^{-1}, total nitrogen (N) 0.11% and available phosphorus (P) 11.7 mg L^{-1}.

The culture management consisted of the application of humic acids (12% humic and 12% fulvic) 15 days after transplantation. Subsequently, application was made every 30 days until the end of the production cycle. A conventional chemical fertilization (100: 60: 60, N: P: K) was applied during the complete production cycle (at 20, 50, 90 and 120 ddt). Also, every 20 days, foliar applications of micronutrients were made with commercial products (13% N, 9% P_2O_5, 12% K_2O, 0.17% Ca, 0.05% Cu, 0.01% Co, 0.07% B, 0.04% S, 0.08% Fe, 0.5% Mn, 0.18% Mg, 0.03% Mo, 0.20% Zn; diluents and conditioners, 64.31%). In addition, at the beginning of flowering and every 20 days afterwards, foliar applications of a commercial product with calcium and boron were made (9% Ca, 2% B, 4% Zn; diluents and conditioners, 85%).

Three treatments were evaluated 1: T22® (*Trichoderma harzianum* strain T22); 2: Mix® (*T. harzianum*, *T. viride*, *T. asperellum*, *T. koningii*); and 3: control (tap water), applied at doses of 0.5% (w/ v), as biostimulants. Also, other three treatments 1: Movento®, at a dose of 250 mL ha^{-1}; 2: the bioinsecticide *Isaria javanica* strain 304® at a dose of 1L ha^{-1}, and 3: control (tap water) were evaluated as insecticides.

Biostimulants were applied monthly to the soil using the drench technique. The solution was directed to the neck of the plant, from the moment of transplantation. For evaluation, fruits were collected in two harvest cuts, at 113 and 123 ddt. In each treatment and replicate, 20 fruits per plant were randomly selected to measure the response variables, individual fruit weight (g), equatorial diameter (cm), polar diameter (cm) of the fruit, and total weight of 20 fruits (g).

The evaluation of insecticides was done at 63 ddt, when the presence of the pest was detected *Myzus persicae* (Hemiptera: Aphididae), commonly known as green aphid, which has spread throughout the crop. A single dose of each insecticide was applied using a 20 L electric sprayer (HYUNDAI®). To avoid cross-contamination between treatments, 2 m high screens between experimental blocks were used as physical barriers. Aphid population monitoring was done before insecticide application and subsequently at 3, 5, 7 and 14 days after application (daa). In each experimental block, the two central plants were sampled, and two leaves per plant were selected (one in the upper part and the other in the middle part). The total number of live aphids was counted on each leaf, recording treatment and repetition, on each evaluation date.

A factorial design was used in randomized complete blocks. In each bed of the macrotunnel, six large blocks of 18 plants were established to distribute the biostimulant treatments, obtaining two replications per bed. Within each large block, insecticide treatments were distributed in small blocks of six plants per treatment, obtaining six replicates per seedbed.

The normality of the data was assessed with the Shapiro-Wilk goodness-of-fit test. Data were transformed to $\sqrt{+0.5}$ to be normalized in order to perform an analysis of variance with interaction. In the cases where significant differences were identified ($p \leq 0.05$), a Tukey mean comparison test was applied with the original means of the data. The InfoStat© version 2020 software was used for data analysis.

RESULTS AND DISCUSSION

The biostimulants applied had a positive effect on the weight and dimensions of the Serrano pepper fruit in the two harvest cuts (Table 1). In the first harvest, both treatments with biostimulants significantly increased ($p=0.0001$) the average weight of the fruit compared to the control. However, the size of the fruit (polar diameter and equatorial diameter) and the total weight of 20 fruits showed a significant increase ($p=0.0001$) only in the treatment with the T22® biostimulant. In particular, the T22® biostimulant increased the average fruit weight by 36% compared to the control fruits, while the Mix® biostimulant achieved an increase equivalent to 6.8% (Table 1).

Table 1. Effect of biostimulants on Serrano pepper fruits produced in protected macrotunnel conditions.

Treatments	Weight (g)	Eq. D (cm)	Polar D. (cm)	Weight of 20 fruits (g)
Cutting 1				
T22®	2.36±0.02[a]	0.80±0.01[a]	5.42±0.07[a]	46.50±0.80[a]
Mix®	1.62±0.03[b]	0.65±0.01[b]	4.98±0.09[b]	34.04±1.00[b]
Control	1.51±0.04[c]	0.66±0.02[b]	4.98±0.11[b]	35.44±0.96[b]
C.V. (%)	14.56	6.89	12.63	8.14
Cutting 2				
T22®	2.04±0.02[a]	0.72±0.01[a]	5.47±0.07[a]	39.63±0.80[a]
Mix®	1.97±0.03[b]	0.72±0.02[a]	5.41±0.06[a]	38.34±0.87[a]
Control	1.78±0.02[c]	0.68±0.01[b]	5.18±0.07[b]	32.28±0.95[b]
C.V. (%)	14.83	7.73	11.74	8.78

D: diameter. Means with common letters are not significantly different ($p>0.05$). Values are presented in mean plus standard error, $\overline{X}$±S.E.

T22® increased the equatorial diameter and polar diameter of the fruits by 17.5% and 8.1% respectively, in addition to increasing the total average weight of a sample of 20 fruits by 23.8% compared to fruits in the control. In the second harvest, with the applications of the two biostimulants, Serrano pepper fruits were obtained on average significantly larger ($p=0.0061$) and heavier ($p=0.0001$) compared to the fruits of plants in the control. With the T22® biostimulant, the average fruit weight increased by 12.7% and with Mix® by 9.6%. In addition, the two products increased the equatorial diameter of the fruits by 5.6%, however for polar diameter, the application of T22® increased it by 5.3%, and with Mix® by 4.3%. Regarding the average weight of the sample of 20 fruits, the applications of T22® increased it by 18.5% and with Mix® by 15.8%, compared to fruits of the plants in the control (Table 1).

The interactions of biostimulants with insecticides improved the weight and size of Serrano pepper fruits. In the first harvest of fruits, significant effects were obtained on the weight ($p=0.0001$), equatorial diameter ($p=0.0027$) and polar diameter ($p=0.0109$) of fruits. With the applications of the T22® biostimulant, to improve plant nutrition and the chemical insecticide Movento® or the biological one *Isaria javanica* for the control of aphids, wider and heavier Serrano pepper fruits were obtained compared to the fruits obtained from control plants or with applications of the biostimulant Mix® (Figure 1A and Figure 1B). Regarding fruit length, only the fruits of plants with applications of the T22® and Movento® products were significantly different from the fruits of the control plants (Figure 1C).

In the second harvest, differences were also recorded among the interactions of the treatments in weight ($p=0.0001$), equatorial diameter ($p=0.0001$) and polar diameter ($p=0.0461$) of the fruits. As in the first fruit harvest, the plants treated with T22® and the insecticides *I. javanica* and Movento® produced wider and heavier Serrano pepper fruits than those in the control plants (Figure 1D and Figure1E). The length of the fruit was improved by the application of the bio-stimulant Mix® and insecticide Movento®

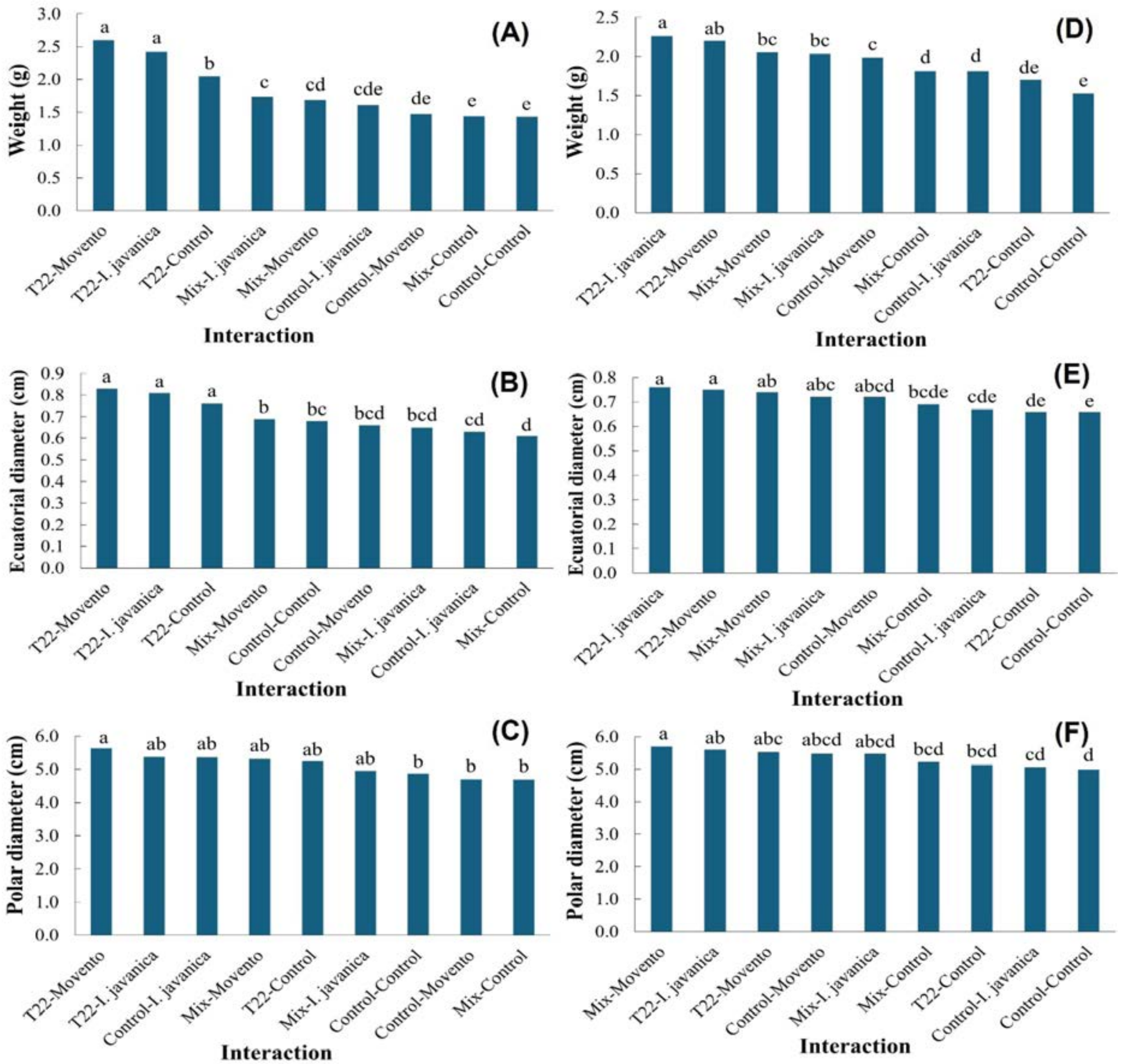

Figure 1. Effect of biostimulant × insecticide interactions on the mean weight (g), equatorial diameter (cm) and polar diameter (cm) in two harvest cuts of Serrano pepper fruits produced under macrotunnel protected conditions. A, B, C: harvest 1 and D, E, F: harvest 2. Means with different letters indicate statistical difference ($p \leq 0.05$).

compared to those interactions of treatments with the control, except control × Movento® (Figure 1F).

Regarding the average weight of samples of 20 fruits, in the first harvest, the interaction of the applications, for aphid control, of T22® plus Movento® or *I. javanica* produced on average fruits of greater weight ($p=0.0221$) compared to all other interactions (Figure 2A). In the second harvest, with the interactions of the applications of T22® plus *I. javanica*, and Mix® plus Movento® or *I. javanica*, it was possible to obtain samples of 20 fruits with significant and greater average weight ($p=0.0103$) than the fruit samples obtained with those interactions from control plants (Figure 2B).

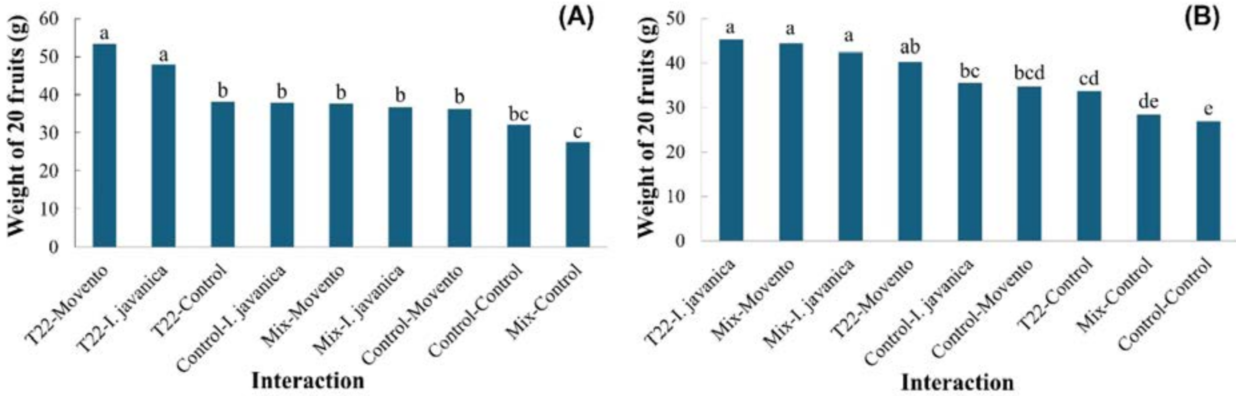

Figure 2. Effect of the biostimulant × insecticide interaction on the mean weight (g) of samples of 20 Serrano pepper fruits in two harvests produced in protected macrotunnel conditions. A: harvest 1 and B: harvest 2. Means with different letters indicate statistical difference ($p \leq 0.05$).

The biostimulants T22® and Mix® showed significant effects in promoting the growth of Serrano pepper fruits compared to the fruits of plants in the control. Similar results are reported in other horticultural crops such as habanero peppers, bell peppers, tomatoes and eggplants, although with variations in efficiency depending on the product and the crop evaluated. For example, the biostimulant T22® showed a greater increase in the weight of habanero pepper and eggplant fruits, while the Mix® product was more effective in bell peppers and tomatoes. In percentage terms, the largest increases were recorded in eggplant (T22®=27.4% and Mix®=17.8%) and habanero pepper (T22®=18.8% and Mix®=17.9%), followed by bell pepper (T22®=10.5% and Mix®=11.4%) and tomato (T22®=2.7% y Mix®=6.9%) (Adame-García *et al.*, 2023; Murillo-Cuevas *et al.*, 2021; 2023).

Compared to the crops reported in other studies, the biostimulant T22® showed a higher increase in the weight of Serrano pepper fruits in the first harvest, and also exceeded the results obtained in tomatoes and bell peppers during the second harvest. These differences could be attributed to the different levels of compatibility between *Trichoderma* spp. strains and host plant species. The efficiency of microbial biostimulants depends largely on the physiological and biochemical compatibility of plant-microorganism interactions, which implies genetic recognition between the two (Cano, 2011).

On the other hand, significant differences were also observed between the effects of the two biostimulants evaluated, particularly in the first harvest. This may be due to the specific formulation of each product: T22® contains a unique strain of *Trichoderma harzianum* (T22 strain), while Mix® is composed of a mixture of several species (*T. harzianum*, *T. viride*, *T. asperellum* and *T. koningii*), which implies a possible variability in the production of bioactive compounds such as auxins, organic acids, or in the solubilization mechanisms of inorganic phosphate, which influence the promotion of growth. This variability in effects between strains was confirmed by Larios *et al.* (2019), who evaluated the efficiency of the co-application of two native strains of *Trichoderma* sp. (SP6 and Clombta), and a commercial product (Tri-HB® with *T. harzianum* and *Bacillus subtilis*) on *Capsicum chinense* var. *Chichen Itza*. Those authors reported that plants treated with the Clombta strain presented better results in height (11 cm), stem diameter (2.6 mm), aboveground biomass (0.8 g fresh and 0.13 g dry), and root volume (0.13 g fresh and 0.4 g dry) than other treatments at 28 days after germination.

The benefits of fungi of the *Trichoderma* genus are related to their ability to control fungal pathogens, as well as a plant growth-promoting effect, by improving nutrition and the development of plants and fruits (Ruiz-Cisneros *et al.*, 2018; Elshahawy & El-Mohamedy, 2019). These effects are due, in part, to the production of phytohormones, peptides, volatile compounds, and secondary metabolites that stimulate root growth and increase nutrient absorption capacity, resulting in increased plant vigor and improvements in plant productivity (Shahnaz *et al.*, 2022).

The results obtained confirm that the T22® and Mix® biostimulants formulated with *Trichoderma* spp. strains have a significant effect on the development of Serrano pepper fruits. The incorporation of these products into biorational management programs can contribute to reduce the excessive use of chemical fertilizers or to make a reduced application more efficient, thus favoring more sustainable production. Espinoza *et al.* (2019) reported positive interactions between *Trichoderma* spp. and Serrano pepper varieties, attributed to the complex chemical activity of volatile secondary metabolites, phytohormones, and antibiotics released into the rhizosphere, which favored plant development, by improving nutrient absorption and increasing phytopathogen control.

Before applying the insecticides (day 0) the average number of live aphids per plant ranged from 21.77 to 23.29, and were statistically equal ($p>0.05$) among the established treatments. At 3 daa, significant differences ($p=0.0009$) were obtained in the average number of aphids per plant. Plants treated with the insecticide Movento® only had an average of 3.69 aphids, which is equivalent to an average reduction of 21.8 aphids (85.5%). Unlike the plants treated with the bioinsecticide *I. javanica*, which had an average reduction of 6.2 aphids (24.4%), which was non-significant compared to the control plants (Table 2).

At 5 daa, plants treated with the insecticides, both chemical and biological, had a significant lower average in the number of aphids ($p=0.0001$) than the control plants (Table 2). With the insecticide Movento® an average reduction of 26.4 aphids (94.3%) was obtained, and with *I. javanica* of 10.7 aphids (38.4%) less than control. At 7 and 14 daa, the reduction of aphids also was significant ($p=0.0001$) compared to control plants (Table 2). The insecticide Movento® maintained the average reductions at 23.5 (95.7%) and 13.6

Table 2. Effect of insecticides on the average number of aphids per plant present in the Serrano pepper crop at 3, 5, 7 and 14 daa under macrotunnel protected conditions.

Treatments	Days after application (DAA)			
	3	5	7	14
Movento®	3.69 ± 1.15^{a}	1.58 ± 0.97^{a}	1.06 ± 0.67^{a}	1.04 ± 0.67^{a}
Isaria javanica	19.25 ± 1.16^{b}	17.21 ± 0.97^{b}	10.69 ± 0.67^{b}	6.15 ± 0.64^{b}
Control	25.46 ± 1.15^{b}	27.94 ± 0.97^{c}	24.52 ± 0.68^{c}	14.68 ± 0.64^{c}
C.V. (%)	15.44	14.98	15.37	15.46

Means with different letters indicate statistical difference ($p\leq0.05$). Values show mean plus standard error, $\bar{X}\pm$S.E.

(92.9%) less aphids than control at 7 and 14 daa. Whereas the bioinsecticide *I. javanica* decreased the average number of aphids by 13.8 (56.4%) and 8.5 (58.1%) at 7 and 14 daa.

In the interaction of insecticides with biostimulants, there were significant differences ($p \leq 0.05$) at 3, 5, and 7 daa (Figure 3). In addition, some interactions of biostimulants without insecticides were statistically different from the zero control in relation to a lower number of aphids per plant (Figure 3). At 3 daa, interactions with Movento® were those that registered on average a lower number of live aphids; these interactions were statistically equal to each other, with or without the addition of biostimulants (Figure 3A). At 5 daa, the Movento® interactions with or without biostimulants further reduced the average number of live aphids per plant, remaining non-significant with each other.

The interaction of *I. javanica* plus the biostimulant T22® significantly decreased the average number of live aphids compared to the other interactions with I. javanica and the zero control (Figure 3B). At 7 and 14 daa, interactions with Movento® continued to be those that presented on average the lowest number of live aphids. However, the interaction of I. javanica plus the biostimulant T22® turned out to be statistically equal to the interactions of Movento® insecticide, with an average of live aphids lower than those obtained from plants in the control (Figure 3C and Figure 3D).

