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Nutritional evaluation of white corn grain for tortilla manufacturing

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ABSTRACT
Objective: Agronomic characteristics such as density, hectoliter weight, grain hardness and nutritional value of maize (*Zea mays* L.) grain in terms of protein, starch and oil content were evaluated.
Design/Methodology/Approach: Using the Dickey John INSTALAB 700® equipment, hybrid and native maize grains from different commercial houses that are planted in Durango, central region of northern Mexico. The seeds evaluated were of the certified type and evaluated in the Agricultural Biology laboratory of the Technological Institute of the Guadiana Valley. The grains were prepared following CIMMYT laboratory protocols in order to evaluate the nutritional and industrial quality of maize.
Findings/Conclusions: Ten genotypes exhibited soft grain hardness, one showed intermediate hardness and six showed hard hardness with values higher than a hectoliter weight of 74 kg hL^{-1}, the latter having the greatest potential to be planted extensively for use in the production of tortilla dough. Conclusions: The protein content in the corn evaluated was not statistically significant, but the starch content varied from 52 to 62% with an oil content of 3.5 to 6.9%.

Keywords: White grain corn, tortillas, starch and hardness

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INTRODUCTION

White grain corn has a *per capita* consumption of 196.4 kg in Mexico, its main use is in the production of tortillas, it represents 20.9% of the total food expenditure, much higher than other grains such as wheat and barley (FIRA, 2016). 7.5 million hectares of white and yellow corn were planted on an annual average from 2010 to 2020, with average yields of 3.5 tons ha^{-1} with an annual production of 26.3 million tons, with white grain corn being the one with the highest production, attaining yields above 21 million tons (SIAP, 2021). To satisfy the domestic demand for white and yellow grain corn, 15 million tons are imported annually (CIMMYT, 2019). In Mexico there are various forms of corn consumption, such as tortillas or processed flours, nonetheless, tortillas represent 64% of a total of 12 million tons per year, the color of the grain can be white, yellow or colored. Approximately, 36% of production is used by the flour industry (Salinas *et al.*, 2011). Corn grain that produces dough with a high degree of humidity, that can be extensible and of appropriate hardness is the best for tortilla production (Arámbula *et al.*, 2001).

(Rangel *et al.*, 2004) reported that creole white corn grain exhibits greater dough hardness and adhesion as well as a faster cooking speed when compared with other enhanced varieties. Tortillas elaborated with blue, red and orange corn are more antioxidant dense than tortillas elaborated with white corn grain (Salinas *et al.*, 2017).

Regarding the nutritional composition of corn, carbohydrates are within a range of 44 to 69%, 72% starch, 10% protein and 4% lipids (Urango, 2018). The commercial quality of corn grain for human consumption is declared in the MNX-034 (2002) standard, which includes the processes of nixtamalized flour fabrication and the elaboration of dough and tortilla.

In both cases, industries prefer corn with the following characteristics: hectoliter weight $\geq$74 kg hL^{-1}, nixtamal humidity between 36% and 42%, flotation index $\leq$40%, remaining pericarp $<$2% and solid loss $<$5%. The hectoliter weight is a factor related to the hardness of the grain, white corn that is intended to produce tortillas must have a minimum hectoliter weight of 74 kg hL^{-1}, if the value is greater than 75 it is considered hard grain, 74 to 75 is considered intermediate grain, and less than 74 is considered soft grain (Palacios, 2018). Generally, texture quality of tortillas decreases after being stored for more than 24 hours, this lapse can be increased by using corn with soft or intermediate hardness (Salinas y Aguilar, 2010). Corn grains generally contain an oil average of 3 to 5%, being oleic and inoleic acids the most representative (62%) and palmitic and stearic acids representing 12.8% on average (Corcuera *et al.*, 2013). The quality of tortillas as well as their shelf life is closely related to the endosperm in the corn; consumers prefer tortillas with a soft texture; this is achieved by using corn grain flours with floury endosperm and intermediate hardness (Osorio *et al.*, 2011). The determination of the nutritional analysis of corn grain can be achieved using the INSTALAB® 700 equipment, which uses NIR technology and a mathematical statistical treatment to predict the percentage concentration of a constituent within a sample. Certain constituents have been proven to absorb light energy at specific wavelengths. For example, moisture absorbs in the 1.94 μm (micrometer) band of near-infrared (NIR) light, protein absorbs in the 2.18 μm band, and oil in the 2.31 μm and 2.33 μm bands. By bombarding a sample with a very narrow band of light at a specific wavelength of NIR light, samples can be analyzed. Thus proteins, cellulose, starch, oil, fats and alcohol can be determined. NIR spectroscopy has been applied to predict some major components of grains, such as moisture, proteins, and lipids. It has demonstrated such a good performance, that it is currently preferred over traditional wet chemistry methods for routine analysis. Ferrari *et al.* (2004) mentions that the determination of the nutritional value of corn using near-infrared NIR spectroscopic techniques generate faster results and avoids the generation of dangerous residues. The objective of this work is to determine the nutritional value of white corn grain using NIR technology, the protein, oil and starch content in different commercial corn grains for use in the tortilla industry, as well as the agronomic variables of hardness and hectoliter weight.