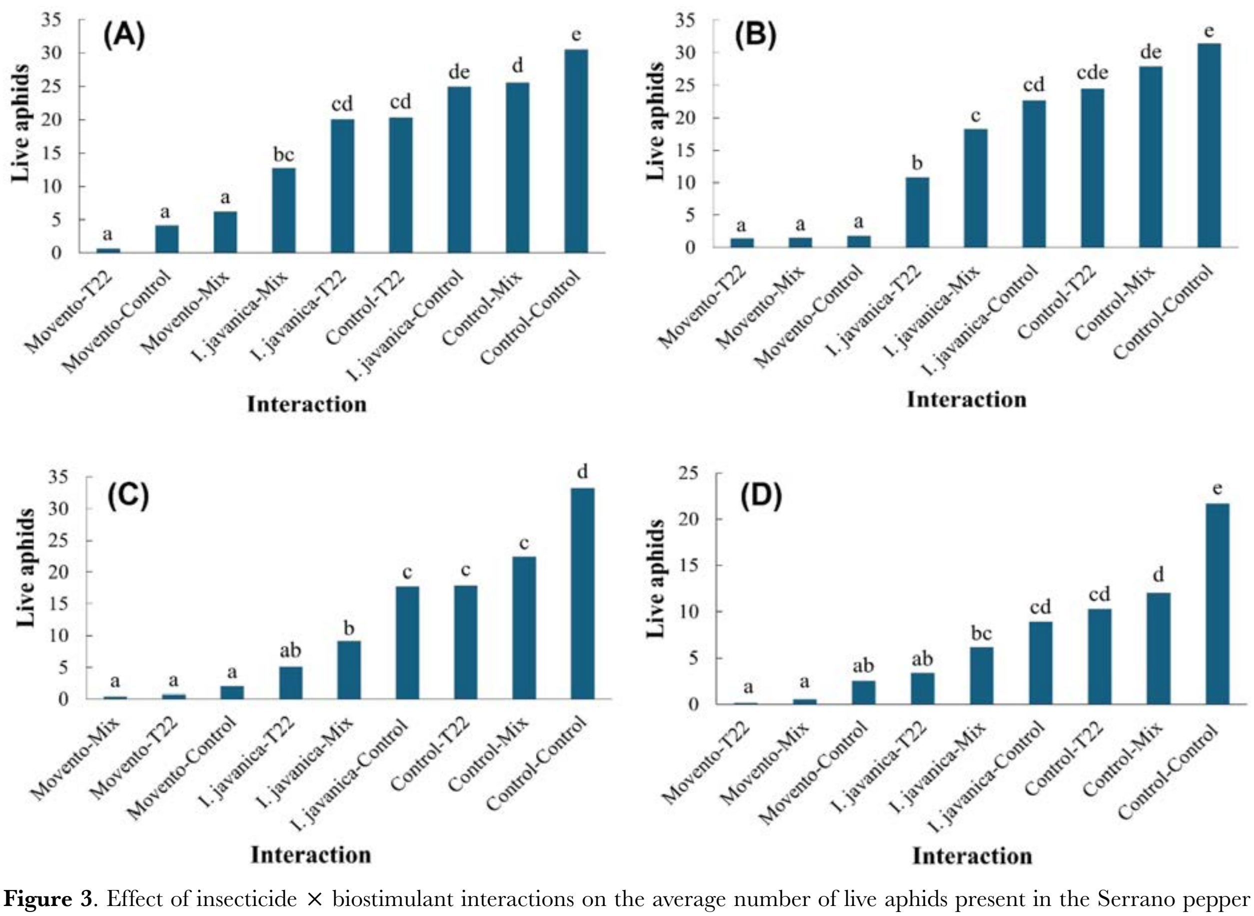

Figure 3. Effect of insecticide × biostimulant interactions on the average number of live aphids present in the Serrano pepper crop under protected macrotunnel conditions. A: 3 daa, B: 5 daa, C: 7 daa, and D: 14 daa. Means with different letters indicate statistical difference ($p \leq 0.05$).

The results obtained corroborate the high efficiency of the insecticide Movento® in the control of green aphids, which is attributed to its active ingredient, spirotetramat, an inhibitor of lipid biosynthesis that mainly affects the juvenile stages of the insect and reduces adult fecundity (Gong *et al.*, 2016). After foliar application, spirotetramatenol, with bimodal allocation through xylem and phloem, reaching both growing shoots and roots. This bidirectional systemic activity ensures the control of hidden sucking pests and protects new shoots (Gong *et al.*, 2016).

The high efficiency of spirotetramat has already been previously documented. Cortez *et al.* (2018) reported 91% and 100% mortality of the yellow sorghum aphid (*Melanaphis sacchari*) at 72 hours, and 120 hours after application. Similarly, Díaz *et al.* (2019) indicated a biological effectiveness of 89.33% in the control of *Aphis gossypii*, the aphid in pumpkin crops, when using the dose recommended by the manufacturer. Recently Murillo-Cuevas *et al.* (2023) recorded a 93.3% reduction of whitefly populations in eggplant crops when spirotetramat was used. These data coincide with our results in this study; we recorded mortalities of 85.5%, 94.3%, 95.7% and 92.9% at 3, 5, 7 and 14 daa respectively, confirming the high efficiency of spiroteramat against *Myzus persicae* in Serrano peppers.

On the other hand, the entomopathogenic fungus *I. javanica* strain 304 showed an important effect on the control of the aphid *M. persicae* in the Serrano pepper crop under macrotunnel protected conditions, since mortalities of 56.4% and 58.1% were reached at 7 and 14 daa. These results are comparable to those reported by Spinel *et al.* (2009) for *Isaria fumosorosea* in the control of whitefly nymphs *Bemisia tabaci* under laboratory conditions, as well as by Mweke *et al.* (2018), who reported, by an Isaria species, a mortality of 64.2% of *Aphis craccivora* in the legume *Vigna unguiculata*. However, in this latter study, it was also observed that *Isaria* caused lower mortality than the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*, with respective efficiencies of 74.7% and 90%.

Under field conditions, it has also been reported that the fungus *I. fumosorosea* presented a lower efficiency compared to *B. bassiana* and *M. anisopliae* in the control of whiteflies in tomato and zucchini crops grown on lands in the open (Murillo-Cuevas *et al.*, 2020). Similarly, in the cultivation of eggplant, *I. javanica* reduced the whitefly population by 75.6%, in contrast to 83.8% achieved with *B. bassiana* (Murillo-Cuevas *et al.*, 2023). These results suggest that, although *I. javanica* has biocontrol capacity, its efficiency may be lower than that of other entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae*.

CONCLUSIONS

The biostimulant formulated with *Trichoderma harzianum* strain T22 presented the greatest potential to a significant improvement of the size and weight of Serrano pepper fruits in a protected agricultural production. The insecticide based on spirotetramat was the one that best controlled the aphid *Myzus persicae* with mortalities greater than 85% and a persistence of up to 14 daa in the Serrano pepper crop in protected production system. These products based on *T. harzianum* strain T22 and spirotetramat can be integrated into a Serrano pepper production scheme with biorational management in protected macrotunnel conditions, since they offer relevant agronomic benefits with a low environmental impact.

The entomopathogenic fungus *Isaria javanica* strain 340 only showed a medium efficiency, reaching mortality percentages close to 60% at 7 and 14 daa. However, its inclusion in integrated pest management strategies in Serrano peppers can contribute to reduce dependence on synthetic insecticides in production systems with biorational management. The interactions between biostimulants and insecticides had positive effects on both fruit development and the evaluated pest control. This emphasizes the potential of their combined use in Serrano pepper production systems with biorational management in protected agriculture conditions.

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Competitiveness in organic agricultural micro-enterprises in the municipalities of La Paz and Los Cabos, Baja California Sur

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ABSTRACT

Objective: to evaluate the level of competitiveness in agricultural micro-enterprises under organic production systems operating in the municipalities of La Paz and Los Cabos, Baja California Sur, Mexico.

Design/Methodology/Approach: through a non-probabilistic sampling technique of an intentional type, socioeconomic information and factors associated with competitiveness were collected in eight micro-enterprises. The level of competitiveness was determined using specific items, implementing the Likert scale in the valuation. Micro-enterprises were characterized and classified into groups through the statistical techniques of principal components analysis and cluster analysis.

Results: owners or representatives of the micro-enterprises averaged 43.8 years of age, 12.9 years of education, and 8.9 years of seniority in the activity, while the main crops produced were vegetables. Micro-enterprises varied in their competitiveness; it is necessary to strengthen aspects such as value chain, innovation management, associativity, and the implementation of public policies for agriculture. The multivariate analysis formed four groups due to their similarity in relation to the level of competitiveness.

Limitations/Implications of the study: results showed the level of competitiveness of the participating micro-enterprises and not of the total population of micro-enterprises. This is due to the lack of an official micro-enterprise registry, and to the reluctant attitude of some owners or representatives to provide information, thus sample size was limited.

Findings/Conclusions: this research was very useful in the identification of areas of opportunity and the definition of strategies intending to increase the competitiveness of those micro-enterprises dedicated to organic agriculture.

Keywords: competitiveness evaluation, Likert scale, competitiveness factors, multivariate analysis, organic agriculture.

INTRODUCTION

Competitiveness allows a company to generate more income than the competition, this through maintaining comparative advantages, in a local or external socioeconomic environment (Saavedra, 2012). In Mexico, micro-enterprises have had a high incidence of

closures due to lack of competitiveness (Castro, 2018; Patino-Galván & Hernández, 2023). This is worrying, since in Mexico there are 4.7 million companies and 95% are classified as micro-enterprises, which generate 10 million jobs and produce an estimate 14.2% of the gross revenue of the sector (INEGI, 2019).

Despite the importance of micro-enterprises, there is a lack of theoretical and empirical studies that allow us to know their level of competitiveness, and thus design strategies that strengthen them (Kotenko *et al.*, 2021). The aforementioned situation is further complicated by the difficulty of public access to financial, accounting, and operational information of micro-enterprises (Allende *et al.*, 2020). In addition, given the lower level of resources available, it is difficult to collect, organize, and analyze information for evaluation and decision-making (Otero & Taddei, 2018). Likewise, it is necessary to understand that the competitiveness of a micro-enterprise is associated with specific cultural and economic contexts. Hence the relevance of developing particular studies, since implementing general models to improve competitiveness could lead more quickly to failure.

Competitiveness is usually evaluated through the application of specific performance perception surveys (Otero & Taddei, 2018) and using the Likert scale, *i.e.*, psychometric instruments where the respondent must indicate their agreement or disagreement on a statement (Matas, 2018). Leal *et al.* (2021) evaluated the factors that influenced the competitiveness of agricultural companies, and obtained results on managers' perception of product quality, compliance with certifications, and innovation capacity. On the other hand, Acosta *et al.* (2020) used specific questionnaires and the Likert scale to assess the factors that affected the competitiveness of agricultural firms, and identified areas of opportunity in innovation management, use of competitive advantages, associativity, implementation of agricultural public policies, as well as in the participation of irrigation districts.

Micro-enterprises dedicated to organic agriculture in Baja California Sur are relevant for their contribution to human health, the conservation of natural resources, and the economy of the state (Villa & Bracamonte, 2013). However, there is a lack of information on various aspects (Iglesias *et al.*, 2021), including the level of competitiveness. Therefore, the objective of this research was to evaluate the level of competitiveness in agricultural micro-enterprises under organic production systems operating in the municipalities of La Paz and Los Cabos (Baja California Sur), Mexico.

MATERIALS AND METHODS

The study was applied to micro-enterprises, that is, economic units that have 1 to 10 employees, with minimal or no modern technology, and a family-type business organization (INEGI, 2019). All of them located in the municipalities of La Paz (24° 08' 37.3" N; 110° 18' 04.3" W) and Los Cabos (22° 53' 41.1" N; 109° 56' 24.5" W), in Baja California Sur, Mexico. Through the intentional non-probability sampling technique (Otzen & Manterola, 2017), eight micro-enterprises were identified, whose producers, owners, or representatives were given a questionnaire to collect socioeconomic information, such as education, age, gender, established crops, seniority in the company, and cultivated area, in addition to considering items to assess competitiveness in relation to the factors described below.

- **Use of competitive advantages**. Value directed toward customers, in the form of lower prices than those offered by competitors, and through the foreseeing of differentiated products whose revenues would exceed the corresponding costs (Romero *et al*., 2020).
- **Innovation management**. Strategies followed for the conception and implementation of significant changes in the product, process, marketing, or organization of the company (Pérez, 2018).
- **Associativity**. Business association or coalition with a common goal, in which each participant maintains their legal and managerial independence (Sánchez & Parra, 2024).
- **Implementation of public policies for agriculture**. Interaction with programs derived from a set of government decisions aimed at solving agricultural and rural society problems, within the framework of the public interest (Velázquez *et al*., 2020).
- **Value chain**. A theoretical model that describes the activities of an organization to generate value for the end customer and for the company (Astudillo *et al*., 2020). Table 1 lists the items considered within each of the competitiveness factors evaluated. The response to each item was evaluated through a Likert scale, using the response options 1=Never, 2=Almost never, 3=Occasionally, 4=Almost always, and 5=Always.

Table 1. Items considered within each factor used to evaluate competitiveness in organic agricultural micro-enterprises.

Factor	Items
Use of Competitive advantage	a. Does the quality of your products meet the standards demanded by the market? b. Do your products have a differentiation that allows them to have a competitive advantage? c. Does the company make a commitment to maintain market demand? d. Are there strategies implemented to reduce production costs as a comparative advantage in sowing, harvesting, or post-harvest?
Innovation management	e. Are there innovations implemented in the products generated by the company at planting, harvesting, or post-harvest? f. Are there innovations implemented in production processes at sowing, harvesting, or post-harvest? g. Are there technological innovations implemented in terms of machinery and equipment for the production of their products at sowing, harvesting, or post-harvest? h. Are there innovations (*e.g.* specialized software) implemented in terms of management strategies for administrative tasks? i. Are there innovations implemented in terms of sales, advertising, and marketing techniques?
Associativity	j. Does the company have the capacity to negotiate with suppliers? k. Does the company have the capacity to deal with customers? l. Does the company collaborate with government agencies to ensure sustainability in the market? m. Is the company committed to associate with one or more producer organizations to develop productive activities? n. Does the company work within or as part of a production chain in rural farming or native communities, thus facilitating cooperation between the companies?
Implementation of agricultural public policies	o. Has the company received technical advice from the Federal or State government? p. Has the company received training from the Federal or State government? q. Has the government supported the company with credits?
Value chain	r. Does the company meet the product specifications demanded by the market? s. Do the company's geographical limitations hinder the relationship with the market? t. Do you consider that the company has adequate means of transport to move their products? u. Are there collaboration and coordination strategies implemented among producers, thus competing fairly in the market?

Based on the interviewees' responses to the socioeconomic questionnaires, averages and standard errors were calculated. Additionally, the values provided by the interviewees to each of the items, within each of the competitiveness factors evaluated, were used to obtain the total sum of factor items (TSFI) value. This variable made it possible to identify the progress of each micro-enterprise within each competitiveness factor. Afterwards, the TSFI values of each micro-enterprise in the five factors were added together to determine the general level of competitiveness (Leal *et al.*, 2021). In addition, the TSFI values of each micro-enterprise within each factor, as well as the level of competitiveness were standardized (Mazziotta & Pareto, 2021), and were subsequently used to characterize and classify micro-enterprises through the multivariate techniques of principal components analysis and cluster analysis, using the Ward method as a grouping criterion (Diaz *et al.*, 2019). Statistical analyses were performed in SAS® version 9.3.

RESULTS AND DISCUSSION

Socioeconomic characteristics

The owners or managers of the units were all male, averaging 43.8 years of age, 12.9 years of education, and 8.9 years of seniority in the company. Planted area averaged 6.9 hectares. In regard to the type of crops produced, we found mainly vegetables, aromatic herbs and fruit trees (Figure 1).

Results showed scarce opportunity for female participation in the representation of micro-enterprises, a situation that agrees with Moy (2023), who has observed this situation as something characteristic in Mexico. Limited women participation derives from factors such as the lack of a functional caregiving system, since complexities of motherhood

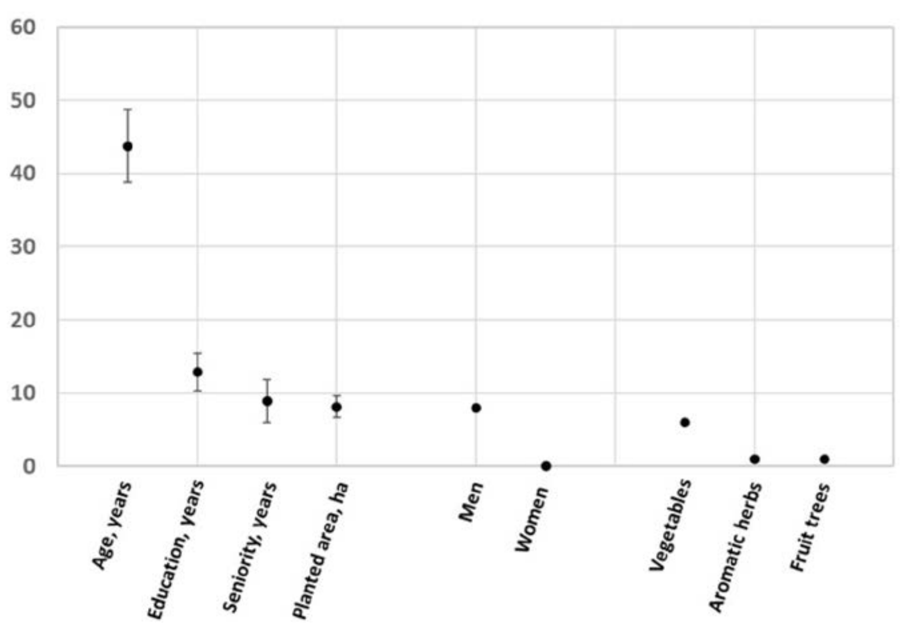

Figure 1. Socioeconomic characteristics (average ± standard error), sex of producers or representatives, and types of crops targeted by organic agricultural production micro-enterprises in the municipalities of La Paz and Los Cabos (Baja California Sur), Mexico.

coincide with the advancement of a professional career, in addition to the lack of flexibility of schedules and resistance to change.

The average age of the owners or representatives of micro-enterprises, as well as the level of seniority, coincides with the trend reported by Alderete & Diez (2014), who stated that newer companies are generally led by young people, generally also with a high level of education. On the other hand, given that age is one of the factors that generates variability in the ways of learning, which can facilitate or hinder the processes of appropriation of technologies and innovations (Acero *et al.*, 2018). To take into account the age as a factor could contribute to the design of strategies, based on the use of andragogy techniques, for capacity building in order to strengthen competitiveness.

Regarding level of education, the value determined for owners or representatives of micro-enterprises participating in this study was below the average of 15.4 years reported for Mexico by the Organization for Economic Cooperation and Development (OECD). Moreover, it is below the average of 18 years reported by that organization, where education is highlighted as a fundamental dimension of competitiveness (Salazar *et al.*, 2023). Additionally, in the detailed analysis of the education level of the interviewees, 62.5% presented 17 years of education, which means being educated up to bachelor's level, while the remaining 37.5% informed six years of education (primary school completed). According to Otero & Taddei (2018) and Farida & Setiawan (2022), schooling is an important factor, since the level of knowledge of producers or representatives entail advantages that can increase productivity, competitiveness and innovation by positively relating to the adoption of technology.

Competitiveness valuation

The valuation of the items, within each competitiveness factor, generated a variant privileged location of micro-enterprises (Table 2). Likewise, the factor that presented the best valuation and less heterogeneity among micro-enterprises due to their average value and their lower dispersion was the use of competitive advantage (4.7 ± 0.4). That is, micro-enterprises presented a high valuation of the quality and differentiation of their products, seeking to maintain market demand and to implement strategies that reduce production costs. The competitiveness factors, successive in the valuation and ranked from highest to lowest, were the value chain (3.6 ± 1.4); innovation management (3.5 ± 1.2); and associativity (3.2 ± 1.7).

The factor with the lowest valuation was the implementation of public policies for agriculture (1.5 ± 0.8). The 'overall competitiveness' variable placed the M2 micro-enterprise as the most competitive, scoring 92 points. Results found are consistent with elements identified by Acosta *et al.* (2020), who through the use of specific questionnaires and the Likert scale, evaluated those factors that affected the competitiveness of agricultural companies. Those authors found areas of opportunity in the factors innovation management, use of competitive advantages, associativity, and the implementation of public policies for agriculture.

Based on the findings above, it is recommended that micro-enterprises implement some improvements per factor. These are, specifically, 1) Innovation management: to implement

Table 2. Valuation of factor items associated with the competitiveness of organic agricultural micro-enterprises (MIEMs).