MATERIALSAND METHODS

17 commercial white corn hybrids that are traditionally planted in Mexico were used (Table 1).

Table 1. Different white corn hybrids and creoles evaluated for tortilla production.

Items	Corn grain	Enterprise
1	Caribú	Asgrow
2	Cristiani 52	Cristiani
3	H 311	Agroferza
4	Creole VS 201	Nativo
5	Creole 3 1/2	Nativo
6	OSSO	Asgrow
7	308 Ángel	Asgrow
8	Ocelote	Asgrow
9	Tigre	Asgrow
10	Garañón	Asgrow
11	Dow 2358	Agroscienses
12	Novasem R 101	Novasem
13	Canelo	Semillas Rica
14	P3015 w	Pionner
15	P2361 w	Pionner
16	P3260 w	Pionner
17	P3057 w	Pionner

The methodology in this study consisted of: Classification of the grain by hybrid and variety, removal of impurities from the grains by passing them through No. 4 sieves (4.75 mm hole diameter), determination of humidity, determination of the hectoliter weight in kg hL^{-1}, weight of 100 seeds in grams, seed viability (additional) by the tetrazolium method in percentage grain hardness, calibration of the INSTALAB 700 equipment, calibration curves for white corn (one for each variety, 17 curves total), measurement of protein, starch and oil values within each variety and hybrid, statistical analysis.

Grain classification and cleaning. Dye was removed from each grain using a 70% alcohol-water mixture, seeds were submerged in the mixture for 45 min, after that, the grains were washed three times with tap water; lastly, the grains were dried for 24 h at room temperature.

Determination of humidity and hectoliter weight. To determine the humidity of the grain, the GAC 2100® equipment from Dickey John was used, grain humidity was determined in percentage directly. The hectoliter weight in kg hL^{-1} for each hybrid and corn creole was determined directly using the same equipment.

Weight of 1000 seeds, this step was carried out separating the impurities in sieves, 100 grains of each genotype were weighed on a digital weighing machine.

Viability of the embryo within the grain, 20 seeds were placed in Petri dishes, submerged with a 2% tetrazolium solution and covered with aluminum foil for 24 h, the result was reported as percentage of viable seeds.

Evaluation of the nutritional value of the corn grain, grains were grounded (the deck was removed leaving only the endosperm) until flour was obtained, this was carried out using a ROMER Series II® mill, each grind was labeled and stored in a polyethylene bag for later evaluation.

Evaluation of protein, oil and starch in corn genotypes, this procedure was conducted as follows: the INSTALB 700® equipment was turned on and allowed to heat up for 45 min until the color indicator turned from yellow to green, it was then calibrated with the corn curves previously loaded, each grain was filled with corn flour separately, grain type is selected, and the measurement is performed. Loading and data collection time takes approximately two minutes per sample.

Experimental design

The experimental design employed to evaluate the response variables of protein, starch and oil content is completely random and if minimal significant differences are detected, the results are subjected to the comparison of DMS means at a probability $p<0.05$. The mathematical model is as follows:

$$Y = U + T_{ij} + E_{ij}$$

Where: Y: dependent variable, U: is the population mean, T_{ij}: Treatments or hybrids evaluated, E_{ij}: Experimental error. The analysis of variance is conducted using the (Olivares, 1994) statistical software.

Table 2. Variables evaluated for density, hectoliter weight and grain hardness in corn hybrids and creoles.

Corn grain		Enterprise	Density g cm^{3-1}	Hectoliter weight kg hL^{-1}	Grain hardness
1	Caribú	Asgrow	1.41	49.20	Soft
2	Cristiani 52	Cristiani	1.42	42.00	Soft
3	H 311	Agroferza	1.44	60.10	Soft
4	Creole VS 201	Native	1.43	62.20	Soft
5	Crole 3 1/2	Native	1.43	62.10	Soft
6	OSSO	Asgrow	1.42	69.30	Soft
7	308 Ángel	Asgrow	1.52	89.90	Hard
8	Ocelote	Asgrow	1.51	83.30	Hard
9	Tigre	Asgrow	1.42	83.20	Hard
10	Garañón	Asgrow	1.43	78.50	Hard
11	Dow 2358	Agrosciences	1.45	80.20	Hard
12	Novasem R 101	Novasem	1.40	74.60	Intermediate
13	Canelo	seeds Rica	1.43	77.20	Hard
14	P3015 w	Pionner	1.43	65.90	Soft
15	P2361 w	Pionner	1.50	57.50	Soft
16	P3260 w	Pionner	1.42	41.80	Soft
17	P3057 w	Pionner	1.41	41.60	Soft

RESULTS AND DISCUSSION

Calculated density, hectoliter weight and grain hardness in evaluated corn hybrids and creoles can be consulted in Table 2.

Density. All the genotypes evaluated exhibited homogeneous density with values between 1.40 and 1.53 g cm^{3-1}, the hectoliter weight ranged from values lower than 41.6, 41.8 for the hybrids P3057 w and P3260 from the Pioneer company (Ordoñez *et al.*, 2012) reported density values between 1.177 and 1.33 g cm^{3-1} in a chemical and physical study of red color hard corn.

Hardness. Regarding grain hardness, hybrids from the Asgrow commercial company were harder, while those from Pioneer were softer. The flour industry prefers grains that present hardness greater than 74 Kg hL^{-1}. In this study, the genotypes 308 Ángel, Ocelote, Tigre and Garañon from Asgrow met this requirement. Dow 2358 from Agroscienses and Novasem R 101 and Canelo from Rica seeds met these conditions. In an evaluation of white grain creole corn subjected to different thermal drying processes, (Garcia *et al.*, 2016) reported grain densities between 0.98 and 1.38 g cm^{3-1}, the hectoliter weight was lower in these corn grains at 74 Kg hL^{-1}, so its classification is soft type grain.

Hectoliter weight. Seven genotypes exhibited hectoliter weights greater than 74, so they are considered suitable for use by the nixtamal industry for the manufacture of tortillas. (Jimenez *et al.*, 2012) evaluated pre-improved white grain corn from the CIMMYT germplasm center and reported hectoliter weights of 82 to 89 Kg hL^{-1}, in this study the evaluated genotypes 308 Ángel, Ocelote, Tigre and Garañon exhibit similar values.

Protein, oil and starch

The analysis of variance for each variable revealed significant statistical differences at $p<0.05$ for protein, oil and starch content, the comparison of means by DMS can be seen in Table 3.

Protein. No significant statistical difference was found, but numerically, but the Cristiani 52 and P3015 w hybrids have the highest protein content, 2.23 and 2.26% respectively. (Mendez *et al.*, 2005) reported protein values between 8.8 and 11.3% in an evaluation of 20 corn suitable for tortilla production. (Martinez, 2022) reported protein content of 6.63% in white grain native corning a study of pigmented native corn with white grain color. In this work, protein content was lower in value, which is attributed to the fact that the majority of the grains were washed and rinsed with alcohol to remove the dye.

Starch. Obtained results indicate that hybrids 1, 2, 10 and 15,16 and 17 corresponding to the white grain corn Caribú, Cristiani 52, Garañón, P2361w, P3260w and P3057w, were the ones that contained the highest amount of starch with 61.5, 60.7, 60.0, 60.6, 60.1, and 60.0% respectively, with Asgrow's Caribú being the one that exhibited the highest amount of starch (61.5%) (Mendez *et al.*, 2005) reported starch percentage values ranging from 55.3 to 70.4, the results obtained in this study coincide, since evaluated hybrids concentrated a greater amount of starch compared to the native creoles (Agama *et al.*, 2013) reported values between 70.9 and 76.2% of starch in white corn grains, they found a relationship that indicates that the higher the starch content in the grain, the lower the protein content,

Table 3. Comparison of means for protein, oil and starch content in 17 white corn grains evaluated for use in the tortilla industry.

	Corn grain	Enterprise	Protein %	Oil %	Starch %
1	Caribú	Asgrow	1.41	4.30 i	61.5 a
2	Cristiani 52	Cristiani	2.23	4.10 j	60.7 ab
3	H 311	Agroferza	1.44	4.50 h	59.4 bc
4	Creole VS 201	Native	1.06	5.20 e	54.6 e
5	Creole3 1/2	Native	0.26	5.00 f	57.2 d
6	OSSO	Asgrow	1.60	6.90a	52.1 f
7	308 Ángel	Asgrow	0.53	6.50 b	54.8 e
8	Ocelote	Asgrow	0.66	5.60 d	56.3 de
9	Tigre	Asgrow	0.86	5.50 d	57.2 d
10	Garañón	Asgrow	2.00	4.70 g	60.0 ab
11	Dow 2358	Agroscienses	1.93	4.90 f	59.7bc
12	Novasem R 101	Novasem	1.80	6.00 c	57.8 cd
13	Canelo	Seeds Rica	1.86	3.50 l	60.00 ab
14	P3015 w	Pionner	2.26	3.50 l	61.70 a
15	P2361 w	Pionner	1.20	3.20 m	60.60 ab
16	P3260 w	Pionner	1.60	4.30 i	60.10 ab
17	P3057 w	Pionner	1.20	3.80 k	60.00 ab
DMS			NS	0.169	1.840