Competitive advantage							**Innovation management**						
MIEM	**a**	**b**	**c**	**d**		**TSFI**	**MIEM**	**e**	**f**	**g**	**h**	**i**	**TSFI**
M7	5	5	5	5		20	M2	5	5	5	4	5	24
M5	5	5	5	5		20	M4	5	5	5	3	4	22
M6	5	5	5	5		20	M1	3	4	4	5	4	20
M4	5	5	5	4		19	M7	3	3	4	4	5	19
M8	5	5	4	5		19	M8	4	4	3	2	4	17
M3	5	5	4	5		19	M5	2	2	2	5	5	16
M2	4	5	5	5		19	M6	3	3	2	5	2	15
M1	4	3	5	5		17	M3	3	3	2	1	1	10
	†4.7±0.4							3.5±1.2					
Associativity							**Implementation of public policies for agriculture**						
MIEM	**j**	**k**	**l**	**m**	**n**	**TSFI**	**MIEM**		**o**	**p**	**q**		**TSFI**
M2	5	5	5	5	5	25	M1		3	3	3		9
M5	5	5	3	5	1	19	M2		3	3	1		7
M7	5	5	1	1	5	17	M4		1	3	1		5
M1	4	4	4	1	3	16	M7		1	1	1		3
M8	5	5	1	3	1	15	M5		1	1	1		3
M4	5	4	1	1	4	15	M6		1	1	1		3
M6	5	5	1	1	1	13	M8		1	1	1		3
M3	3	4	1	1	1	10	M3		1	1	1		3
	3.2±1.7							1.5±0.8					
Value chain							**Overall competitiveness**						
MIEM		**r**	**s**	**t**	**u**	**TSFI**	**MIEM**						
M2		5	3	4	5	17	M2	92					
M1		5	1	5	5	16	M1	78					
M4		5	3	4	4	16	M4	77					
M7		5	1	5	5	16	M7	75					
M5		5	2	4	5	16	M5	74					
M6		5	1	4	5	15	M6	66					
M8		4	1	4	3	12	M8	66					
M3		3	1	3	1	8	M3	50					
	3.6±1.4												

TSFI: total sum of factor items for a specific participating micro-enterprise. MIEM: micro-enterprise. Items: a to u, as described in Table 1; M1, M2…M8: participating micro-enterprises. † Mean ± standard deviation.

innovations in the products generated, as well as in production processes, machinery and equipment to obtain their products, in addition to implementing innovations in terms of sales, advertising and marketing techniques; 2) Associativity: to implement strategies that increase the level of negotiation with suppliers and customers. As well as, to participate with government agencies to be sustainable in the market, associating with organizations of producers, and working as part of a production chain; 3) Value chain: to implement strategies that contribute to compliance with the product specifications demanded by the market; also, that can reduce geographical limitations with the market, have adequate means to transport their products, and increase collaboration among producers in order to compete in the market. 4) Implementation of public policies for agriculture: it is recommended to approach government institutions in the search for technical advice, training or credits.

Thus, the valuation of competitiveness made it possible to identify aspects that require greater intervention by micro-enterprises, which has been reported as a valuable element in the implementation of this type of research (Rodríguez *et al.*, 2019; Leal *et al.*, 2021). On the other hand, even though this evaluation did not include parameters related to the measurement of the level of capabilities of the totality of human resources or the efficiency of the organizational structure of micro-enterprises, it is necessary to implement strategies that can strengthen these elements in order to increase their competitiveness (Latifah, 2021; Farida & Setiawan, 2022).

Multivariate characterization of organic agricultural micro-enterprises

The data on the valuation of factors associated with competitiveness were characterized by a principal component analysis (Table 3). The first two principal components (CP1 and CP2) were more relevant due to their eigenvalue greater than one (Allee *et al.*, 2022) for a total cumulated variance of 92.2%. CP1 was positively associated with greater competitiveness in micro-enterprises due to better innovation management, implementation of agricultural policies, associativity, and value chain. CP2 was positively associated with the competitive advantages of micro-enterprises.

Figure 2A shows the distribution of micro-enterprises between the two principal components of importance. The principal component analysis made it possible to reduce the dimensionality of data, minimizing information loss of and facilitating data

Table 3. Pearson correlation coefficients (r) and eigenvectors (E) associated with competitiveness factors in organic agricultural micro-enterprises.

Competitiveness factors	Principal components			
	PC1		PC2	
	r	E	r	E
Competitive advantage	−0.32	−0.16	0.93**	0.75
Innovation management	0.94**	0.47	0.05	0.04
Implementation of agricultural public policies	0.74**	0.37	−0.64	0.26
Associativity	0.84**	0.42	0.33	−0.51
Value chain	0.86**	0.43	0.32	0.26
Competitiveness	0.98**	0.49	0.17	0.14
Eigenvalue	3.9		1.5	
Variance proportion, %	66.5		25.7	
Accumulated variance, %	66.5		92.2	

PC1: first principal component; PC2: second principal component; ** Significant at ($p \leq 0.01$).

interpretation, thus fulfilling PCA implementation purpose (Jolliffe & Cadima, 2016). On the other hand, cluster analysis (Figure 2B) facilitated the formation of four groups based on their similarity (Everitt *et al.*, 2011) in relation to the level of competitiveness. The most competitive micro-enterprise was M2, followed by M1, as well as by the group composite of M4, M5, M6, M7 and M8; finally, the micro-enterprise with the least competitiveness was M3. The distribution of M3 and M1 based on the influence of the second principal component (Figure 2A) also denotes the relevance of both micro-enterprises working on the implementation of practices that increase their competitive advantages.

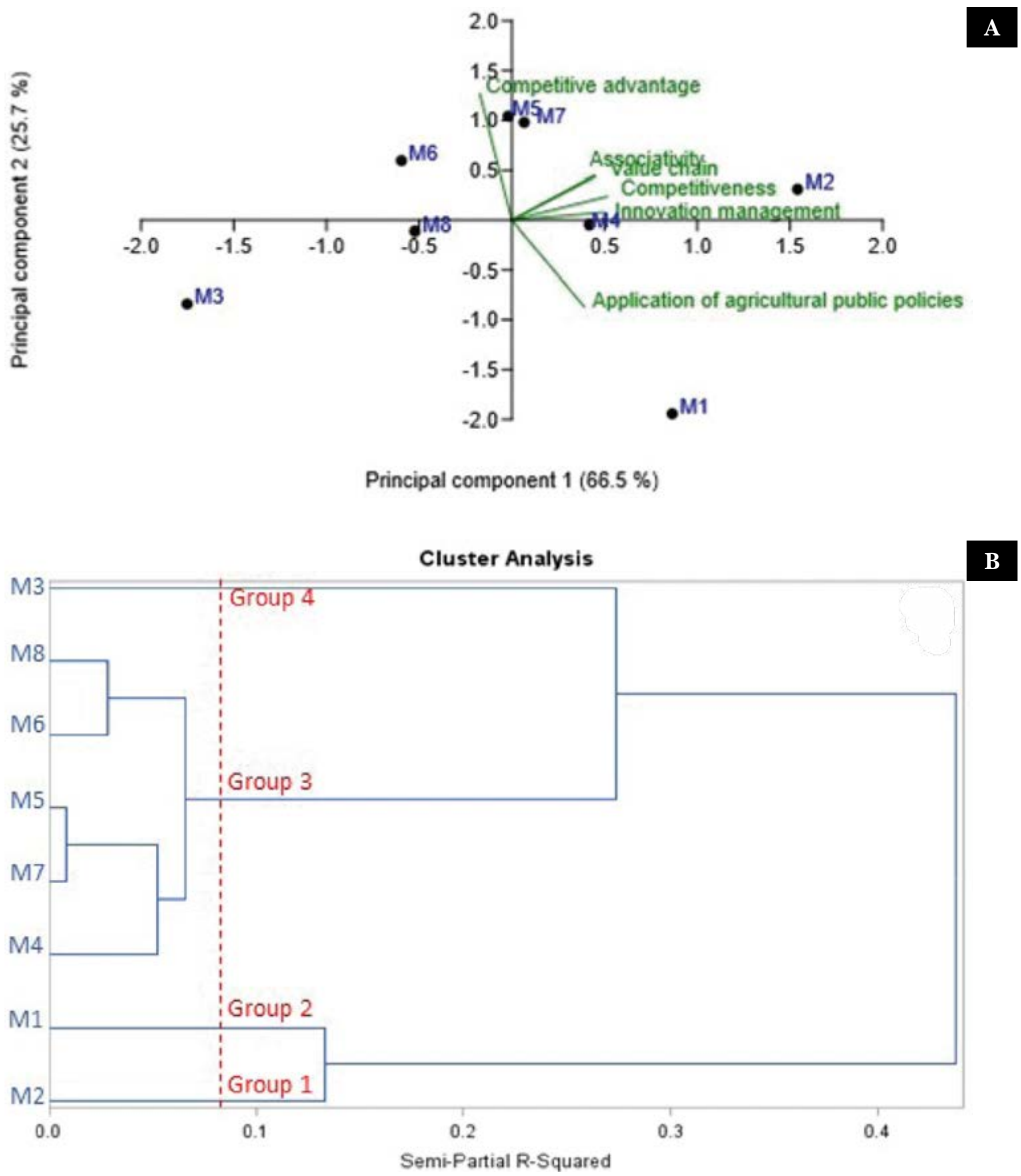

Figure 2. A: distribution of competitiveness factors and organic agricultural micro-enterprises between the two principal components of importance. B: cluster analysis applied to organic agricultural micro-enterprises.

CONCLUSIONS

The level of competitiveness was variable among organic agricultural micro-enterprises. The factor associated with competitiveness with the best valuation was competitive advantage, while with an intermediate level of improvement area, the factors value chain, innovation management and associativity were identified. The factor in which greater effort is required by micro-enterprises was the implementation of public policies for agriculture.

It is then recommended to approach government institutions in the search for technical advice, training and credits. For future evaluations of competitiveness, it is recommended to consider employees of micro-enterprises in the sampling. That is, to know the capabilities of human resources and the efficiency of the organizational structure, which can lead to the design of strategies to strengthen competitiveness.

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Colegio de Postgraduados

Evaluation of the antioxidant activity of aqueous and organic extracts of edible insects to different cooking temperatures and pH conditions

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ABSTRACT

Objective: To evaluate heat treatment and pH on the antioxidant activity of extracts obtained from edible insects endemic to Mexico. Insects known as jumiles, grasshoppers (adults and nymphs), red maguey (*Agave* sp.) wormfrom the maguey penca, white maguey worms from the pineapple, and chicatanas were used.
Design/methodology/approach: Different solvents (water, methanol, ethanol, NaCl, and acetic acid) were used to prepare the extracts. These were characterized by X-ray diffraction, and the effect of temperature and pH on their antioxidant activity was analyzed. The methods used were DPPH and FRAP, and total polyphenols and flavonoids were quantified.
Results: There are presence of antioxidant molecules characterized as quercetin, hesperetin, and DL-α-lipoic acid. The extracts with the highest content of flavonoids and total phenols were from adult grasshoppers in water, chicatanas in 50% ethanol, and maguey worms from the penca in water. However, the antioxidant activity of these extracts was negatively affected by elevated pH and temperature.
Study limitations/implications: The main limitations of the study were the availability of the insects, which are endemic species of Mexico and most are seasonal.
Findings/conclusions: Molecules with antioxidant capacity were identified, and their antioxidant activity decreased when exposed to high pH values.

Keywords: Edible insects, Antioxidants, *Sphenarium purpurascens*, *Atta mexicana*.

INTRODUCTION

Antioxidants are substances that prevent the oxidation of other compounds. The use of antioxidants has become a necessity for food products, which are sensitive to this

type of chemical change. Widely used synthetic antioxidants are effective (*e.g.*, BHA, BHT, TBHQ). Currently, there is a growing consumer demand for the use of natural ingredients in processed foods. Therefore, new sources of natural compounds with antioxidant activity are being sought (Hęś *et al.*, 2019). Some natural antioxidants have a more potent effect than artificial antioxidants (Valenzuela and Pérez, 2016). Naturally occurring antioxidants are mainly found in plants, microorganisms, fungi, and animal tissues (Gallegos *et al.*, 2013).

Polyphenols are organic chemical compounds that occur naturally in plants and constitute one of the most abundant and diverse groups of natural compounds in the plant kingdom. These compounds are classified as secondary metabolites, produced through the phenylpropanoid-derived shikimate pathway and/or the polyketide pathway. There are approximately 80,000 identified polyphenols, with molecular weights reaching up to 30,000 Da. Polyphenols are classified as flavonoids, phenolic acids, lignans, and stilbenes, each defined by unique structural features (Sejbuk *et al.*, 2024). Polyphenols share common phenolic structural features and exhibit structural diversity with several subgroups. Polyphenols are categorized by the number of phenolic rings they contain and by the structural elements that link the rings. The four primary structural groups of polyphenols are flavonoids, phenolic acids, lignans, and stilbenes; these are present in fruits, vegetables, tea, and wine. Caffeic and ferulic acids are prevalent in coffee and grains. Lignans are found in seeds and whole grains, while stilbenes, such as resveratrol, are found in grapes and berries. This wide variety of phenolic structural types forms complex biological functions (Jalouli *et al.*, 2025).

Food processing aims to extend shelf life, improve organoleptic properties, and increase the availability of bioactive compounds present in raw materials. However, some methods can result in the loss of polyphenols. Therefore, the final amount of these compounds depends on the processing technique used, the exposure time, and the characteristics of the ingredients (Marín *et al.*, 2015).

There are various processing strategies; for example, the use of heat is one of the most common. This method includes techniques such as boiling, steaming, frying, baking, dehydration, pasteurization, sterilization, canning, and roasting. The effect of heat on polyphenols varies depending on the specific procedure; in some cases, high temperatures promote their absorption and breakdown of cellular structures, while in others, they can cause degradation through oxidation (Minatel *et al.*, 2017). This study aimed to evaluate the effect of heat treatment and pH on the antioxidant activity of extracts from edible insects endemic to Mexico.

MATERIAL AND METHODS

Samples

The samples were obtained from the San Juan exotic meat market in Mexico City. The insects used were: jumiles (*Euschistus taxcoensis*), grasshoppers (*Sphenarium purpurascens*) adults and nymphs (fifth instar), red maguey worms (*Comadia redtenbacheri*), white maguey worms (*Aegiale hesperiaris*), and chicatanas (*Atta mexicana*).

Extracts obtained

The extracts of the insects above were obtained with five different solvents: distilled water, 0.6 M sodium chloride, 50% ethanol, 50% methanol, and 30% acetic acid. The methodology proposed by Mendoza *et al.* (2013) and Calderón *et al.* (2016) was used. Table 1 shows the coding. All extractions were performed by liquefying 5 g of the insect samples and 50 mL of the solvents. The mixtures with distilled water and 0.6 M NaCl were boiled and stirred for 30 min, while the other samples were stirred at 90 rpm for 120 hours at 24 °C. Finally, the extracts were filtered through Whatman No. 1 paper.

Concentration of the different extracts

The ethanol, methanol, and acetic acid extracts were concentrated in a rotary evaporator at 20 rpm for 20 min and 45 min, respectively, at a temperature of 50 °C. All extracts were then frozen with liquid nitrogen and lyophilized (Labconco FreeZone® 2.5, Houston, USA); they were then stored in Mylar bags and kept at room temperature until use.

Quantification of total polyphenols

Quantification was performed using the Folin-Ciocalteu spectrophotometric method proposed by Lin and Tang (2007), with some modifications. A reaction was carried out by mixing 100 μL of extract with 2.8 mL of water and then adding 100 μL of 50% Folin-Ciocalteu reagent for 5 min. Subsequently, 2 mL of 2% sodium carbonate was added, and the mixture was left to stand for 40 min at room temperature, all in the dark. Finally, the absorbance at 750 nm was measured using a spectrophotometer (Genesys 105 UV-VIS Thermo Scientific). Gallic acid was used as a standard for the 0-200 μg/mL standard curve.

Flavonoid quantification

The reaction was carried out using the methodology proposed by López (2010), with the following modifications. The reaction was carried out in 250 μL of extract, 1.25 mL of water, and 75 μL of 5% sodium nitrite. The mixture was then allowed to stand at room temperature for 6 min. 150 μL of 2% aluminum trichloride was then added for 5

Table 1. Coding of edible insects and solvents *.

Edible insects		Solvents	
Jumiles	JU	Water	01
Chicatanas	CH	Sodium chloride 0.6M	02
Grasshoppers in the fifth nymph stage	CC	Ethanol 50%	03
Adult grasshoppers	CG	Methanol 50%	04
Maguey worms from the penca	PE	Acetic acid 30%	05
Pineapple maguey worms	PI		

The extracts were named after the edible insect code. For example, JU01 corresponds to the water extract of jumiles. *Only the following extracts were prepared: PI01, CG01, PE01, CC01, CH02, JU02, CH03, PI04, CC05, PE05, and JU05, according to the preliminary results obtained by the working group.

min, followed by the addition of 500 μL of 1 M sodium hydroxide. The final volume was completed to 2.25 mL with distilled water and incubated for 50 min at room temperature. The reaction was carried out in the dark. The absorbance at 510 nm was then measured in a spectrophotometer (Genesys 105 UV-VIS Thermo Scientific). A standard was routinely used to prepare the calibration curve, ranging from 0 to 250 μg/mL.

Effect of heat treatment and pH on antioxidant activity

The extracts were treated under different pH and heat treatment conditions as shown in Table 2. Subsequently, total polyphenols, flavonoids, and antioxidant activity, FRAPP, and DPPH were quantified.

Ferric Reduction Antioxidant Potential (FRAP)

Antioxidant activity was measured using the FRAP method, following the methodology described by Calderón *et al.* (2016). A reaction was performed with 100 μL of extract and 750 μL of FRAP reagent (López, 2010) and incubated for 5 min at 37 °C. A total of 150 μL was withdrawn and analyzed using a plate reader (Synergy HT BioTek Instruments, Vermont, USA) at 593 nm. A standard curve was created using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) dissolved in 50% methanol in a concentration range of 0-400 μg/mL.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical reducing activity

Kinetics were performed to determine the percentage of DDPH radical reduction in the presence of the extracts and the reference antioxidant (0.0078-0.125 mg/mL of gallic

Table 2. A combination of pH and temperature of the different treatments.

Treatment	pH	Temperature/time
Control	Without modification	No treatment
1		64 °C / 30 min
2		72 °C / 15 s
3		95 °C / 30 min
4	4	No treatment
5		64 °C / 30 min
6		72 °C / 15 s
7		95 °C / 30 min
8	6	No treatment
9		64 °C / 30 min
10		72 °C / 15 s
11		95 °C / 30 min
12	8	No treatment
13		64 °C / 30 min
14		72 °C / 15 s
15		95 °C / 30 min

acid). The process was carried out with measurements every 5 min for 30 min at 25 °C, using a plate reader (Synergy HT BioTek Instruments, Vermont, USA) at a wavelength of 517 nm. The reactions were analyzed using the methodology of Mendoza *et al.* (2013), with the following modifications: 5 μL of the sample and 195 μL of a 4×10^{-5} M methanolic DDPH solution were added. For grasshopper samples in 50% ethanol, a concentration of 8×10^{-5} M DPPH was used. In addition, various controls were prepared to verify that the solvents or extracts did not absorb at the wavelength used: 1) Equipment blank (200 μL of water); 2) Solvent blank (5 μL of solvent in which the extract was dissolved and 195 μL of water); 3) Extract blank (5 μL of extract and 195 μL of water); 4) DPPH solvent blank (200 μL of methanol). Finally, plate wells were used to measure the absorbance of the DPPH radical without the presence of an antioxidant.

The DPPH reproduction percentage was calculated using the following equation:

$$\%\,of\ reduction\ DPPH = \frac{\left(A_R - A_{bpR}\right) - \left(A_m - A_{bp} - A_{mp}\right)}{A_R - A_{bp}} \times 100\%$$

Where: A_R=Absorbance of the DPPH radical; A_m=Absorbance of the extract; A_{bpR}=Absorbance of the radical solvent blank − Absorbance of the equipment; A_{bp}=Absorbance of the solvent blank − Absorbance of the equipment; A_{mp}=Absorbance of the extract blank − Absorbance of the equipment.

X-ray diffraction

The analysis was performed in the X-ray laboratory of the Division of Basic Sciences and Engineering at UAM-Iztapalapa.

Statistical analysis

All determinations were performed in triplicate, and the results are presented as the mean and standard deviation. Statistical analyses were performed using XLSTAT software version 2014.5.03 (Addinsoft) using an alpha threshold of 0.05. Results were analyzed using one-way analysis of variance (ANOVA) and the Tukey method for comparison of means between treatments for each of the techniques used. Principal component analysis (PCA) was used for the quantification tests of total polyphenols and flavonoids.

RESULTS AND DISCUSSION

The results indicate that all the samples analyzed may have antioxidant activity; however, it has been reported that the phenolic content in some plants is not related to antioxidant activity, but rather to the action of the polyphenols present (Gutiérrez *et al.*, 2008). For example, some polyphenols are widely distributed in the plant kingdom and are important for antioxidant capacity. The phenolic compounds in legume seeds are particularly flavonoids, phenolic acids, and procyanidins, and the antioxidant capacity of legumes is influenced by their composition of active polysaccharides, proteins, amino acids, vitamins, and microelements (Zhao *et al.*, 2014).

The activity of natural antioxidants depends on the participation of phenolic hydrogen in reaction with radicals, the stability of the antioxidant radical formed during radical reactions, and the chemical substitutions present in the structure. Substitutions in the structure are likely the most significant contribution to the capacity of a natural antioxidant, playing a crucial role in controlling radical reactions and facilitating the formation of resonance-stabilized antioxidant radicals (Figure 1).

The electron-donating capacity of methyl, ethyl, and tertiary-butyl substitutions at the ortho and para positions of hydroxyl groups is enhanced by the antioxidant activity of phenol. Furthermore, hydroxyl group substitutions at these positions improved antioxidant activity (Pokorny *et al.*, 2001).