NS: Not significant. Means with different letters are statistically different at $p<0.05$.

for this study the highest value in starch was observed in Asgrow's Caribú corn with 61.5, no genotype exceeded 62%.

Oil. Torres *et al.*, 2010, reported values ranging from 4.3 to 4.7% of oil in Comiteco corn grains, in this study, Asgrow's OSSO hybrid presented the highest oil content with 6.90%, (Guzmán *et al.*, 2015) reported oil contents from 4.11 to 6.29% in cónico Norteño creole corn, in the present work, the two-creole evaluated exhibited oil values between 5.0 and 5.20%.

CONCLUSIONS

The density of the evaluated corn types presented homogeneous densities, most corn types exhibited soft hardness, with only a few presenting hard hardness of interest for the dough and tortilla industries, being the hybrids from Asgrow the ones with the highest hardness and hectoliter weight values. Regarding the use of creole white grain corn in the dough and tortilla industries, it is limited to only being used in the communities of origin due to the steep costs of transport to production centers of high demand. The starch content was acceptable, with the greatest amount being concentrated on the endosperm; the corn hybrids evaluated had a higher starch content compared to the native corn. All genotypes displayed uniform values in oil content. These results allow farmers to identify and plant the types of white corn that the industry requires in order to provide higher quality tortillas and longer shelf life.

AKNOWLEDGMENTS

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Morphology, density and population fluctuation of new records of orthopterans associated with four agroecosystems

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ABSTRACT

Objective: To determine orthopterans taxonomically, to calculate the population dynamics and fluctuation based on collections carried out in four agroecosystems in the Experimental Unit of the "Presidente Juárez" Agrobiology School in Uruapan, Michoacán, Mexico.

Design/methodology/approach: The research was conducted from August to December 2019. The agroecosystem was divided into four sectors, and for each sector a hectare of land with different crops was assigned. A completely randomized experimental design was established, with four treatments and five repetitions. Five collections were made in each sector, every 14 days. The field information was analyzed with the SAS software ver. 3.8, with Tukey's means comparison test (0.05).

Results: Two thousand hits were carried out with an entomological net and 2024 orthopterans were collected. The outstanding genera and species were: *Schistocerca nitens*, *Sphenarium purpurascens* and *Achurum sumichrasti*.

Limitations on study/implications: Only five months of the year were evaluated, so the species determined can vary in their relative abundance.

Findings/conclusions: New records were found in seven families, 19 genera and 16 species: *Acanthorintes tauriformis*, *Achurum sumichrasti* (Saussure, 1861); *Aidemona azteca* (Saussure, 1861); *Arphia conspersa* Scudder, 1875; *Brachystola mexicana* Bruner, 1906; *Ceuthophilus pallidipes* Tomás, 1872; *Conocephalus cinereus* Thunberg, 1815; *Dichromorpha prominula* (Bruner, 1904); *Ducetia japónica* (Thunberg, 1815); *Gryllus* spp., Linnaeus, 1758; *Melanoplus differentialis* (Thomas, 1865); *Neoconocephalus triops* (Linnaeus, 1758); *Rhammatocerus viatorius* (Saussure, 1861); *Schistocerca nitens* (Thunberg, 1815); *Sphenarium purpurascens* (Charpentier, 1841); *Stilpnochlora azteca* (Saussure, 1859); *Taeniopoda stali* Bruner, 1907; *Pyrgocorypha* spp., Stal 1873; and *Stenopelmatus* spp., Burmeister, 1838.

Keywords: Acridididae, Tettigoniidae, Pyrgomorphidae, *Schistocerca*, *Sphenarium*, *Achurum*.

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INTRODUCTION

Insects are ideal indicators for environmental monitoring, since it is possible to understand the conservation and/or deterioration of natural environments, diversity and taxonomic wealth, through them (Guzmán *et al.*, 2016). Scientifically, Othoptera have received greater attention as pest species (O'Neill *et al.*, 2003). From the agricultural point of view, grasshoppers and locusts

(Orthoptera: Acrididae) make significant attacks on various crops in many parts of the world (Maiga *et al.*, 2008). In the crops, they defoliate plants, thus reducing their photosynthetic capacities (Easwaramoorthy *et al.*, 1989). In livestock production, grasshoppers are the most abundant herbivores in grasslands, and they often consume more vegetation than the livestock (Branson and Haferkamp, 2014). From the forestry point of view, *Gryllus assimilis* (F. 1775) (Orthoptera: Gryllidae), is a pest of *Eucalyptus* spp. (Myrtaceae) (Silva *et al.*, 2013), where adults and nymphs commonly damage young plants (Barbosa *et al.*, 2009), and occasionally the seeds (Silveira *et al.*, 2014). As urban pests, knowledge about biodiversity of orthopterans is limited, although they represent a potential risk since they attack sport golf fields and gardening areas in hotel facilities (Artabe *et al.*, 2009). For this reason, the objective of this study was to determine the species of Orthoptera morpho-taxonomically, as well as the fluctuation and population density in four agroecosystems, in the Experimental Unit of the "Presidente Juárez" Agrobiology School, which depends on the Universidad Michoacana de San Nicolás de Hidalgo, México.

MATERIALS AND METHODS

Geographic location

The study was carried out from August to December 2019, in the Experimental Unit of the "President Juárez" Agrobiology School, municipality of Uruapan, Michoacán, Mexico; located between 19° 22' 31" North and 102° 01' 33" West at 1610 masl (Google Earth, 2024) (Figure1).

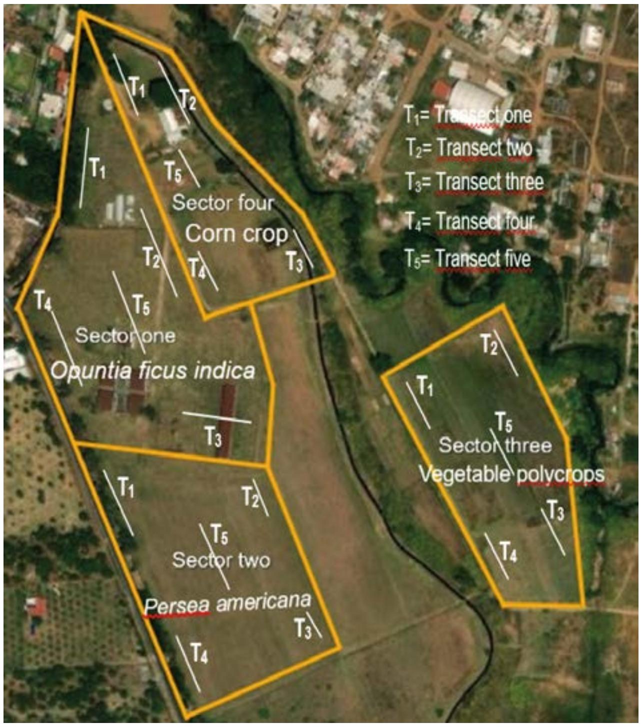

Figure 1. Geographic location of the Experimental Unit of the "Presidente Juárez" Agrobiology School and the sectors for collection (Google Earth, 2024).

Experimental site

Through a completely randomized experimental design, four sectors were chosen. Sector I: with nopal crop (*Opuntia ficus-indica* L.); sector II: orchard with avocado (*Persea americana* Mill. Var Hass); sector III: vegetable polycrops broccoli (*Brassica oleracea* var. Itálica P.), bean (*Phaseolus vulgaris* L.), squash (*Cucurbita pepo* L.); sector IV: corn crop (*Zea mays* L.). In each sector, a systematic sampling was conducted every 14 days during a period of five months (Castillo-Márquez, 2002).

Collection of orthopterans

Five transects were randomly marked in each sector (Figure 1), each transect measured ten meters, and one hit was made with an entomological net in each meter. There were 500 hits per sector, with a total of 2000 hits in the four sectors during each collection. For the collection of orthopterans, entomological vials were used with screw tap, alcohol at 70%, and lethal chamber with ethyl acetate. The samples were labeled and then transported to the laboratory BIO-CHRYS S. P. R. of R. L. of C. V., for their taxonomic determination.

Processing of orthopterans

In the BIO-CHRYS laboratory, the specimens collected were mounted and labeled with entomological pins of number one and two. In some specimens, the male genitalia were obtained. Stereoscopic and biological microscopes were used, both of Zeigen® brand, and a RoHS® digital microscope. To macerate the tegmina and genitalia, a glass vial with metal cap was used, and 5 mL^{-1} of potassium hydroxide at 10% were added, and then the bain-marie technique was applied for 15 min.