The extracts were evaluated by D-DX and compared with different antioxidant compound structures. It was observed that all the insects contained ascorbic acid, gallic acid, and quercetin dihydrate, of which gallic acid (GA) represents the primary class of phenolic acids. In contrast, quercetin dihydrate (QDH) is the main representative of the flavanol group of flavonoids. Gallic acid is available in various parts of plants (fruits, seeds, leaves, and wood). Fruits, such as blackberries, blueberries, strawberries, walnuts, grapes, plums, and mangoes. QDH is a bioflavonoid found in fruits and vegetables. Vegetables include onions, kale, broccoli, and peppers. Fruits include apples, tomatoes, blueberries, cherries, red grapes, raspberries, and cranberries (Bibila Mayaya Bisseyou *et al.* 2024).

On the other hand, jumiles and chicatanas do not contain ferulic acid, which has generated worldwide interest due to its antioxidant capacity, meaning they can potentially protect DNA and lipids from oxidation through reactive oxygen species; its activity has been linked to anti-inflammatory, anti-aging, anticancer, antidiabetic, antihypertensive, and neuroprotective effects. Ferulic acid is found in many plants, including vegetables, coffee, nuts, and cereals (Han & Mackie, 2023). Another antioxidant compound absent in chicatanas is hesperitin, a flavone present in citrus fruits, and is also present in its glycosidic form as hesperidin (Tanwar *et al.*, 2020). Flavanones are distinguished among flavonoids by the absence of the C2-C3 double bond, forming a non-planar C-ring (Amisha and Arati, 2023). On the other hand, hesperetin is a promising potential anticancer compound for inhibiting the aromatase enzyme, which acts as an important therapeutic agent for breast cancer in postmenopausal women (Iqbal *et al.*, 2018).

Lactic acid is not synthesized in grasshoppers at the nymph stage, compared to the adult stage and other insects. Molecules such as halite are also observed, but this was only observed in samples extracted with NaCl and distilled water. Finally, the compounds meso-tartaric acid and β-D-mannose were found in maguey worms extracted with water

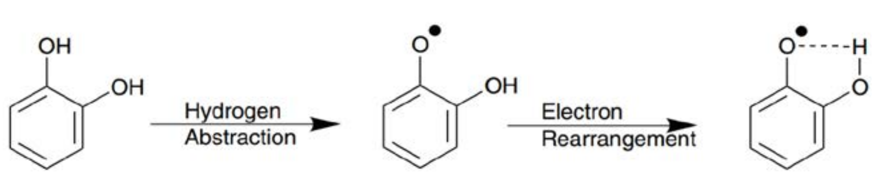

Figure 1. Baum and Perun proposed intramolecular hydrogen bonding of ortho-substituted phenols. Taken from Pokorny *et al.* (2001).

(PE01); these compounds are highly soluble in water. On the other hand, several authors have mentioned some antioxidant compounds present in edible insects such as: gallic acid and quercetin in *Polyrhachis vicina*, *Acheta domesticus*, *Holotrichia parallela*, *Alphitobius diaperinus*, *Tenebrio molitor* (Zhang *et al.*, 2022; Nino *et al.*, 2021; Liu *et al.*, 2012; Gumul *et al.* 2023), additionally quercetin is reported in grasshoppers such as *Dissoteira carolina*, caterpillars: *Rondotia menciana* and *Bombyx mori* worms (Hopkins and Ahmad, 1991; Hirayama *et al.*, 2013; Kurioka and Yamazaki. 2002). Gumul *et al.* (2023) reported levels of 0.45, 7.23, and 0.54 mg/100g of ferulic acid for *A. diaperinus*, *A. domesticus*, and *T. molitor*, respectively. Fu *et al.* (2021) reported the presence of hesperitin in the moth *Antheraea pernyi*.

Based on the quantification of total polyphenols and flavonoids, a Principal Component Analysis (PCA) was performed to select the extracts with the highest concentration of potential antioxidant molecules (Figure 2). The PCA accounted for 100% of the sample variability. The samples with the highest concentrations of total phenols and flavonoids were found in penca maguey worms in water (PE01), adult grasshoppers in water (CG01), and chicatanas in 50% ethanol (CH03).

Table 4 shows the reducing capacity using the FRAP technique. The PE01 extract from treatment 15 showed a significant increase compared to the control (176.43±2.082 and 56.81±0.267, $P<0.05$, respectively). Phenolic compounds had greater reducing capacity because they formed a blue complex with tripyridyltriazine at acidic pH, reducing

Table 3. Antioxidant compounds in edible insect extracts by D-RX.

Name of antioxidant compound	Chemistry Formula	Edible insect extract										
		JU02	JU05	CC01	CC05	PI04	PI04	PE01	PE05	CH02	CH03	CG01
Cinnamic Acid	$C_9H_80_3$	X	X	X	X	X		X	X	X		X
Gallic acid hydrate	$C_7H_6O_5 \bullet H_20$	X					X			X		.
Quercetin dihydrate	$C_{15}H_{14}O_7 \bullet 2H_20$	X	X		X	X	X	X	X	X	X	X
Rutin	$C_{27}H_{30}O_{16}$	X		X	X					X		X
Hesperetin	$C_{28}H_{34}O_{15}$	X	X		X	X	X	X	X			X
B-Carotene	$C_{40}H_{56}$	X	X	X		X		X			X	X
Folic acid	$C_{19}H_{19}N_7O_6$	X	X			X	X	X	X	X	X	X
Ascorbic acid	$C_6H_8O_6$	X	X		X	X	X	X	X	X	X	X
Glutathione	$C_{10}H_{17}N_3O_6S$	X		X		X	X	X	X	X	X	X
dl-a-Lipoic acid	$C_6H_{14}O_2S_2$	X	X	X	X	X	X		X	X	X	X
Halite	NaCI	X		X	X					X		X
Ferulic acid	$C_{10}H_{10}O_4$			X	X	X	X	X	X			X
acid o-coumaric acid	$C_9H_8O_3$			X				X			X	X
Vitamin B12	$C_{63}H_{88}CoN_{14}O_{14}P \bullet 16H_20$			X								
Rhodium, platinum	(Rh, Pt)					X	X					
Meso Tartaric acid	$C_4H_6O_6$							X				
13-D-Mannose	$C_6H_{12}O_6$							X				X

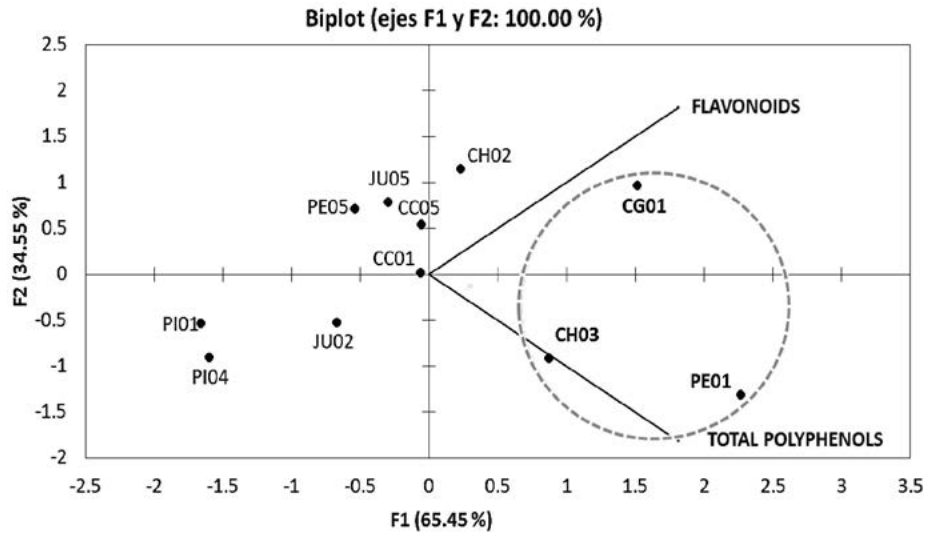

Figure 2. Principal component analysis of insect extracts.
Variables analyzed: total phenols and flavonoids. Extracts with the highest concentrations were grouped into clusters marked with circles.

Table 4. Antioxidant activity and quantification of antioxidant molecules after treatment with pH and temperature of the PE01 extract.

Treatment	Antioxidant activity		Antioxidant molecules, quantification	
	FRAP[1]	DPPH (%)	Polifenols total[2]	Flavonoids[3]
Control	56.81±0.27[C]	58.15±3.72[BC]	13.46±0.62[F]	41.92±5.20[ABC]
1	18.78±0.29[G]	28.55±0.98[F]	24.75±1.32[BC]	35.25±9.43[A,BC]
2	27.9±1.63[F]	52.43±7.21[BCD]	20.92±0.80[D]	26.95±4.74[BC]
3	62.99±1.75[B]	108.37±2.19[A]	27.17±0.52[AB]	53.58±14.14[AB]
4	15.95±0.09[GI]	37.36±4.92[DEF]	13.71±0. 44[F]	34.83±1.77[ABC]
5	17.21±0.24[G]	35.97±2.95[DEF]	26.25±0.66[AB]	59.14±11.09[A]
6	59.25±1.57[BC]	58.92±9.18[B]	20.92±0.80[D]	20.25±4.74[B]
7	61.34±1.98[B]	108.06±2.12[A]	26.75±1.56[AB]	25.25±7.07[C]
8	12. 63±0.35[HI]	51.35±7.36[BCD]	12.71±0.61[F]	40.67±4.12[ABC]
9	17.64±0.53[G]	34.97±3.94[EF]	27.17±1.61[AB]	34.42±1.18[ABC]
10	40.20±1.41[D]	47.48±3.48[BCDE]	17.42±2.04[E]	25.25±2.33[C]
11	33.80±0. 72[E]	96.00±4.59[A]	24.92±0.88[ABC]	61.92±11.79[A]
12	8.78±0.55[J]	43.47±0.97[CDEF]	13.75±0.55[F]	46.64±8.09[ABC]
13	9.65±0.213[I,J]	43.39±0.33[CDEF]	27.92±1.01[A]	46.92±2.35[ABC]
14	29.71±2.095[F]	49.18±2.45[BCD]	14.92±0.63[EF]	22.48±1.93[C]
15	176.43±2.082[A]	49.65±5.35[BCD]	22.58±0.38[CD]	32.47±5.09[BC]

[1] mg TROLOX equivalent/g extract, 2 mg gallic acid/g extract, 3 mg rutin/g/g extract. Superscript letters represent significant differences between rows within the same column ($p<0.05$).

Fe^{3+} to Fe^{2+} (Benzie and Strain, 1996). Treatments 3 and 7 also had a greater increase than the control (62.99±1.751, 61.34±1.976 and 56.81±0.267, P<0.05, respectively); in these treatments there was a reduction in the DPPH radical, the highest value was 108.37±2.19% for PE01 (treatment 3), and 108.06±2.12% for PE01 (treatment 7), This was due to the total phenol content (27.17±0.52 and 26.75±1.56, respectively), but the extract from treatment 7 had a decrease in flavonoid content compared to the control;. In contrast, treatment three did not have a significant difference (25.25±7.07, 41.92±5.20, 53.58±14.14, P>0.05, respectively). The important difference was in treatment 3, where the thermal effect favored the persistence of flavonoids.

Table 5 shows the antioxidant activity values for the extract of adult grasshoppers in water (CG01), treatment 5 (pH 4, 64 °C/30 min), had the highest antioxidant activity in ferric reduction (FRAP) with 23.24±1,050 mg TROLOX equivalent/g of extract (p<0.05) *vs*. Control and other treatments. There are no references on the antioxidant power with ferric reduction (FRAP). In the case of crickets (Orthoptera), there is a report by Gumul *et al.* (2023). In *Acheta domesticus*, the value was 71.15±0.5 mM Fe/kg, lower than that reported by Kurdi *et al.* (2021). *Gryllus bimaculatus* showed 300±100 mM Fe/kg; this value was because the crickets were previously defatted. DPPH showed higher levels in the Control group and Treatment 4 (pH 4, without heat treatment), 47.7±1.35 and 54.75±6.34 mg of gallic acid/g of extract, respectively (P<0.05). The increase in

Table 5. Antioxidant activity and quantification of antioxidant molecules after pH and temperature treatment of the CG01 extract.

Treatment	Antioxidant Activity		Antioxidant molecules, Quantification	
	FRAP[1]	DPPH (%)	Polifenols total[3]	Flavonoids[4]
Control	15.50±0.660^{CD}	47.7±1.35^{DE}	7.46±0.14^{ABCD}	63.31±9.66^{DE}
1	15.28±0.496^{CD}	24.20±3.10^{AB}	7.50±0.87^{ABCD}	80.53±8.67^{EF}
2	16.16±0.496^{D}	17.16±7.13^{AB}	6.83±0.38^{ABC}	30.25±4.71^{A}
3	19.32±0.717^{E}	28.07±1.35^{ABC}	8.92±0.38^{CDE}	91.92±2.35^{F}
4	13.36±0.402^{BC}	54.75±6.34^{E}	7.42±0.56^{ABCD}	65.25±4.41^{DE}
5	23.24±1.050^{F}	20.57±3.09^{AB}	7.44±0.44^{ABCD}	51.08±3.54^{BCD}
6	18.66±0.538^{E}	20.30±0.77^{AB}	6.00±0.43^{AB}	71.77±7.28^{DEF}
7	19.11±0.580^{E}	27.84±3.04^{ABC}	13.08±1.18^{F}	63.59±4.72 ^{DE}
8	15.79±0.255^{D}	20.16±5.21^{AB}	5.29±1.58^{A}	58.59±1.18^{CD}
9	15.46±0.317^{CD}	12.93±1.93^{A}	6.00±0.45^{AB}	55.67±3.54^{BCD}
10	16.04±0.797^{D}	18.39±4.63^{AB}	8.08±1.38^{BCD}	41.92±7.07^{ABC}
11	17.38±1.244^{DE}	24.52±3.28^{AB}	11.42±0.80^{EF}	58.59±16.50^{CD}
12	15.51±0.433^{CD}	30.39±3.54^{BC}	7.50±0.69^{ABCD}	66.92±5.89^{DE}
13	11.22±0.616^{B}	32.29±3.09^{BCD}	5.75±0.78^{AB}	61.64±6.79^{CDE}
14	13.33±1.281^{BC}	40.34±13.30^{CDE}	8.42±1.63^{BCD}	35.25±4.71^{AB}
15	6.85±0.840^{A}	19.07±6.36^{AB}	10.08±1.01^{DE}	28.29±2.35^{A}

[1] mg equivalente de TROLOX/g de extracto, 2 mg de ácido gálico/g de extracto, 3 mg de rutina/g de extracto. Las leras como superíndice representan diferencia significativa entre los renglones de la misma columna (p<0.05).

temperature and pH decreased the antioxidant activity. On the contrary, Kurdi *et al.* (2021) observed a temperature of 135 °C and 15 min increased DPPH activity in *Gryllus bimaculatus* from 0.07±0.08 to 45.51±2.64%. For the quantification of antioxidant molecules, there were differences ($P<0.05$) between the Control group *vs*. Treatment 7, having a higher content of total polyphenols, while Treatments 1, 3, and 6 were for the flavonoid content.

Table 6 shows the antioxidant activity values for the chicatanas extract with 50% ethanol (CH03), the highest FRAP activity was in Treatments 5 (pH 4, 64 °C/30 min), and 8 (pH six and without heat treatment) of 15.37±0.02 and 14.93±0.42 mg TROLOX equivalent/g of extract, respectively ($P<0.05$), in the literature no references were found on the antioxidant power for chicatanas, Campos *et al.* (2014) reported DPPH% activity with a concentration of 50 μg/mL of *Melipona orbignyila* propolis (75±3.5%), being higher than the insect of the Hymenoptera order (71.78±1.67%). Regarding antioxidant molecules, information was only found in total polyphenols from methanolic extracts of beetles *Pharochilus punctiger* and *Titoceres jaspideus* (7.81±0.21 and 6.44±0.30 mg gallic acid/g, respectively) and flavonoids (6.88±0.20 and 5.34±0.27 mg quercetin/g, respectively); these values were lower than in another study (Kibet *et al.*, 2024). On the other hand, increases in pH and temperature decreased FRAP activity; meanwhile, DPPH activity at 95 °C increased compared to pH 4.

Table 6. Antioxidant activity and quantification of antioxidant molecules after pH and temperature treatment of the CH03 extract.

Treatment	Antioxidant Activity		Antioxidant molecules, Quantification	
	FRAP[1]	DPPH (%)	Polifenols total[2]	Flavonoids[3]
Control	13.22±0.35^{CDE}	59.33±9.13^{ABC}	9.13±0.19^{AB}	113.46±8.24^{G}
1	13.77±0.34^{EFG}	62.62±2.38^{ABC}	9.50±0.41^{AB}	44.57±4.74^{CDEF}
2	14.74±0.01^{FG}	54.67±7.08^{ABC}	22.59±0.85^{D}	46.79±8.66^{CDEF}
3	14.80±0.52^{FG}	59.85±2.56^{ABC}	21.54±2.09^{D}	54.30±5.89^{F}
4	13.51±0.22^{DEFG}	70.91±3.91^{C}	9.23±0.20^{AB}	34.02±5.55^{BCD}
5	15.37±0.02^{G}	61.84±6.23^{ABC}	9.50±0.44^{AB}	37.07±3.47^{BCDE}
6	11.37±1.84^{BCD}	69.53±6.44^{BC}	21.25±1.47^{D}	39.30±1.18^{BCDEF}
7	12.64±0.13^{CDEF}	71.78±1.67^{C}	21.88±2.05^{D}	49.29±3.54^{DEF}
8	14.93±0.42^{G}	56.91±8.72^{ABC}	10.17±0.62^{AB}	54.29±6.51^{F}
9	13.11±1.04^{CDEFG}	54.67±4.32^{ABC}	10.71±0.53^{B}	46.24±8.22^{CDEF}
10	13.74±0.75^{EFG}	53.80±9.89^{ABC}	22.42±1.58^{D}	46.79±7.27^{CDEF}
11	13.83±1.65^{EFG}	52.51±2.57^{ABC}	22.79±2.19^{D}	52.63±5.89^{EF}
12	11.14±0.34^{BC}	46.89±3.93^{A}	7.25±0.44^{AB}	26.24±4.59^{AB}
13	12.42±0.43^{CDE}	50.35±8.64^{AB}	6.92±0.50^{A}	32.63±3.54^{ABCD}
14	9.81±0.24^{AB}	57.26±6.77^{ABC}	15.00±0.69^{C}	16.80±2.35^{A}
15	8.80±0.53^{A}	57.69±11.37^{ABC}	15.05±0.80^{C}	30.13±0.00^{ABC}

[1]mg TROLOX equivalent/g extract, 2 mg gallic acid/g extract, 3 mg rutin/g/g extract. Superscript letters represent a significant difference between rows in the same column ($p<0.05$).

CONCLUSION

Molecules with antioxidant capacity were identified and quantified in insects endemic to Mexico, particularly grasshoppers, maguey worms, and chicatanas. However, it was observed that their antioxidant activity is susceptible to changes in pH and temperature.

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Yield and adaptability in corn genotypes evaluated in the northern region of Tamaulipas, Mexico

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ABSTRACT

Objective: To estimate the performance and adaptability parameters of simple corn hybrids in the northern region of Tamaulipas, Mexico.
Design/methodology/approach: During the 2022-2023 F-W agricultural cycle, 49 corn genotypes were evaluated across two environments in Tamaulipas. In both locations, experiments were conducted using a 7×7 lattice design with three replicates. The study variables included yield, plant and corncob height, days to male and female flowering, grain moisture, root and stem lodging, corncob appearance, and husk coverage. A combined analysis of variance was performed across locations.
Results: The study revealed significant differences among genotypes and environments for most variables, except for root and stem lodging. Regarding genotype × environment interaction, the only significant variable was grain moisture. The experimental hybrids GEN 34, GEN 15, GEN 16, and GEN 24 achieved yields above 8.4 t ha^{-1} and showed good adaptability compared to transnational materials. The evaluated agronomic characteristics indicated wide genetic diversity.
Limitations on study/implications: Water scarcity and high temperatures in the region limited the hybrids' ability to reach their maximum yield potential. The four outstanding hybrids are recommended for adoption in the northern region of Tamaulipas. The data generated are essential for guiding selection strategies in breeding programs and for facilitating the rapid development of new genotypes.

Keywords: *Zea mays* L., Hybrids, agronomic parameters, environments.

INTRODUCTION

In plant breeding programs, the final stage involves the evaluation of hybrids or varieties, from which those with the greatest yield potential and desirable agronomic traits are selected and commercialized. In Mexico, in 2024, a total of 6.5 million hectares of corn were planted, with a production of 18.2 million tons and an average yield of 3.6 t ha^{-1} (SIAP, 2025). Of the total corn area planted in the country, 75.0% corresponds to landraces or native varieties, while 25.0% corresponds to improved seed (Espinosa-Calderón *et al.*, 2012; Gaytán-Bautista *et al.*, 2009; Tadeo-Robledo *et al.*, 2015). However, the cost of hybrid seed is high across the different agroecological zones, with the additional limitation that it is marketed in packages of 60,000 seeds per hectare. For this reason, farmers seek alternatives from public institutions or research centers, in order to use their improved corn varieties with the objective of reducing production costs and, consequently, increasing profitability in agricultural production (Ramírez *et al.*, 2024). In this context, the contribution of geneticists in assessing grain yield and other agronomic parameters requires conducting field experiments that involve experimental hybrids, as well as including well-established genotypes in the market, which are used as checks (King *et al.*, 2024).