Identification of orthopterans

The orthopterans were grouped by morphospecies, to later perform their genus and species determination, based on the information in the database Orthoptera Species File (Otte, 1994), as well as the use of taxonomic keys of different sources of information: Karny (1938); Hebard (1941); Chopard (1951); Emsley (1970); Thomas (1873); McNeill (1897); Scudder (1897); Naskrecki (2000); Pomares (2002); Rowell (2013); and Gorochov and Cadena (2016). The taxonomic determination was the responsibility of Dr. Francisco Javier Avendaño Gutiérrez and Engineer Leticia Vázquez Ortiz. Photographs were captured of the traits with taxonomic value with the microscopes previously mentioned. The photographic camera used was AmScope® version x64, 2020, as well as a RoHS® 500X digital microscope.

Population fluctuation and dynamics

To analyze the density of orthopterans, the field information was captured in the Microsoft Excel software version 23H2 and a database was generated. The number of orthopterans collected was interpreted by sector and capture dates. Finally, with the help of the SAS Version 3.8 software, the Analysis of Variance was calculated with Tukey's means comparison test with α=0.05.

RESULTS AND DISCUSSION

Taxonomic determination of orthopterans

The number of Ensifera and Caelifera collected was 2024, which were distributed in seven families, 19 genera and 16 species (Figure 2) (Table 1).

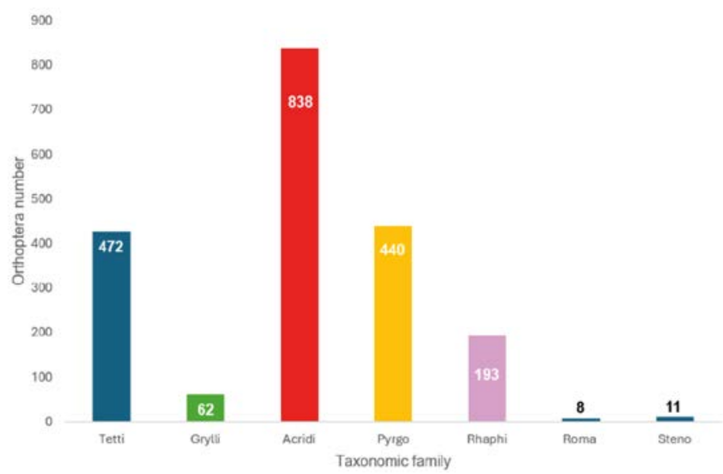

Figure 2. Families of orthopterans collected. Tetti=Tettigoniidae, Grylli=Gryllidae, Acridi=Acrididae, Pyrgo=Pyrgomorphidae, Rhaphi=Rhaphidophoridae, Roma=Romaleidae, and Steno=Stenopelmatidae.

Table 1. Genera and species of Orthoptera collected in the Experimental Unit of the Agrobiology School, 2019.

Location	Taxonomic distribution of orthoptera			
	Suborder	Family	Genus	Species
Experimental unit	Ensifera	Tettigoniidae	*Acanthorintes*	*tauriformis*
			Conocephalus	*cinereus*
			Ducetia	*japonica*
			Neoconocephalus	*triops*
			Stilpnochlora	*azteca*
			Pyrgocorypha	spp.
		Gryllidae	*Gryllus*	spp.
	Caelifera	Acrididae	*Achurum*	*sumichrasti*
			Arphia	*conspersa*
			Aidemona	*azteca*
			Dichromorpha	*prominula*
			Melanoplus	*differentialis*
			Rhammatocerus	*viatorius*
			Schistocerca	*nitens*
		Pyrgomorphidae	*Sphenarium*	*purpurascens*
		Rhaphidophoridae	*Ceuthophilus*	*pallidipes*
		Romaleidae	*Taeniopoda*	*stali*
			Brachystola	*mexicana*
		Stenopelmatidae	*Stenopelmatus*	sp.

Next, the morphotaxonomic characteristics of 15 genera and 13 species were exemplified.