On the other hand, Duvick (2005) noted that evaluations of new hybrid combinations are frequently carried out by plant breeders to monitor progress in crop production and to estimate the genetic gain of each hybrid. Recently, several studies have evaluated different agronomic traits in a large number of corn hybrids, where hybrids or varieties with high yield potential are identified and selected, with the aim of releasing them commercially so that they can be adopted by farmers (García-Mendoza *et al.*, 2024; Hernández-Martínez *et al.*, 2024; Ni *et al.*, 2024; Sanaev *et al.*, 2024; Shojaei *et al.*, 2024). However, the materials under study differ in certain characteristics, such as their genetic origin, growth cycle, adaptability, and genetic response to climate and soil conditions (Andriienko *et al.*, 2024). Despite extensive research on the evaluation of corn genotypes, no ideal genotype exists for all agricultural regions, particularly when it comes to new hybrids. Therefore, conducting such studies in each region of interest is essential. For this reason, the objective of this study was to estimate the performance and adaptability parameters of simple corn hybrids in the northern region of Tamaulipas, Mexico. This will provide farmers with better alternatives to select the material of their interest, thereby taking advantage of the hybrid's high potential and achieving higher income.

MATERIALS AND METHODS

Base genetic material: As female parents, 217 white-grain corn lines were used, and the male parent was line T-42. These lines were developed within the Corn Breeding Program of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) at the Río Bravo Experimental Station (CERIB), Tamaulipas.

Formation of Single or Hybrid Crosses: In an isolated plot, the 217 corn lines and an elite line (T-42) with good combining ability were planted. The planting was done in a 4:2 ratio, *i.e.*, 4 female rows and 2 male rows. Each line was sown in a single 5.0 m-long row, with a row spacing of 0.80 m. The hybrids were formed during the spring-summer (S-S) 2022 cropping season at INIFAP-CERIB. At flowering, the female lines were detasseled manually by field personnel, leaving the male line intact. Out of the 217 expected combinations, 43 single hybrids were successfully obtained.

Location and Evaluation of the Hybrids: The evaluation was carried out in two environments. The first was at a cooperating farmer's field in the municipality of Díaz Ordaz, Tamaulipas, located at 26°16' N latitude and 98° 32' W longitude, with an altitude of 50-200 m above sea level. The climate is classified as hot, dry, and extreme (Bshw), with temperatures ranging from 22 to 24 °C and an average annual rainfall of 400-600 mm (INEGI, 2025). The second site was at the Río Bravo Experimental Station, Tamaulipas, located at 25° 23' N latitude and 97° 51' W longitude, with an altitude of 50-500 m above sea level. The climate is classified as very hot, semi-dry (Bshw), with temperatures ranging from 20 to 24 °C and annual rainfall of 400-700 mm (INEGI, 2025). A total of 49 genotypes were evaluated, of which 44 were white-grain single or hybrid corn lines from the CERIB-INIFAP Corn Breeding Program, and five were commercial hybrids from the region used as checks (Table 1).

Sowing and Management of the Experiment. In the Díaz Ordaz environment, sowing was carried out on February 4, while in the Río Bravo location it was carried out on February 20. Both experiments were conducted under irrigation during the Fall-Winter 2022-2023 cropping season. Agronomic management in both sites followed the recommendations of INIFAP-CERIB (INIFAP, 2017).

Experimental Design and Plot Unit: In both locations, the experiments were established using a 7×7 lattice design with three replications. The experimental plot consisted of a single row 5.0 m in length, with a row spacing of 0.8 m and plant spacing of 0.20 m, resulting in a population density of 62,500 plants ha^{-1}. At harvest, the effective experimental unit consisted of 40 fully competitive plants.

Study Variables: Grain yield (GY) was estimated based on the field weight of each plot, adjusted for shelling percentage, and extrapolated to kg ha^{-1} at 14% moisture content. Plant height and conrcob height (PH and CH) were measured in centimeters (cm), from the base of the stem to the insertion of the flag leaf (PH) and to the insertion of the main Conrcob (CH). For both variables, five representative plants from each plot were sampled to record the average height. Days to anthesis and silking (DA); the number of days required for the plant to exhibit the male inflorescence (tassel; DA-M) and the female inflorescence (silks; DA-F). In both cases, data were recorded visually when 50% of the plants showed dehiscent anthers (DA-M) and receptive silks (DA-F). Grain moisture (GM); a 100 g grain sample was collected, and measurements were taken with a Dickey-John moisture tester, expressed as a percentage. Root and stalk lodging (RL and SL); the number of plants with an inclination angle equal to or greater than 30° from the vertical position of the plant (RL) and the number of plants lodged per plot, considering as lodged those with stalks completely broken below the main conrcob (SL). Both variables were expressed as percentages. Corncob appearance (CA); corncob quality was evaluated based on the set of cobs in each experimental unit, expressed as a percentage. A scoring scale from 1 to 5 was used, where 1=good and 5=poor. Poor coverage (PC); number of ears in the experimental unit with poor husk coverage, expressed as a percentage.

Table 1. White-grain single corn hybrids evaluated for yield and agronomic traits in two environments of Tamaulipas, F-W 2022-2023 season.

Genotype	Genealogy	Genotype	Genealogy
GEN1	P12GB#-298-5×T42	GEN26	P17GB#-303-18×T42
GEN2	P12GB#-298-8×T42	GEN27	P17GB#-303-23×T42
GEN3	P12GB#-298-11×T42	GEN28	P17GB#-303-26×T42
GEN4	P12GB#-298-12×T42	GEN29	P17GB#-303-29×T42
GEN5	P12GB#-298-13×T42	GEN30	P17GB#-303-30×T42
GEN6	P13GB#-299-6×T42	GEN31	P18GB#-304-2×T42
GEN7	P13GB#-299-9×T42	GEN32	P18GB#-304-13×T42
GEN8	P13GB#-299-12×T42	GEN33	P18GB#-304-16×T42
GEN9	P13GB#-299-20×T42	GEN34	P18GB#-304-19×T42
GEN10	P13GB#-299-21×T42	GEN35	P18GB#-304-29×T42
GEN11	P14GB#-300-6×T42	GEN36	P19GB#-305-2×T42
GEN12	P14GB#-300-8×T42	GEN37	P19GB#-305-3×T42
GEN13	P14GB#-300-11×T42	GEN38	P19GB#-305-15×T42
GEN14	P14GB#-300-12×T42	GEN39	P20GB#-306-1×T42
GEN15	P14GB#-300-19×T42	GEN40	P20GB#-306-2×T42
GEN16	P15GB#-301-3×T42	GEN41	P20GB#-306-5×T42
GEN17	P15GB#-301-8×T42	GEN42	P20GB#-306-7×T42
GEN18	P15GB#-301-23×T42	GEN43	P20GB#-306-8×T42
GEN19	P15GB#-301-25×T42	GEN44	P20GB#-306-12×T42
GEN20	P16GB#-302-5×T42	GEN45	H-440*
GEN21	P16GB#-302-11×T42	GEN46	P3051W&
GEN22	P16GB#-302-13×T42	GEN47	NB722&
GEN23	P16GB#-302-21×T42	GEN48	P3057W&
GEN24	P16GB#-302-23×T42	GEN49	AG2525W&
GEN25	P16GB#-302-23×T42		

*=CERIB-INIFAP check; &=Regional check.

Statistical analysis. A combined analysis of variance across locations was performed, and mean comparisons were conducted using Tukey's test ($p \leq 0.05$) for the study variables. The linear additive model for the combined analysis was:

$$y_{ijk} = \mu + A_j + k(j) + C_i + (CA)_{ij} + e_{ijk}$$

To carry out the combined analysis of variance, SAS® software version 9.4 was used (SAS Institute Inc., 2016).

RESULTS AND DISCUSSION

The study revealed significant differences ($p \leq 0.01$) among genotypes for the variables grain yield (GY), grain moisture (GM), days to anthesis (DA-M), days to silking (DA-F), plant height (PH), corncob height (CH), root lodging (RL), corncob appearance (CA), and poor husk coverage (PC). However, for stalk lodging (SL), no significant differences were observed (Table 2). The differences found among the evaluated hybrids indicate the presence of broad genetic diversity, which can be attributed to the fact that the F1 progenies originated from different genetic backgrounds. Therefore, it is recommended that breeders broaden the genetic base in their breeding programs and evaluate hybrid combinations to gain knowledge of their performance across various traits such as yield (Cervantes-Ortiz *et al.*, 2018). Similar studies agree with these findings, reporting significant differences in yield and agronomic traits in their analysis of variance (García-Mendoza *et al.*, 2021; Santiago-López *et al.*, 2023; Martínez *et al.*, 2024).

Regarding environments, significant differences ($p \leq 0.01$) were observed for most variables, except for RL and SL. This indicates that the environments under study differ in soil, temperature, and precipitation, which primarily influenced these two variables. In this regard, Ponce-Encinas *et al.* (2022) noted that environments vary from year to year, even within the same locations or regions; therefore, they recommend testing diverse genotypes, including both experimental and commercial hybrids. Similarly, García *et al.* (2020) reported that the performance of each genotype depends on the environmental conditions present in each study location.

Regarding the genotype per environment interaction (G×E), the variable showing significant differences was grain moisture (GM). Therefore, it can be inferred that there are genotypes with good stability and adaptability across both environments (Table 2). These results contrast with Rodríguez-Ortega *et al.* (2024), who reported in their combined analysis that the evaluated materials differ in each ecological niche due to the conditions of each environment. The G×E interaction is essential for plant breeders, as its main objective is to evaluate different genotypes across two or more environments in order to determine the genetic potential of the materials under study. This information is used to identify the genotypes with the highest likelihood of success in each region (García *et al.*, 2020). In this regard, this study reveals that there are single hybrids suitable for commercial use that could be successful in the northern region of Tamaulipas. The mean yield of the evaluated single hybrids was 7,515.5 kg ha^{-1}, higher than the national production of 3,600.0 kg ha^{-1} (SIAP, 2025).

According to Tukey's test ($p \leq 0.05$), the best white-grain hybrids were those that demonstrated high efficiency in yield and agronomic parameters in the Díaz Ordaz and Río Bravo locations, Tamaulipas, Mexico (Table 3). In this regard, the experimental hybrids performed similarly in yield compared to the commercial checks; however, the genotypes that stood out were GEN 34, GEN 15, GEN 16, and GEN 24, showing not only adaptability across both environments but also a clear advantage over the transnational materials (P3051W, NB722, P3057W, AG2525W), as well as the hybrid developed by CERIB-INIFAP (H-440). In this regard, the difference in yield between the best experimental hybrid (GEN 34) and the best commercial check (AG2525W) was 814.8 kg ha^{-1}. However, when comparing GEN 34 with the CERIB-INIFAP check, the difference was 4,149.6 kg ha^{-1}. These results validate the superiority of the experimental genotypes and support their recommendation for adoption in the northern region of

Table 2. Mean squares and significance from the analysis of variance of 49 white-grain corn hybrids based on yield and agronomic parameters, evaluated in two environments of Tamaulipas, Mexico, F-W 2022-2023.

S.V.	D.F.	GY	GM	DA-M	DA-F	PH
Environment (E)	1	39251414.2**	148.4**	8959.6**	9070.3**	87535.1**
R/E	4	3156137.4	5.6**	3.2	5.7	259.9
Subb./Rep.×E	30	1143400.0	2.2*	1.3	1.7	123.6
Genotypes (G)	48	3470324.6**	8.8**	30.2**	37.3**	264.8**
G×E	42	2225290.1	2.1*	3.1	1.3	121.9
Error		1794609.2	1.3	2.2	3.2	117.9
C.V. (%)		17.8	8.4	2.0	2.3	4.8
Media		7515.5	13.7	73.7	75.2	223.2
S.V.	**D.F.**	**CH**	**RL**	**SL**	**CA**	**PC**
Environment (E)	1	67628.1**	18.1	2.7	1.3**	2102.9**
R/E	4	148.5	202.6**	22.2	0.0	398.9**
Subb./Rep.×E	30	47.6	25.0	19.7	0.0	16.4
Genotype (G)	48	177.6**	48.5	103.8**	0.2**	40.5**
G×E	42	97.5	29.0	32.0	0.1	28.7
Error		67.1	29.1	28.2	0.1	22.2
C.V. (%)		8.8	169.7	108.6	26.0	110.1
Everage		92.5	3.1	4.8	1.3	4.2

*, **: significantly different at $p \leq 0.05$ and 0.01, respectively. S.V: sources of variation, D.F: degrees of freedom, GY: grain yield, GM: grain moisture, DA-M: days to anthesis male flowering, DA-F: days to anthesis female flowering, PH: plant height, CH: corncob height, RL: percentage of root lodging, SL: percentage of stem lodging, CA: corncob appearance, PC: poor coverage, R/E: repetition within environment, Subl/Reps × E: subblocks within repetitions by environment, G×E: genotype by environment interaction, C.V: coefficient of variation in percentage.

Tamaulipas, as higher productivity implies greater profitability for farmers in this region. These results are similar to those reported by Hernández *et al.* (2024), who found an experimental hybrid (LEARB9×UAY113) with a yield of 9,600.0 kg ha^{-1}, which was also competitive with the commercial checks. These findings suggest that the selection and release of experimental hybrids can translate into competitive advantages over commercial materials. However, before recommending a new hybrid, it is advisable to evaluate it across multiple agroecological environments to identify genotypes that excel in yield, adaptation, and stability (Paz *et al.*, 2018; Crossa *et al.*, 2006).

The grain moisture (GM) variable is crucial in hybrids because it directly affects the quality and yield of the harvested grain. In this context, the hybrids with the highest GM content were GEN 27, GEN 42, GEN 34, and GEN 4, with values above 15.0%. However, genotypes GEN 48, GEN 45, GEN 46, GEN 6, and GEN 10 presented less than 12.0% GM (Table 3). The difference in GM is mainly attributed to the diverse environmental conditions (temperature, relative humidity, and precipitation during crop development) and the genetic characteristics of each genotype. Hybrids with lower GM are recommended for regions with high environmental humidity, as they facilitate postharvest handling

Table 3. Means of 49 white-grain corn hybrids based on yield and agronomic parameters, evaluated in two environments of Tamaulipas, Mexico, F-W 2022-2023.

Hybrids	GY	GM	DA-M	DA-F	PH	CH	PAL	PSL	CA	PC
GEN34	9184.0 a	15.3 abc	75.3 a-g	77.0 a-e	226.1 abc	95.0 abc	4.4 a	6.3 a-e	1.0 a	4.0 ab
GEN15	8656.3 a	14.2 a-g	76.5 abc	78.1 abc	225.5 abc	96.6 abc	5.7 a	5.4 a-e	1.3 a	5.9 ab
GEN16	8443.1 a	13.9 a-h	72.0 g-m	74.1 c-i	219.5 abc	86.0 abc	4.6 a	0.0 e	1.5 a	7.0 ab
GEN24	8435.3 a	14.0 a-g	75.3 a-g	76.8 a-f	217.0 abc	95.8 abc	3.0 a	4.2 b-e	1.0 a	1.1 ab
GEN49[&]	8369.1 a	14.7 a-e	74.1 b-j	75.1 a-i	215.5 abc	85.8 bc	0.0 a	1.5 de	1.4 a	0.1 b
GEN14	8304.0 a	14.8 a-e	73.8 b-k	74.6 b-i	225.3 abc	95.3 abc	0.7 a	12.5 a-d	1.1 a	4.6 ab
GEN11	8239.3 a	14.0 a-g	73.0 d-k	74.0 c-j	215.5 abc	89.3 abc	2.0 a	4.4 b-e	1.0 a	9.2 ab
GEN47[&]	8219.6 a	12.5 d-j	72.1 f-m	72.8 e-k	223.6 abc	87.5 abc	0.0 a	2.0 de	1.6 a	6.3 ab
GEN32	8212.0 a	14.5 a-e	72.6 e-l	74.3 b-i	228.1 abc	94.0 abc	4.3 a	15.9 ab	1.0 a	5.5 ab
GEN46[&]	8204.1 ab	11.3 hij	69.0 mn	69.6 kl	236.3 a	81.3 c	0.0 a	2.4 de	1.4 a	0.6 ab
GEN7	8109.3 ab	12.5 d-j	70.5 klm	71.6 ijk	218.5 abc	91.8 abc	1.3 a	0.5 de	1.4 a	5.7 ab
GEN12	8069.1 ab	14.5 a-e	74.5 b-h	76.1 a-h	228.6 abc	98.1 abc	2.2 a	5.2 a-e	1.4 a	11.4 a
GEN5	8035.6 ab	13.1 a-j	76.0 a-e	76.5 a-h	206.5 c	93.5 abc	7.0 a	5.4 a-e	1.1 a	3.7 ab
GEN13	8025.5 ab	13.9 a-h	73.8 b-k	75.0 b-i	219.8 abc	91.1 abc	2.6 a	11.6 a-e	1.2 a	3.1 ab
GEN28	7977.5 ab	13.7 a-i	73.3 b-k	74.8 b-i	226.1 abc	92.3 abc	3.6 a	2.4 de	1.1 a	2.2 ab
GEN3	7935.5 ab	14.7 a-e	76.6 ab	78.5 ab	215.3 abc	97.1 abc	3.6 a	5.6 a-e	1.1 a	7.4 ab
GEN31	7866.0 ab	13.5 a-i	74.0 b-j	75.8 a-i	235.8 a	97.8 abc	2.0 a	2.2 de	1.2 a	4.5 ab
GEN40	7814.0 ab	13.1 a-j	75.3 a-g	76.8 a-g	228.0 abc	105.1 a	0.7 a	1.3 de	1.1 a	4.5 ab
GEN25	7754.6 ab	14.0 a-h	74.6 a-g	76.3 a-h	225.5 abc	94.1 abc	0.7 a	1.5 de	1.2 a	6.5 ab
GEN1	7737.8 ab	14.6 a-e	75.5 a-f	76.6 a-f	212.6 abc	85.6 bc	9.1 a	4.6 b-e	1.2 a	3.0 ab
GEN26	7723.3 ab	14.5 a-e	73.8 b-k	75.0 b-i	224.0 abc	88.3 abc	0.6 a	1.9 de	1.2 a	2.7 ab
GEN10	7616.0 ab	11.7 f-j	70.3 j-m	72.3 h-k	227.0 abc	87.6 abc	1.2 a	4.4 b-e	1.2 a	5.7 ab
GEN30	7593.1 ab	14.2 a-g	74.5 b-h	76.8 a-f	226.0 abc	96.1 abc	3.4 a	3.2 cde	1.6 a	3.4 ab
GEN23	7585.5 ab	13.2 a-j	73.1 c-k	74.6 b-i	222.3 abc	91.0 abc	9.8 a	10.7 a-e	1.1 a	1.9 ab
GEN27	7512.5 ab	15.7 a	75.0 a-g	77.0 a-e	229.8 abc	95.6 abc	0.0 a	1.1 de	1.2 a	1.0 ab
GEN2	7510.6 ab	14.4 a-f	76.1 a-d	77.5 a-d	212.5 abc	93.1 abc	0.7 a	7.4 a-e	1.4 a	2.7 ab
GEN29	7482.8 ab	14.1 a-g	74.3 b-i	76.0 a-h	231.6 abc	95.5 abc	2.4 a	1.2 de	1.3 a	3.0 ab
GEN18	7456.6 ab	14.7 a-e	73.8 b-k	75.5 a-i	224.6 abc	94.1 abc	0.6 a	4.6 a-e	1.2 a	7.7 ab
GEN17	7437.6 ab	12.9 b-j	73.3 b-k	75.1 a-i	224.3 abc	86.0 abc	2.5 a	3.0 cde	1.5 a	4.7 ab
GEN9	7422.1 ab	13.6 a-i	71.1 h-m	72.5 g-k	227.6 abc	87.8 abc	9.1 a	0.7 de	1.5 a	3.1 ab
GEN42	7418.1 ab	15.4 ab	76.1 a-d	78.0 abc	229.3 abc	100.3 abc	2.7 a	3.0 de	1.2 a	2.1 ab
GEN33	7400.0 ab	14.9 a-e	75.1 a-g	76.8 a-f	222.6 abc	92.3 abc	7.4 a	3.7 b-e	1.3 a	2.0 ab
GEN48[&]	7399.1 ab	10.6 j	69.5 lm	69.8 jkl	220.1 abc	95.3 abc	0.0 a	1.2 de	1.6 a	5.1 ab
GEN22	7218.1 ab	14.8 a-e	74.0 b-j	75.6 a-i	216.5 abc	89.1 abc	3.9 a	1.7 de	1.3 a	6.3 ab
GEN4	7138.5 ab	15.0 a-d	78.0 a	79.3 a	221.8 abc	94.3 abc	12.3 a	5.1 a-e	1.1 a	1.1 ab
GEN44	7028.5 ab	12.9 b-j	73.1 c-k	74.3 b-i	223.6 abc	93.6 abc	3.5 a	7.2 a-e	1.1 a	1.4 ab
GEN19	6964.6 ab	13.1 a-j	73.8 b-k	75.8 a-i	224.5 abc	84.3 bc	0.6 a	2.4 de	1.3 a	6.2 ab
GEN35	6943.3 ab	12.5 d-j	75.1 a-g	77.0 a-e	227.6 abc	95.6 abc	5.0 a	17.1 a	1.3 a	6.7 ab
GEN38	6941.6 ab	13.9 a-h	75.1 a-g	76.8 a-f	223.0 abc	103.0 bc	3.2 a	7.7 a-e	1.2 a	1.9 ab
GEN43	6859.6 ab	13.5 a-i	74.3 b-i	76.6 a-g	224.5 abc	91.0 abc	0.6 a	1.2 de	1.3 a	7.5 ab

Table 3. Continues...

Hybrids	GY	GM	DA-M	DA-F	PH	CH	PAL	PSL	CA	PC
GEN37	6754.0 ab	14.5 a-e	75.3 a-g	77.8 a-d	232.5 ab	90.1 abc	2.9 a	3.8 b-e	1.5 a	2.3 ab
GEN8	6745.0 ab	12.6 c-j	71.0 i-m	72.6 f-k	218.1 abc	95.1 abc	7.5 a	8.9 a-e	1.1 a	4.1 ab
GEN41	6705.6 ab	13.9 a-h	74.5 b-h	76.1 a-h	231.5 abc	98.0 abc	0.0 a	6.1 a-e	1.4 a	4.0 ab
GEN6	6687.3 ab	11.5 g-j	72.6 e-l	73.6 d-k	222.0 abc	93.0 abc	3.0 a	2.5 de	1.7 a	6.0 ab
GEN21	6679.1 ab	14.8 a-e	73.8 b-k	75.5 a-i	217.6 abc	90.3 abc	1.1 a	3.5 b-e	1.1 a	5.4 ab
GEN39	6590.0 ab	13.5 a-i	74.8 a-g	76.6 a-g	226.1 abc	101.1 ab	4.1 a	15.4 abc	1.2 a	2.6 ab
GEN36	6413.5 ab	12.2 e-j	73.6 b-k	75.8 a-i	227.5 abc	90.0 abc	2.5 a	5.9 a-e	1.6 a	1.0 ab
GEN20	6408.8 ab	13.2 a-j	74.0 b-j	76.0 a-h	223.3 abc	84.3 bc	1.3 a	2.1 de	1.5 a	8.9 ab
GEN45*	5034.6 ab	11.1 ij	66.0 n	66.8 l	209.3 bc	83.3 bc	4.7 a	5.8 a-e	1.8 a	1.3 ab
SHD	3141.3	2.7	3.4	4.2	25.4	19.2	12.6	12.4	0.8	11.07

a-n Values with different letters within the same column are significantly different ($P<0.05$).
GY: grain yield, GM: grain moisture, DA-M: days to anthesis male flowering, DA-F: days to anthesis female flowering , PH: plant height, CH: corncob height, RL: percentage of root lodging, SL: percentage of stem lodging, CA: corncob appearance, PC: poor coverage, SHD: significant honest difference. *: CERIB-INIFAP check; [&]: regional check.

and reduce the need for drying, which in turn decreases costs and improves efficiency at harvest. The varieties and grain moisture (GM) content are key factors that affect the mechanical properties of corn. Therefore, it is essential to understand the influence of these relationships at the time of shelling in order to avoid losses during field harvest (Zhu *et al.*, 2023; Li *et al.*, 2018). In this regard, it is suggested that corn harvest in the field should occur when grain moisture is between 14.0 and 18.0%.

Regarding days to male flowering (DA-M) and female flowering (DA-F), the experimental hybrids exhibited variability in both variables. In this sense, the earliest hybrid was GEN 45, with values of 66.0 DA-M and 66.8 DA-F. On the other hand, GEN 15 and GEN 4 were classified as late materials for both DA-M and DA-F. These results allow the identification of early-, intermediate-, and late-cycle materials (Table 3). The data differ from Canales-Islas *et al.* (2024), who reported maize varieties with male flowering at 81 days and female flowering at 82 days, classifying them as late-cycle, while also identifying early-cycle materials with 73 days for male flowering and 75 days for female flowering. Early-cycle hybrids complete their cycle in a shorter period, allowing less time for grain filling and biomass accumulation, which translates into lower productivity. However, these characteristics are important in regions with early frosts or scarce rainfall, or where there is a need to clear agricultural land for the preparation of the next sowing. Therefore, it is necessary to consider these crosses in a breeding program aimed at earliness (Cervantes-Ortiz *et al.*, 2018). In contrast, late-cycle materials have longer developmental periods, which allow them to take better advantage of the available environmental factors and achieve greater accumulation of assimilates during grain filling. As a result, this is reflected in higher yield values. Nevertheless, it is important to consider the most suitable sowing dates for maize growth, particularly early sowings, since crop development may coincide with rainfall, lower temperatures, and increased relative humidity, which affect both the quantity and quality of grain yield (Odhaib and Hassan, 2024).

The hybrid with the lowest plant height (PH) was GEN 45 with 209.3 cm, while the cross with the greatest height was GEN 46 with 236.3 cm. However, for conrcob height (CH), GEN 46 registered 81.3 cm and GEN 40 reached 105.1 cm (Table 3). A wide diversity was observed among the experimental hybrids, which may have been influenced by the evaluation environments as well as the genetic constitution of each hybrid. Plant height (PH) and conrcob height (CH) are traits closely related to planting density and corn lodging resistance. Therefore, appropriately reducing PH and CH can contribute to increased planting density and yield, as well as improve lodging resistance, which in turn facilitates mechanical harvesting in the field during shelling (Li *et al.*, 2025). In this regard, some authors mention that an ideal plant for harvest should have a plant height ranging from 200 to 240 cm and an ear height between 100 and 150 cm (Conceição dos Santos *et al.*, 2019; Hernández and Esquivel, 2004).

Regarding root lodging (RL), the experimental hybrids performed similarly to the hybrids used as checks. The values for this variable ranged from 0.0 to 9.8%. For stalk lodging (SL), differences were observed among the hybrids, indicating genetic divergence for this trait. The SL range in this study was 0.0 to 17.1%, which impacted the yield of each genotype (Table 3). Lodging in plants can be caused by a combination of factors, including genetic conditions, root and stalk diseases (Ramírez-Diaz *et al.*, 2018), and adverse climatic events such as hail, strong winds, and heavy rainfall (Lindsey *et al.*, 2024). These traits are particularly relevant in the northern region of Tamaulipas, where climatic events are unpredictable. Therefore, when recommending the adoption of new materials for this region, it is advisable to prioritize those that exhibit lower lodging incidence, thus ensuring more stable and profitable production under the agroecological conditions of this region.

For the trait conrcob appearance (CA), no significant differences were observed among the hybrids under study; values ranged from 1.0 to 1.7 (Table 3). This indicates that the experimental crosses were similar compared to the checks used. Regarding poor husk coverage (PC), the hybrid with the lowest value was GEN 49 (0.1%), while the cross with the highest PC was GEN 12 with 11.4%. Similar results were reported by Martínez and De León (1996) in the Veracruz region; however, they did not find differences among the evaluated hybrids. There is a close relationship between these two variables, as having hybrids with poor husk coverage negatively affects ear appearance, compromising grain health and reducing the commercial and agronomic quality of the ear.

CONCLUSIONS

There is wide genetic variability among the evaluated genotypes. This diversity indicates potential for recommending materials with superior agronomic traits and higher yield potential, such as GEN 34, GEN 15, GEN 16, and GEN 24, which demonstrated not only adaptability across both environments but also an advantage over transnational materials. The data generated are essential for guiding selection strategies in a breeding program, facilitating the rapid development of new genotypes.

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Colegio de Postgraduados

Preliminary study of the hormetic effect of nitrate and chloride of cerium on the micropropagation of *Stevia rebaudiana* Bertoni

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ABSTRACT

Objective: This study aimed to evaluate the effect of cerium nitrate and cerium chloride on morphophysiological and biochemical variables in *S. rebaudiana* grown *In vitro*.

Design/methodology/approach: *S. rebaudiana* explants were grown for 30 days on MS medium supplemented with cerium nitrate or chloride at 0, 10, 20, 30, 40 and 50 μM concentrations, respectively.

Results: It was observed that cerium nitrate at a concentration of 10 μM stimulated shoot proliferation (10.55 ± 1.11) and plant growth (12.12 ± 0.51 cm), whereas cerium chloride at the same concentration favoured plant height (13.25 ± 0.53 cm). Cerium nitrate at a concentration of 30 μM significantly increased chlorophyll content (0.957 ± 0.005 mg g^{-1} FW). In addition, a hormetic effect was observed with both cerium salts, *i.e.*, biostimulation at low concentrations and lethal toxicity at the maximum concentration evaluated (50 μM), showing a greater toxic effect with the chloride counterion.

Limitations on study/implications: This study constitutes a preliminary *in vitro* research; although cerium has a beneficial effect during the micropropagation of *S. rebaudiana*, its effectiveness must be evaluated through additional *ex vitro* studies.

Findings/conclusions: Controlled and adequate doses can be used to obtain the highest biostimulant effect on *S. rebaudiana* shoot proliferation *In vitro*.

Keywords: Biostimulant, Stevia, Hormesis, Micropropagation, Toxicity.

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INTRODUCTION

S. rebaudiana is the only species in the *Stevia* genus with non-caloric steviol glycosides up to 300 times more sweetness than sucrose (Ahmad *et al.*, 2020). Industrially, *S. rebaudiana* plants are used as raw material to extract sugar substitutes, which according to medical studies, improves health and corrects metabolic disorders such as diabetes (Patel and

Navale, 2024). In addition, it presents bioactive components with therapeutic activity in various human health conditions (Jahangir *et al.*, 2020).

Currently, a large number of propagules are required to establish commercial plantations. Conventional propagation of *S. rebaudiana* is limited by genetic variation, low seed viability and complicated rooting of vegetative cuttings, which generates a limited number of individuals (Khalil *et al.*, 2014; Ramírez-Mosqueda *et al.*, 2016; Simlat *et al.*, 2020). The growing demand for *S. rebaudiana* propagules worldwide generates the need to establish techniques for its mass propagation, as well as to improve the physiological vigour and potentiate the productivity of this crop (Angelini *et al.*, 2018; Abdelsattar *et al.*, 2023).

Plant tissue culture (PTC) is a biotechnological strategy that allows obtaining many commercial propagules in a short time and space (Negi *et al.*, 2024). This technique has been used in *In vitro* micropropagation of *S. rebaudiana* to establish commercial plantations (Sivasankarreddy *et al.*, 2021). Ramírez-Mosqueda and Iglesias-Andreu (2016) reported *In vitro* micropropagation of *S. rebaudiana* through the thin-layer method. Meanwhile, other authors established the massive micropropagation of *S. rebaudiana* through temporary immersion systems (Ramírez-Mosqueda *et al.*, 2016; Medorio-García *et al.*, 2024). Currently, natural alternatives with lower environmental impact are being sought, which can replace or minimize the use of synthetic plant growth regulators (Le *et al.*, 2020; Yang *et al.*, 2021). Sridhar and Aswath (2014) increased the number of shoots in *S. rebaudiana* using casein hydrolysates as a biostimulant. Similar results have been obtained with yeast extracts, alginates (Bayraktar *et al.*, 2016) and silver nanoparticles (Castro-González *et al.*, 2019).

Biostimulants are substances and microorganisms that induce metabolic mechanisms and responses that lead to improved plant development, nutrient uptake, stress tolerance, and vigour (Du Jardin, 2015; Trejo-Téllez and Gómez-Merino, 2023). Some light lanthanides (La^{3+}, Pr^{3+}, Nd^{3+}, $Eu^{3+}/^{2+}$, $Ce^{3+}/^{4+}$) stand out as inorganic biostimulants because they exhibit a beneficial effect at low doses (Trejo-Téllez and Gómez-Merino, 2023). However, these compounds can induce toxic effects at high concentrations, adjusting to the response to a hormesis curve (Bello-Bello *et al.*, 2024). In plant biology, hormesis is a biphasic dose-response phenomenon in which low stressor concentrations induce beneficial effects, such as biostimulation or eustress on morphophysiological variables. In contrast, high concentrations cause inhibition or toxicity. (Godínez-Mendoza *et al.*, 2023; Erofeeva, 2022). This phenomenon has potential applications in plant biotechnology, since the statistical analysis of the hormetic curve allows for identifying the maximum stimulation dose (M) and, simultaneously, to establish the stimulation limit (LDS), which facilitates optimizing the positive response and minimizing adverse effects (Belz and Duke, 2022). Several studies detail the biostimulant effect of lanthanides on plants (Öztürk *et al.*, 2023). For example, in soybean plants, lanthanum application increased nutrient uptake and biomass production (de Oliveira *et al.*, 2015). Zhang *et al.* (2013) reported that biostimulation of medicinal plants with lanthanum, cerium, neodymium or europium can regulate secondary metabolism. Likewise, dosing with lanthanum on tobacco seedlings infected by mosaic virus decreased adverse symptoms (Yongsheng *et al.*, 2012). Cerium also has a regulatory and antagonistic effect on Cu^{2+} detoxification in *Dendrobium nobile*

(Li *et al.*, 2023) and *Spinacia oleracea* under Mg^{2+} deficiency stress (Yuguan *et al.*, 2009). However, light lanthanides present a hormetic effect; at low concentrations, they present stimulation and at high concentrations, a toxic effect, depending on the species of plant and the availability of the metal (Jiang *et al.*, 2023; Öztürk *et al.*, 2023). Cerium exhibits chemical characteristics like Ca^{2+} (Lutz *et al.*, 2012). These properties favour ionic competition (mainly with Ca^{2+} and Mg^{2+}) for organic molecules such as chlorophylls (He *et al.*, 2020; Song *et al.*, 2021), proteins and other organic ligands (Chen *et al.*, 2000; Liu *et al.*, 2011; Dressler *et al.*, 2014; Li *et al.*, 2024). While the stimulating effect of lanthanides can modulate the expression of antioxidant proteins, photosynthetic activity and the homeostasis of micronutrients and phytohormones (Grosjean *et al.*, 2024; Li *et al.*, 2024), their toxic effect activates specific defense mechanisms, such as the synthesis of chelating compounds (phytochelatins, metallothioneins and organic acids) that facilitate the subsequent sequestration of lanthanides in the vacuole (Sharma *et al.*, 2016). Increasing interest has been in adding biostimulants to the *In vitro* culture medium to improve micropropagation efficiency and minimize production costs (Carmo *et al.*, 2021). Recently, lanthanides have emerged as a sustainable alternative, due to their biostimulant effects at concentrations in the range of 1-500 μM (Öztürk *et al.*, 2023; Cruz-Cruz *et al.*, 2024). Studies evaluating the effect of light lanthanides in the micropropagation of plant species are limited. Therefore, this study aimed to evaluate the effect of $Ce(NO_3)_3$ and $CeCl_3$ on the morphology and photosynthetic pigment synthesis during *In vitro* propagation of *S. rebaudiana*.

MATERIALS AND METHODS

Plant material and cerium salts

In vitro-grown seedlings of *Stevia rebaudiana* cv. Morita II, maintained through three subcultures, were obtained from the collection of the Laboratory of Teaching and Services in Biotechnology and Plant Cryobiology at the Faculty of Chemical Sciences, Universidad Veracruzana. Cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) and cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$) were procured from Merck Co. (Darmstadt, Germany).

Treatments for the evaluation of the effect of $Ce(NO_3)_3$ and $CeCl_3$ in *In vitro* micropropagation of *S. rebaudiana*

Nodal segments measuring 3 cm in length and containing a single axillary bud were used as explants. Culture flasks were each supplemented with 30 mL of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), with three explants per flask constituting an experimental unit, and three replicates per treatment. Various concentrations (0, 10, 20, 30, 40, and 50 μM) of cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) and cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$) were evaluated. The medium's pH was adjusted to 5.7 using 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, and solidified with 3 g L^{-1} of Phytagel®. Sterilization was carried out via autoclaving at 121 °C and 115 kPa for 20 minutes. All cultures were incubated in a controlled growth chamber for 30 days at 25±2 °C, under an irradiance of 68 μmol m^{-2} s^{-1} and a photoperiod of 16/8 h (light/dark).

Evaluation of morphometric variables

After 30 days of culture, the number of shoots per explant was recorded. Plant height (cm) and root length (cm) were measured using millimeter paper. Additionally, survival rate (%) and root induction (%) were assessed. For dry weight determination, 0.5 g of plant tissue was dehydrated in Petri dishes within a drying oven at 70 °C for 48 hours. The dried samples were then weighed using an analytical balance (OHAUS PA224).

Evaluation of photosynthetic pigment synthesis

Chlorophyll a, chlorophyll b, and total chlorophyll content were quantified following the method described by Harborne (1973). For each sample, 0.1 g of fresh leaf tissue was weighed, frozen with liquid nitrogen, and ground before being macerated in 10 mL of 80% acetone. The extracts were stored at −4 °C for 24 hours. Subsequently, the samples were filtered using Whatman No. 1 filter paper and the final volume was adjusted to 20 mL with 80% acetone. A 2 mL aliquot was transferred to quartz cuvettes and analyzed using a Genesys™ 10S UV-Vis spectrophotometer. Absorbance readings were taken at wavelengths of 645 nm and 663 nm.

Statistical analysis

A completely randomized experimental design was employed, with all treatments replicated three times. Data were recorded and statistically analyzed using R software version 4.5.0 (https://www.r-project.org/). An analysis of variance (ANOVA) was performed, followed by Tukey's *post hoc* test at a significance level of $p \leq 0.05$. For dose-response variables exhibiting a hormetic pattern, polynomial regression models were applied (Equation 1).

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \ldots + \beta_n x^n + \varepsilon \quad (1)$$

The dataset was randomly divided into training (80%) and testing (20%) subsets for model validation. Polynomial models were fitted and evaluated based on the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and residual analysis. Predictive performance was assessed using the Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and the coefficient of determination (R^2). Additionally, the maximum stimulation dose (M) and the Limiting Dose for Stimulation (LDS) were estimated from the fitted models. Finally, a Spearman correlation analysis was conducted across all evaluated variables.

RESULTS AND DISCUSSION

After 30 days of treatment, significant morphometric differences were observed across the evaluated response variables (Figure 1). A 100% survival rate was recorded for treatments with cerium salt concentrations ranging from 0 to 40 μM. In contrast, the 50 μM concentration of both $Ce(NO_3)_3$ and $CeCl_3$ exhibited toxic effects, resulting in 0% survival and complete inhibition of growth across all measured parameters.

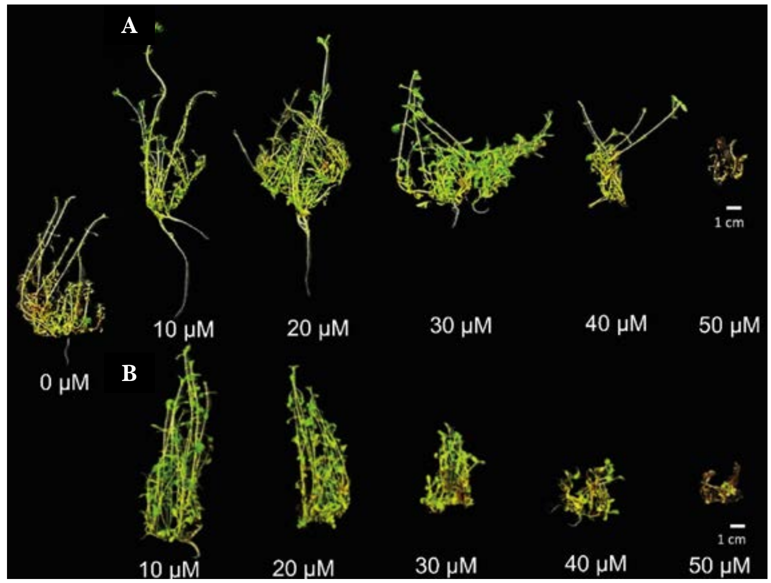

Figure 1. Effect of $Ce(NO_3)_3$ and $CeCl_3$ on *S. rebaudiana* micropropagation after 30 days. A) $CeCl_3$ treatments; B) $Ce(NO_3)_3$2 treatments.