Acanthorintes tauriformis

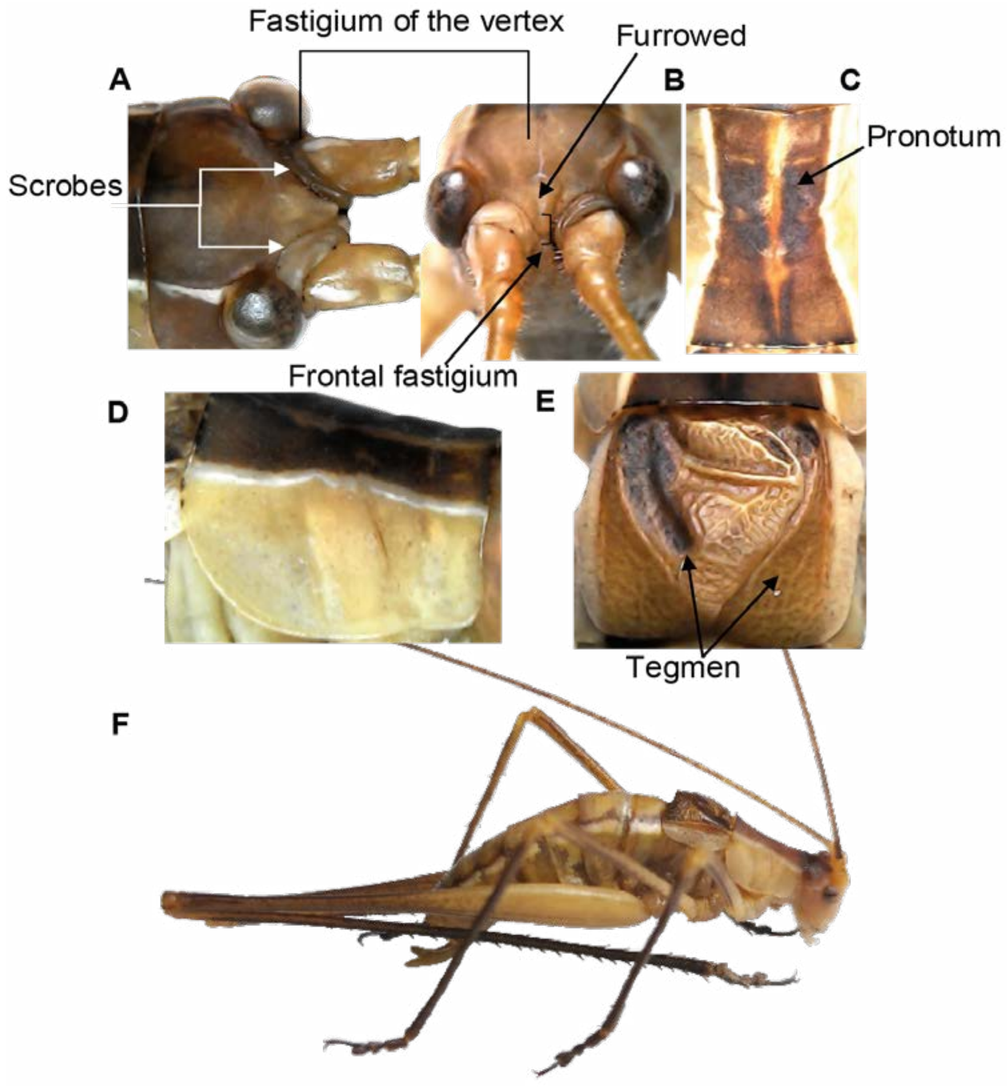

Figure 3. A-F: *Acanthorintes tauriformis*. Male (♂): A. Scrobes and head with declining occiput towards the fastigium, B. Fastigium of the vertex, slightly compressed, with the weakly furrowed dorsum, without touching the frontal fastigium, C. Dorsal view of the pronotum, D. Lateral view of the pronotum, E. More reduced tegmina than the pronotum, F. Moderately thin body shape, lateral view.