A significant increase in shoot number was observed at the 10 μM $Ce(NO_3)_3$ treatment, with an average of 10.5±1.11 shoots per explant, compared to the control (5.7±0.52 shoots). This was followed by the 10 μM $CeCl_3$ and 20 μM $Ce(NO_3)_3$ treatments, which produced 9.1±1.16 and 7.8±0.77 shoots per explant, respectively (Figure 2A). Conversely, a decline in shoot formation was recorded at 40 μM concentrations of both $Ce(NO_3)_3$2and $CeCl_3$, yielding 3.5±0.33 and 3.1±0.11 shoots per explant, respectively. At the highest tested concentration (50 μM), both cerium salts completely inhibited shoot production.

These findings are consistent with previous studies on the biostimulant activity of rare earth elements. Xu *et al.* (2016) reported a 150% increase in shoot formation compared to the control during *In vitro* micropropagation of *Anoectochilus roxburghii* treated with $La(NO_3)_3$ and $Ce(NO_3)_3$. Similarly, Liu *et al.* (2021) documented enhanced shoot proliferation in *Dendrobium aphyllum* following treatment with 100 μM $Ce(NO_3)_3$. Corresponding trends were observed by Liu *et al.* (2018) in *Dianthus caryophyllus* cultured *In vitro* and exposed to lanthanum, cerium, or neodymium at concentrations ranging from 50 to 150 μM.

Significant differences in plant height were observed among the treatments (Figure 2B). The highest increase in height was recorded at 10 μM $CeCl_3$, reaching 13.2±0.53 cm, compared to the control (8.8±0.95 cm). Although the 10 μM $Ce(NO_3)_3$ treatment produced an average height of 12.1±0.51 cm higher than the control this increase was not statistically significant. In contrast, plant growth was inhibited at 40 μM concentrations of both cerium salts, with $CeCl_3$ (5.2±0.52 cm) showing significant suppression relative to the control. These findings demonstrate that low concentrations of cerium promote plant elongation, whereas higher concentrations exert inhibitory effects. Furthermore, plant height and shoot number exhibited a strong positive correlation (ρ=0.9) (Figure S1).

The marked increase in vegetative elongation at low concentrations aligns with typical responses within the hormetic dose-response framework, as reported in sugarcane

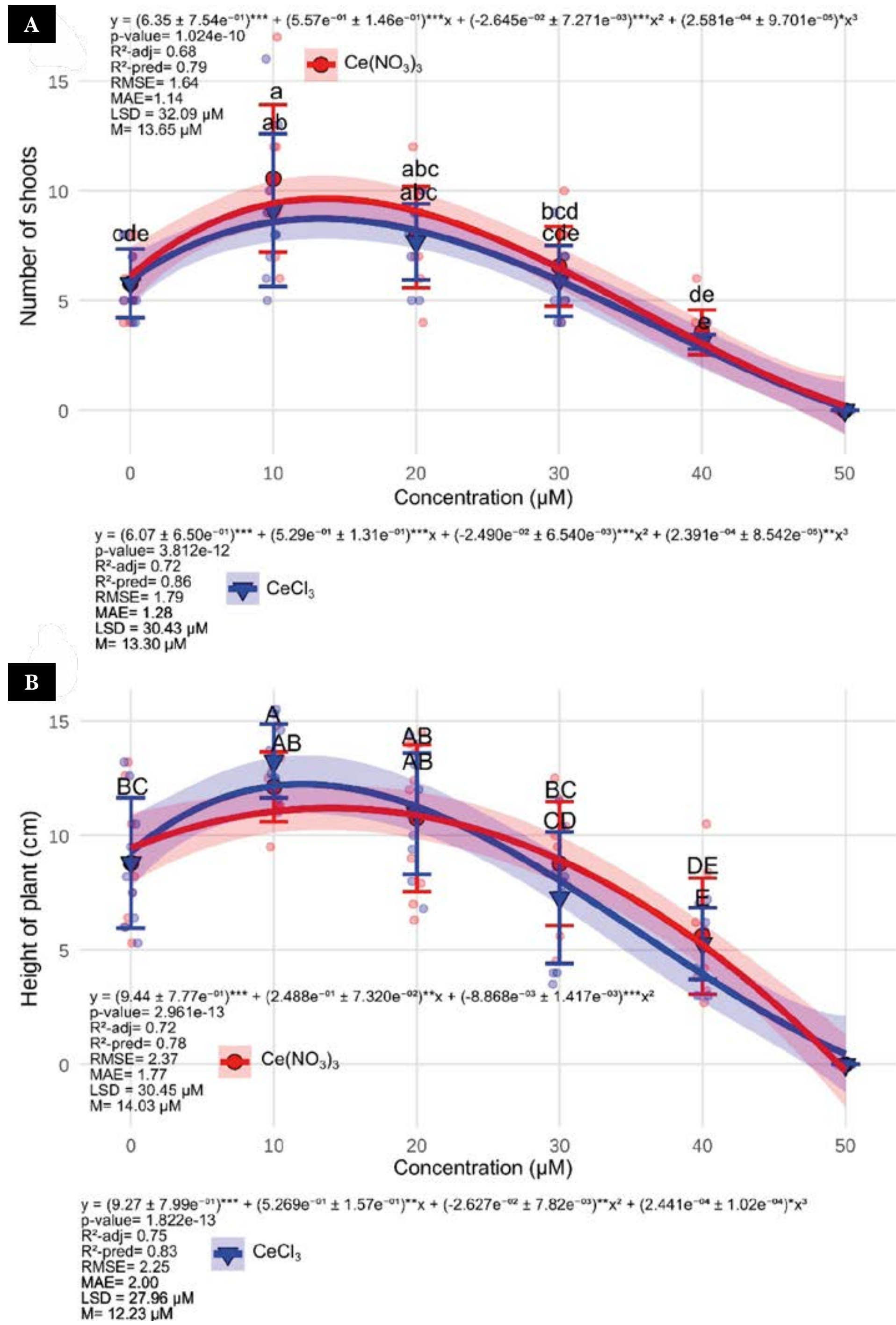

Figure 2. Hormetic dose-response effects of cerium salts on shoot proliferation (A) and plant height (B) in *S. rebaudiana* micropropagation after 30 days. Statistical significance is indicated as $p<0.05$*, $p<0.01$**, $p<0.001$***.

(Ramírez-Antonio *et al.*, 2023), rice (Ramírez-Olvera *et al.*, 2018), and other species (Rico *et al.*, 2014; Sobarzo-Bernal *et al.*, 2021), likely due to enhanced nutrient uptake and elevated metabolic activity (Yang *et al.*, 2019). Based on the shoot proliferation data, the polynomial model estimated an optimal stimulation dose (M) of 13.65 μM for $Ce(NO_3)_3$ and 13.30 μM for $CeCl_3$, while the Limiting Dose for Stimulation (LDS) was 32.09 μM and 30.43 μM, respectively. Beyond these thresholds, shoot production declined sharply. For plant height, the model estimated M=14.03 μM and LDS=30.45 μM for $Ce(NO_3)_3$, and M=12.23 μM and LDS=27.96 μM for $CeCl_3$. Both variables followed a similar pattern, with a pronounced stimulatory effect at low concentrations and marked inhibition at higher doses. Hormesis, characterized by a biphasic dose-response, occurs when low doses of a stressor (*e.g.*, biostimulants, toxic agents, UV radiation) elicit beneficial effects, while high doses result in toxicity (Erofeeva, 2023; Godínez-Mendoza *et al.*, 2023). This phenomenon has been widely documented in plants, such as *Astragalus membranaceus* treated with yeast extract (Park *et al.*, 2021), *Cucumis sativus* with chitosan (Jogaiah *et al.*, 2020), *Salvia sclarea* with zinc (Moustakas *et al.*, 2022), and *Capsicum annuum* with silicon (Trejo-Téllez *et al.*, 2020). The phytotoxicity of a compound depends on multiple factors, including its chemical nature, concentration, physicochemical properties, mechanism of action, interaction with the environment (*e.g.*, absorption and translocation), and plant-specific biological characteristics (Madanayake and Adassooriya, 2021). Recently, the phytotoxic mechanisms of lanthanum at elevated concentrations (0.1-5 mM) were elucidated in rice (Jiang *et al.*, 2023). Therefore, identifying optimal biostimulant doses within the hormetic zone is crucial and must consider plant species and environmental or chemical factors that influence lanthanide bioavailability (Erofeeva, 2022; Wiche and Pourret, 2023).

Regarding root length, low concentrations of cerium did not yield statistically significant differences (Table 1). However, the highest root length (3.46±0.50 cm) was observed at 30 μM $Ce(NO_3)_3$. At higher concentrations (40-50 μM), both cerium salts significantly inhibited root development compared to the control (1.92±0.79 cm). Root induction percentage improved in the 10-30 μM range for both salts, ranging from 66% to 100%, relative to the control (55%). In contrast, treatments at 40-50 μM resulted in a marked reduction in root induction, falling below 22%.

Luo *et al.* (2008) reported enhanced root development during the *In vitro* rooting stage of *Dendrobium densiflorum* treated with $Nd(NO_3)_3$. In contrast, studies in *Arabidopsis thaliana* demonstrated that lanthanum exposure inhibited primary root meristem growth due to the overproduction of reactive oxygen species (ROS), which led to increased lateral root formation and a restructured root system. These effects were associated with disrupted auxin signaling and the upregulation of genes involved in auxin biosynthesis (Liu *et al.*, 2016).

Regarding dry weight (Table 1), an increase over the control (0.0511±0.002 g) was observed in treatments with 40 μM $CeCl_3$ and 10 μM $Ce(NO_3)_3$, reaching 0.0600±0.005 g and 0.0565±0.006 g, respectively; however, these differences were not statistically significant. Conversely, a significant reduction in dry weight was noted at 20, 30, and 10 μM $CeCl_3$, with values of 0.0315±0.003, 0.0325±0.011, and 0.0370±0.002 g, respectively, relative to the control and the $Ce(NO_3)_3$ treatments at 10-40 μM. Notably, root induction ($\rho=-0.81$) and root length ($\rho=-0.58$) were negatively correlated with dry weight, suggesting a trade-off in resource allocation for root system development at cerium concentrations between 10 and 30 μM. These findings also indicate a more pronounced toxic effect of cerium in its chloride form compared to its nitrate counterpart.

Table 1. Effect of $Ce(NO_3)_3$ and $CeCl_3$ on *S. rebaudiana* micropropagation after 30 days of treatments on morphometric and biochemical variables.

Cerium salt	Concentration (μM)	Root length (cm)	Root induction (%)	Dry weight (g)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)
Control	0	1.92±0.79cde	55.5	0.0511±0.002a	0.404±0.019b	0.194±0.001b	0.599±0.018b
$Ce(NO_3)_3$	10	2.28±0.69a	66.6	0.0565±0.006a	0.207±0.015d	0.107±0.010de	0.315±0.025d
	20	1.61±0.50abc	66.6	0.0521±0.003a	0.364±0.010bc	0.169±0.005bc	0.533±0.016bc
	30	3.46±0.50bcd	100	0.0510±0.001a	0.630±0.000a	0.327±0.005a	0.957±0.005a
	40	0.58±0.39de	22.2	0.0560±0.007a	0.209±0.009d	0.104±0.018de	0.314±0.008d
$CeCl_3$	10	1.94±0.55ab	77.7	0.0370±0.002b	0.218±0.017d	0.107±0.008de	0.326±0.026d
	20	1.98±0.50abc	88.8	0.0315±0.003b	0.331±0.013c	0.146±0.003cd	0.477±0.017c
	30	2.80±0.44cde	100	0.0325±0.011b	0.181±0.021d	0.088±0.013e	0.270±0.034d
	40	0.16±0.16e	11.1	0.060±0.005a	0.186±0.001d	0.118±0.009de	0.305±0.008d

Higher dry weights were recorded in *In vitro* seedlings treated with 10-40 μM $Ce(NO_3)_3$. This increase may be attributed to cerium accumulation in cell walls, chelating molecules, and calcium-binding sites, which, in turn, may promote nutrient uptake through induced systemic endocytosis (Lai *et al*., 2006; Cheng *et al*., 2012; Kovaříková *et al*., 2019). On the other hand, seedlings exposed to 10-40 μM $CeCl_3$ exhibited reduced dry weight, possibly due to the counterion effect. While chloride acts as a micronutrient involved in osmoregulation, elongation growth, and nitrate/water efficiency (Geilfus, 2018a; Hu *et al*., 2023), elevated chloride levels may disrupt nitrate assimilation (Geilfus, 2018b). Significant differences in photosynthetic pigment content were also observed among treatments (Table 1), with strong positive correlations ($\rho \geq 0.85$) among pigment variables (Figure S1). The highest chlorophyll a content (0.630±0.000 mg g^{-1} FW) was obtained at 30 μM $Ce(NO_3)_3$, significantly higher than the control (0.404±0.019 mg g^{-1} FW). In contrast, significantly reduced chlorophyll a levels were observed in the 30 μM $CeCl_3$, 40 μM $CeCl_3$, 40 μM $Ce(NO_3)_3$, and 10 μM $CeCl_3$ treatments, with values of 0.181±0.021, 0.186±0.001, 0.209±0.009, and 0.218±0.017 mg g^{-1} FW, respectively.

For chlorophyll b, the 30 μM $Ce(NO_3)_3$ treatment also showed a significant increase (0.327±0.005 mg g^{-1} FW) over the control (0.194±0.001 mg g^{-1} FW), whereas the greatest decrease was observed at 40 μM $CeCl_3$ (0.088±0.013 mg g^{-1} FW). Regarding total chlorophyll, the highest content was recorded in the 30 μM $Ce(NO_3)_3$ treatment (0.957±0.005 mg g^{-1} FW), which was significantly greater than the control (0.599±0.018 mg g^{-1} FW). In contrast, treatments with 30 μM $CeCl_3$, 40 μM $CeCl_3$, and 40 μM $Ce(NO_3)_3$ showed significant reductions, with values of 0.270±0.034, 0.305±0.008, and 0.314±0.008 mg g^{-1} FW, respectively. These results indicate that cerium nitrate enhances photosynthetic pigment synthesis, whereas cerium chloride either has no effect or exerts a negative impact.

Several studies have reported the biostimulatory effects of light lanthanides on pigment biosynthesis through the upregulation of photosynthesis-related enzymes (Yuguan *et al*., 2009; Sobarzo-Bernal *et al*., 2021). Additionally, nitrate ions (the counterion in $Ce(NO_3)_3$ serve as a direct nitrogen source, reduced to nitrite $\left(NO_2^-\right)$ and subsequently to ammonium $\left(NH_4^+\right)$ via nitrate and nitrite reductase enzymes, thus promoting chlorophyll and amino acid synthesis (Wang *et al*., 2012). In contrast, $CeCl_3$ reduced pigment levels, likely due to chloride's antagonistic effect on nitrate uptake (Geilfus, 2018b). Related toxic effects of lanthanides have also been observed in rice (*Oryza sativa*), where lanthanum exposure increased foliar Cu, Mn, and Zn levels, while decreasing Mg content and photosynthetic pigment concentrations. Photosystem activity was impaired due to reduced electron transport and increased energy dissipation, attributed to structural damage in proteins, chloroplasts, and thylakoid membranes (Jiang *et al*., 2023).

In this study, a significant increase in the number of shoots per explant was observed in *In vitro* plants exposed to low concentrations of cerium salts. In contrast, high concentrations of lanthanides suppressed shoot formation, likely due to the disruption of membrane proteins and the activation of metabolic reprogramming to mitigate stress induced by elevated cerium ion levels (Erofeeva, 2023; Jiang *et al*., 2023). Thus, precise and controlled dosing is essential to maximize the biostimulant potential of cerium for promoting shoot proliferation in *S. rebaudiana* under *In vitro* conditions.

CONCLUSION

The biostimulant effects of $Ce(NO_3)_3$ and $CeCl_3$ were clearly demonstrated during the *In vitro* micropropagation of *S. rebaudiana*, with significant enhancements in shoot proliferation and plant height. Notably, $Ce(NO_3)_3$ at concentrations of 10-20 μM produced the greatest increases in shoot number and dry weight. A hormetic response was identified, wherein low concentrations of lanthanide salts stimulated plant growth, while higher concentrations (40-50 μM) exerted phytotoxic effects. These findings underscore the critical importance of optimizing biostimulant dosages to maximize physiological benefits within the hormetic range.

Further research is warranted at additional evaluation levels such as in temporary immersion bioreactors and *ex vitro* conditions to validate and fine-tune the effective concentrations for large-scale applications. Moreover, the integration of omics approaches could provide valuable insights into the molecular mechanisms underpinning the biostimulant activity of cerium-based compounds.

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Supplementary material

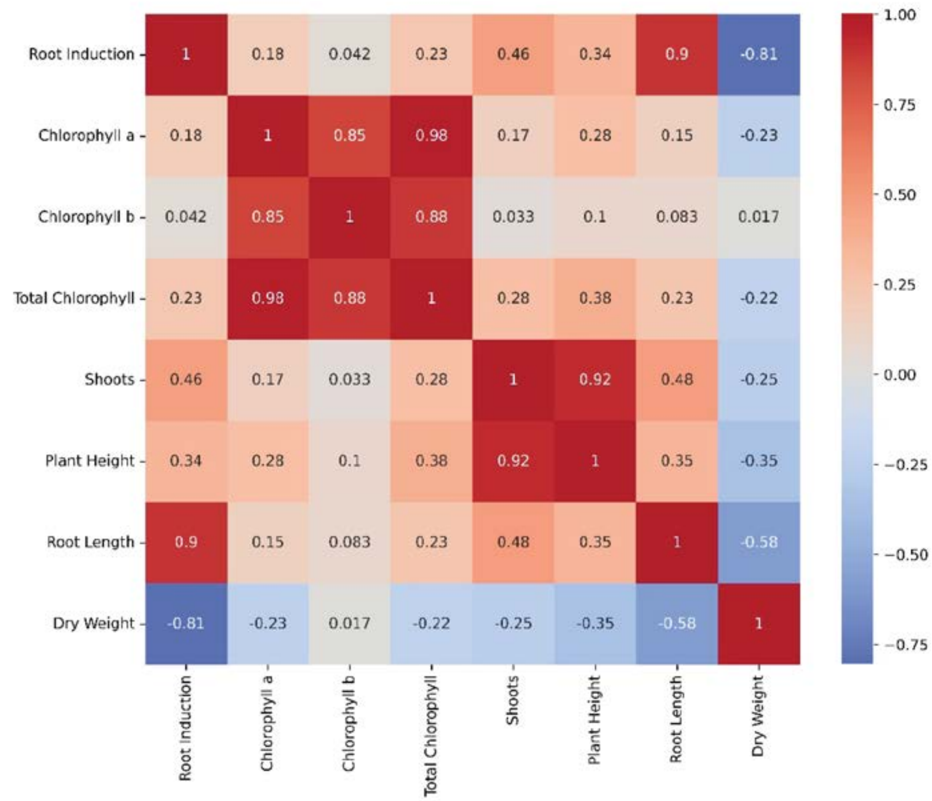

Figure S1. Spearman correlation matrix of morphophysiological and biochemical variables in *S. rebaudiana* micropropagation after 30 days of Ce$(NO_3)_3$ and $CeCl_3$ treatments.

Colegio de Postgraduados

Use of vermicompost in the production of crops with emphasis on *Coffea arabica* L.

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ABSTRACT

Objective: To obtain a comprehensive view of the impact and applications of the use of vermicompost in agriculture in different types of crops and especially in the production of *Coffea arabica* L.

Design/methodology/approach: Data were obtained from the Scopus data metabase, using the concepts "crop", "vermicompost" and "coffee" to search in the title, abstract and keywords, and considering scientific articles and book chapters. The main journals, countries and institutions that have published on the subject were recognized. The data was analyzed using the VOSviewer software to determine the co-occurrence of the terms.

Results: India contributed 47% of the publications, followed by China with 4% and Mexico with 3%. Research on vermicompost focuses on the characterization of its physicochemical properties, and evaluations for use as substrates, organic amendments and organic fertilizer. It is an agent of biological control of pests and diseases transmitted by the soil; it is a technological alternative for in situ stabilization of heavy metals; and, in addition, it is a viable option to mitigate greenhouse gases: ammonia (NH_3) and carbon dioxide (CO_2).

Study limitations/implications: This study did not consider other academic search engines as Google Scholar, Science Direct, among others.

Findings/conclusions: Vermicompost is increasingly used to improve soil nutrition and fertility in horticultural systems. In coffee (*Coffea arabica* L.), it is used in seed germination and as a substrate for seedlings in nursery.

Keywords: soil organic carbon (SOC), fertility, organic fertilizers, nutrient recycling.

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INTRODUCTION

In the presence of a growing demand for quality foods within the context of climate change, loss of arable lands, increase and degradation of soils, it is essential to generate alternatives that strengthen a more sustainable agricultural and forestry production.

Vermicompost is a stable product that is generated through recycling of organic residues by way of using earthworms with organic matter feeding habits, such as: *Eisenia andrei*, *Eisenia fetida*, *Dendrobaena veneta*, *Eudrilus eugiensis*, *Perionyx ceylanesis*, *Lumbricus rubellus*, *Lampito mauritii*, among others [1,2,3]. These grind and enrich organic matter through

their digestive tract with many beneficial microorganisms that support the regeneration and improvement of soil fertility [3].

In addition to its agronomic value, vermicompost has been considered a viable option to mitigate CO_2 emissions, since it promotes retention, stabilization and an increase in soil organic carbon (SOC). It also contributes to decreasing the volatilization of greenhouse gases such as carbon dioxide (CO_2) and ammonia (NH_3) [4].

Other studies have demonstrated the potential of vermicompost to control nematodes of the root knots (*Meloidogyne incognita*) through antagonist bacteria, such as *Peribacillus frigoritolerans* and *Lysinibacillus fusiformis*; these microorganisms produce chitinase and protease that prevent the normal development of eggs and second-stage juveniles [5]. Vermicompost has been studied to reduce and relieve the dissemination of resistance genes to antibiotics generated by animal feces in agricultural production due to their microbial load [6]. It has also been used in the remediation of lands contaminated by pesticides, such as atrazin which is degraded by *Proteobacteria*, *Firmicutes* and *Actinobacteria* present in vermicompost [5,7]. It has also been used as a technological alternative to stabilize heavy metals in situ such as cadmium, lead, chrome, arsenic, in contaminated soils, minimizing their negative effects on the production of grains and foods of global importance [8,9]. In addition, it has been proven to be useful for the reduction of salinity and the increase of drought tolerance, when improving the stability of cellular membranes and antioxidant enzymatic activity [10,11]. Because of this, a review was conducted to define an integral view of the impact and applications of the use of vermicompost in agriculture in different types of crops, primarily in the production of *Coffea arabica* L.

MATERIALS AND METHODS

This literature review followed a quantitative analysis, using two methods: scientific mapping by using bibliometric software and yield through the analysis of publications in function of authors, countries and institutes. The metasearch engine chosen was Scopus because of its broad coverage to recover information. The study was conducted with words such as "vermicompost" and "crop" for a general search, and "vermicompost" and "coffee" for the specific search, referring to scientific articles and book chapters in title, abstract and keywords. No time constraints were established, since the intention was to visualize these two concepts in the entire period. In the general exploration, 1533 references were found and 41 in the specific one (1990 by September 23, 2024) (Figure 1).

For the bibliometric study and the performance analysis of journals, countries and institutes, the cutting point was 10 publications. Likewise, 10 articles were selected that referred to the application of vermicompost on main crops and fruit trees, highlighting the "dose" and "benefits" that it contributes to production. Similarly, according to the specific search, 10 articles were selected that referred to the application of vermicompost in coffee production (*Coffea arabica* L.).

Content analysis

For each search (general and specific), a co-occurrence analysis was conducted. For the general analysis, the frequency value of ≥ 10 registries was used as minimum value

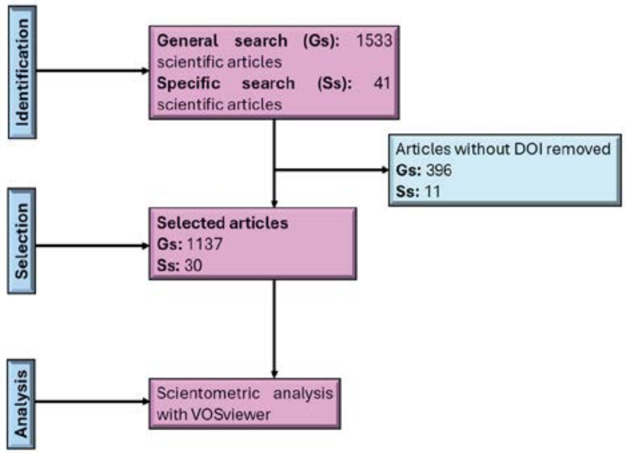

Figure 1. Flowchart of the bibliometric review (prepared by the authors).

and cluster size of 15 [12]. For the specific analysis, a frequency value of 5 was used. To refine terms in both processes, concepts related to the research process were eliminated (for example, statistical design, method, etc.). The algorithm is designed to place the terms that appear in the same year closer one to another, and for those with higher frequency value to be placed in circles with greater diameter. The terms that were not relevant for the map were eliminated [13].

RESULTS AND DISCUSSION

The number of publications analyzed was 1137, which addressed the use of vermicompost in crop nutrition in the period of 1990-2024.

Analysis of the study period

Publications in the period of 1990 to 2006 were fewer than 20 per year. Starting in 2010, an increase in manuscripts addressing the topic begins, and the maximum peak was observed in the years 2022 and 2023 with an average of 150 publications per year (Figure 2). For the entire period, the mean was 52.86±49.58 articles, with a minimum of 1 (1990-1999) and a maximum of 151 (2022).

Table 1 shows the ten main journals, countries and institutes with publications on "vermicompost" and "crop". From a total of 160 journals, the ones that concentrated the largest number of publications were Indian Journal of Agricultural Sciences, Indian Journal of Agronomy, Communications in Soil Science and Plant Analysis, Plant Archives, and Ecology Environment and Conservation, representing 20% of the total.

Regarding the countries that have developed more research in the topic from 1990-2024, they were: India (890), China (88), United States (84), Iran (69), and Mexico (65).

The contributions from the authors cover 160 institutions, with India standing out as one of the main countries in this field. Among the institutions, there are the following: ICAR - Indian Agricultural Research Institute, New Delhi, Indian Council of Agricultural Research, CCS Haryana Agricultural University, Banaras Hindu University, and Bidhan Chandra Krishi Viswavidyalaya, which lead with the largest number of publications.

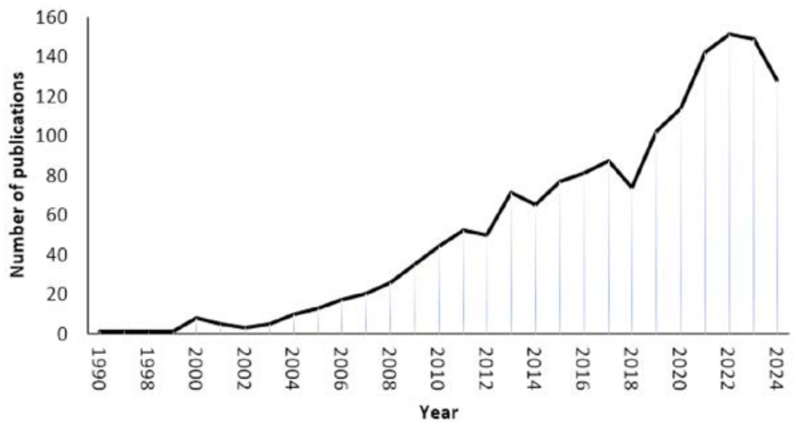

Figure 2. Behavior of publications on "vermicompost" and "crop" (1990-2024).

Scientific cartography

The co-occurrence analysis provided a general view of the research areas, identifying the key themes addressed. This analysis was carried out using the VOSviewer software. The co-occurrence network of keywords "vermicompost" and "crop" shows 5 clusters: organic residues used and technological innovation in the process of vermicomposting (yellow), integrated management of nutrients: combination of vermicompost with nitrogenous synthetic fertilizers in the application on crops (red), its effects on the morphology and physiology of plants according to the variables considered (green), enzymatic activity (purple), and microbial biomass and its beneficial effects on the rhizosphere of plants (blue) (Figure 3).

The concept where the four clusters intercept is "soils Meath", because the use of vermicompost improves the quality and the health of soils [14,15,16].

Table 1. Performance analysis: main journals, countries and institutes that have carried out research on "vermicompost" and "crop" (1990 to September 23, 2024).

Place	Megazine	Pub.	Country	Pub.	Institute	Pub.
1	Indian Journal of Agricultural Sciences	58	India	890	ICAR - Indian Agricultural Research Institute, New Delhi	70
2	Indian Journal of Agronomy	55	China	88	Indian Council Of Agricultural Research	60
3	Communications in Soil Science and Plant Analysis	51	United States	84	CCS Haryana Agricultural University	37
4	Plant Archives	33	Iran	69	Banaras Hindu University	36
5	Ecology Environment and Conservation	32	Mexico	65	Bidhan Chandra Krishi Viswavidyalaya	31
6	Agronomy	30	Pakistan	49	Central Institute of Medicinal and Aromatic Plants India	28
7	Journal of Plant Nutrition	24	Spain	49	ICAR - Research Complex for North Eastern Hill Region, Umiam	28
8	Bioresource Technology	22	Saudi Arabia	37	Annamalai University	27
9	Legume Research	20	Turkey	35	Krishi Vigyan Kendra	24
10	Annals Of Biology	19	Bangladesh	34	Tamil Nadu Agricultural University	24

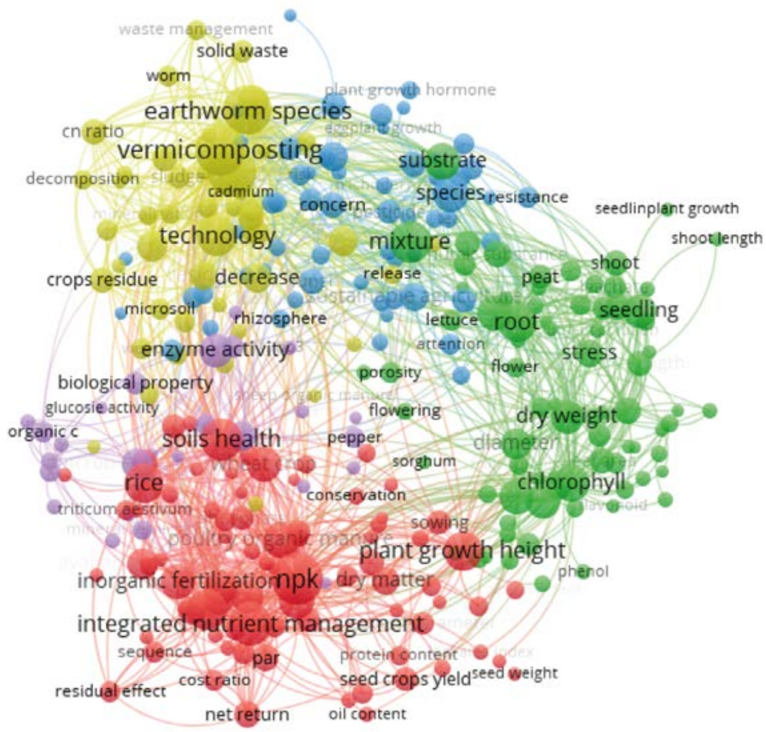

Figure 3. Superimposed visualization of VOSviewer for "vermicompost" and "crop" from 1990 to 2024.

In the specific search "vermicompost" and "coffee", with a minimum frequency value of 5, the co-occurrence analysis shows 3 clusters: the use of residues from the coffee industry in vermicomposting (red), incorporation of vermicompost as substrate for the production of coffee seedlings (blue), and its effect on morphological and physiological development (green) (Figure 4).

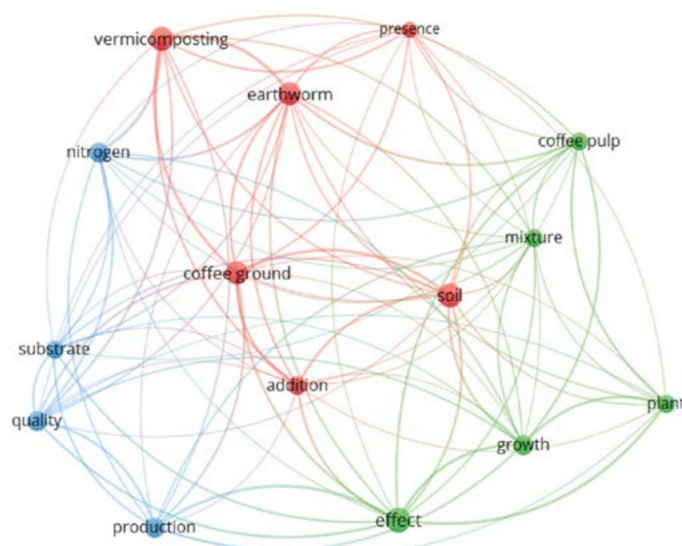

Figure 4. Superimposed visualization of VOSviewer for "vermicompost" and "coffee" from 1990 to 2024.

In the vermicomposting process of organic residues derived from the coffee agroindustry, the earthworms used are *Eisenia fetida*, *Eisenia andrei* and *Dendrobaena veneta* [2,17,18]. Bio-residues from the agroindustry, such as coffee grounds, mostly worry because of their damaging effects due to their high degradability and content of harmful compounds, such as phenols, caffeine, and tannins [19].

Because of this, options to reuse them have been sought, in order to decrease their environmental impact through vermicompost production for nutrient recycling and exploitation in the agricultural sector [18,20].

In the agricultural sphere, vermicompost is presented as a sustainable alternative used in various agricultural and forestry crops as substrate, organic amendments, soil improver, and organic fertilizer [4,21,22]. The general review revealed that a significant percentage of the articles refer to its use in vegetable production, due mainly to its contribution in organic carbon, beneficial micronutrients and microorganisms, which favor the increase in microbial biomass, higher availability of nutrients, and metabolic activity of plants [16,23,24]. In addition, vermicompost has positive effects on the soil health, improving its resilience while facing adverse conditions such as stress from drought, salinity and contamination by heavy metals: Cadmium (Cd), Chromium (Cr), Nickel (Ni), Lead (Pb), Copper (Cu), Arsenic (As), and silver (Ag) [9, 25, 26]. In addition, it has been studied for its significant contribution of N and micronutrients, considering it as a nitrogenous fertilizer [15,19,27]. However, its combined use with synthetic fertilizers is common, since they complement each other effectively to achieve higher yields, improving the quality of foods and regenerating the soil health [26,28,29].

It has also been used in the production of cereals, mainly in basic foods, such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and basic grains like bean (*Phaseolus vulgaris* L.) in Mexico. Its use happens in combination with synthetic fertilizers (NPK), with biofertilizers (*Azospirillum*, *Trichoderma*) and different composts based on the crop's requirements and the soil fertility [26,30,31]. Table 2 lists some studies that refer to these strategic crops, presenting the benefits that vermicompost contributes in general, and the doses with which experiments have been performed.

This organic amendment has been scarcely used in fruit production fields. Some of the causes that can be interfering with it to develop this way is the amount needed to tend to one hectare, since a greater proportion is required compared to vegetables and cereals. Another reason can be the long time necessary to evaluate the variables, as well as the increase in total costs of the studies; to evaluate the impact of vermicompost on fruit quality and yield in fig production (*Ficus carica* L.), 10 kg/tree/year [32] were needed, at the same time that in plum (*Prunus salicina* L.) nutrition, 22.5 kg/tree/year [33] were necessary to increase the fruit yield.

The use of vermicompost in coffee production (*Coffea arabica* L.) has been scarcely studied. Of the 41 articles found through the search with the concepts "vermicompost" and "coffee", only 3 address its use as substrate for germination, development and growth of the seedlings in nursery [39,40,41]. In addition, no article was found that analyzes its impact on the nutritional management of coffee plantations established in the field, or about its

Table 2. Main crops and fruit trees where the use of vermicompost as soil improver and source of organic nutrition has been evaluated.

Crop	Benefits	Dose	Reference
Corn (*Zea mays* L.)	Significantly improved growth characteristics, quality parameters, and green forage yield attributes.	50% farm manure and 50% vermicompost.	[34]
	Improved the physical and chemical properties of the soil. Increased grain yield, straw yield, and harvest index (41.53%).	62.5N-60P-30K kg ha^{-1} more 10 t ha^{-1} of farmyard manure, 2 t ha^{-1} of vermicompost, and Azotobacter.	[24]
Rice (*Oryza sativa* L.)	It improved soil properties, increased the diversity and composition of the bacterial community, mitigated the adverse effects of Cd on plants, and increased rice grain yield (38%).	3 and 6 t ha^{-1}	[8]
	It improved soil properties (COS=78.7%) and increased grain and straw yields by 74.5% and 46.1%, respectively.	Equivalent to 80 kg N ha^{-1}, 2.5 t ha^{-1} of wheat crop residue and biofertilizers.	[31]
Bean (*Phaseolus vulgaris* L.)	Improved the physical and chemical properties of the soil, improved the physiological properties of the plant, and increased grain yield.	10 t ha^{-1} (75% vermicompost, 25% organic carbon)	[35]
	It had a positive impact on development, growth, and productivity.	50%, 75% y 100% of the contents of the pot	[36]
Wheat (*Triticum aestivum* L)	Saline and non-saline soil: increased the physical and chemical properties of the soil (COS by 52%, 18%), improved physiological characteristics, increased grain yield (50%, 44%), and increased CO_2 emissions.	11.8 t ha^{-1}	[37]
	It improved morphological, physiological, and biochemical characteristics under drought stress conditions.	4, 6 and 8 t ha^{-1}	[11]
Plum (*Prunus salicina* Lindl.)	It improved the physical and chemical properties of the soil (18% increase in COS) and increased fruit quality.	70% of the recommended dose of N per tree + 22.5 kg of vermicompost + Jeevamrit (2 liters per tree).	[33]
Coconut (*Cocos nucifera* L.)	It improved the physical and chemical properties of the soil and increased the microbial population. It increased the yield of nuts per plant per year (19%).	250N-320P-120K grams per plant + 50% of N supplied with vermicompost.	[38]

effects on the morphological growth, physiological, and physicochemical properties of the soil, the yields and the quality of fruits in producing coffee trees.

Although Mexico is among the main producing countries of organic coffee in the world, there is very little or no information regarding this topic [42]. These results can be attributed to the fact that different composts foreign to vermicompost are being used, and there is a lack of knowledge or documentation of those studies.

From the articles found in the specific search, 88% refer to nutrient recycling of organic residues from the coffee agroindustry (pulp, parchment, and coffee grounds), through vermicomposting and conventional composting, where different proportions of this type of residues are mixed with residues from different crops (banana leaves, palm fiber, straw, etc.), food waste, and excretes from different animals (sheep, goats, cattle, horses), to research their physicochemical and biological properties, their impact on yields and the quality of foods, as well as their impact on soil health [2,18,44] (Table 4).

Table 3. Use of vermicompost in the nutrition of *Coffea arabica* L. and *Coffea canephora*.

Coffee (seedlings)	Benefits	Dose	Reference
Coffea arabica L.	The morphological and physiological development of the seedlings improved.	40% of the total volume of the bag.	[39]
Coffea arabica L.	It improved the morphological variables of the seedlings (number of leaves, stem diameter, root diameter, total number of secondary roots).	10% concentration (liquid).	[40]
Coffea arabica L., *Coffea canephora*	Improved soil chemical properties (CO, total P, and available N) and plant biomass.	0.14 kg/pot	[41]
Coffea canephora	It improved the chemical properties of the soil (total nitrogen) and the morphological variables of the seedlings (height, number of leaves, root length).	100%	[43]

Table 4. Nutrient recycling through vermicomposting of residues from the coffee agroindustry and its use in food production.

Waste from the coffee agroindustry	Crop	Benefits	Dose	Reference
Coffee pulp	Chili pepper (*Capsicum annuum* L.)	It improved the physical and chemical properties of the soil and the morphological characteristics of the plants. It increased yield by 97.86%.	15 t ha^{-1}	[2]
Coffee pulp	Tomato (*Solanum lycopersicum* L.)	Seed germination improved, possibly due to increased microbial diversity.	40%	[45]
Coffee grounds	Crop (*Zea mays* L.)	It improved seed germination and enriched the physicochemical properties of vermicompost.	100%	[46]
Coffee grounds	Lettuce (*Lactuca sativa*)	It improved morphological characteristics and increased biomass. It decreased the Zn, Cu, and Fe content in plants.	7.5%	[47]
Coffee husk and pulp	Not applicable	Vermicomposting reduced the concentration of caffeine, chlorogenic acid, and tannins. It increased the microbial load of microorganisms that promote plant growth.	Not applicable	[1]
Coffee grounds	Not applicable	Recycling coffee grounds through vermicomposting substantially reduced caffeine content and increased P, K, and Mg content.	Not applicable	[20]
Coffee grounds	Not applicable	Vermicompost made from coffee grounds exhibited favorable physical and chemical properties for use as organic amendments to agricultural soils.	Not applicable	[19]

CONCLUSIONS

Vermicompost is valued for its contribution to total N, micronutrients, and organic carbon in organic agriculture. It is frequently used as biofertilizer due to its high microbial load. In addition, it is recommended for vegetable production since it contains different beneficial fungi and bacteria that solubilize P and mineralize N. It also contributes to the bioremediation of contaminated soils and the decrease of salinity of the soils, among others.

Vermicompost not only improves the quality of foods, but also maintains and even increases the yields per hectare throughout time.

This review emphasizes the potential of vermicompost as an innovative and sustainable alternative in agricultural systems, offering solutions in the presence of challenges in the agrifood sector.

The advantages vermicompost offers due to the microbial populations present represent an area of opportunity for future studies. In subtropical and tropical countries, there is the potential to evaluate its properties in various crops under changing environmental conditions, and to promote nutrient recycling strategies through vermicomposting of organic residues, which would contribute to the agroecological management of the crops.

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