Acanthorintes tauriformis

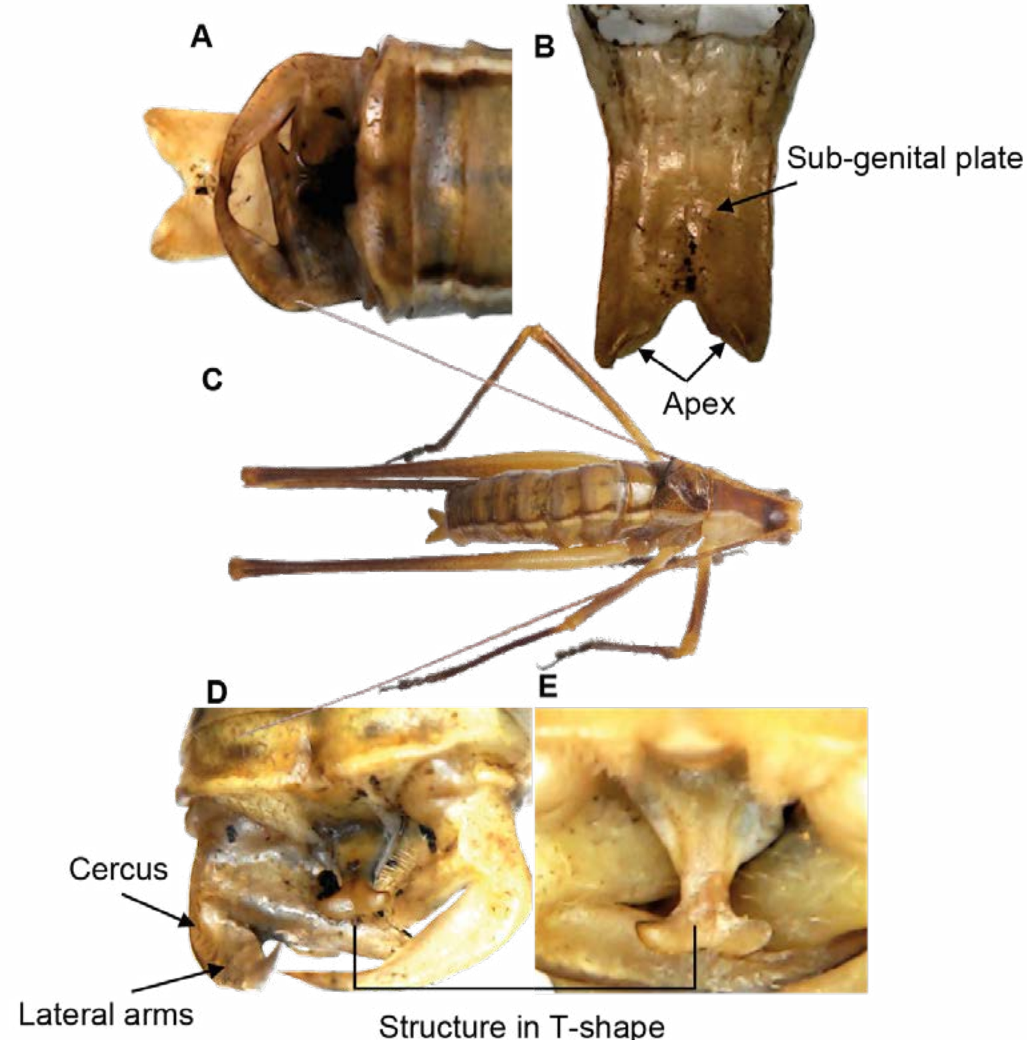

Figure 4. A-E: *Acanthorintes tauriformis*. Male (♂). A. Apex of the abdomen, dorsal profile, B. Subgenital plate, with long concave lateral margins and "doubly emarginated" apex, slightly convex internal margins of the apical slot, C. Complete dorsal body view, D. The cercus with strongly upwards lateral arms, E. Structure in T-shape in the proximity of the apex of the supra-anal plate.

Conocephalus cinereus

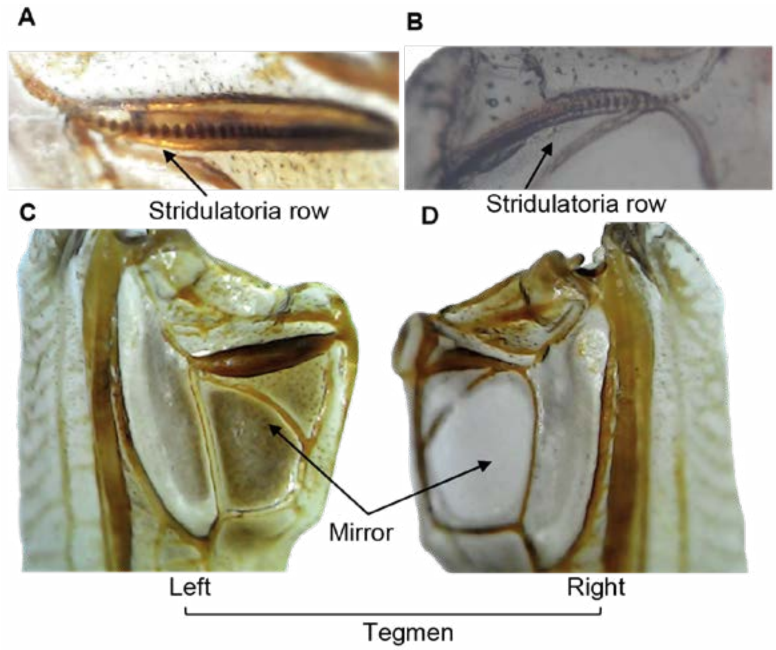

Figure 5. A-D: *Conocephalus cinereus*. Male (♂): A. Stridulation row with 44-45 thick teeth, B. Stridulation row of the right tegmen ventral view, C. Left mirror of the stridulatory apparatus, D. Right tegmen.

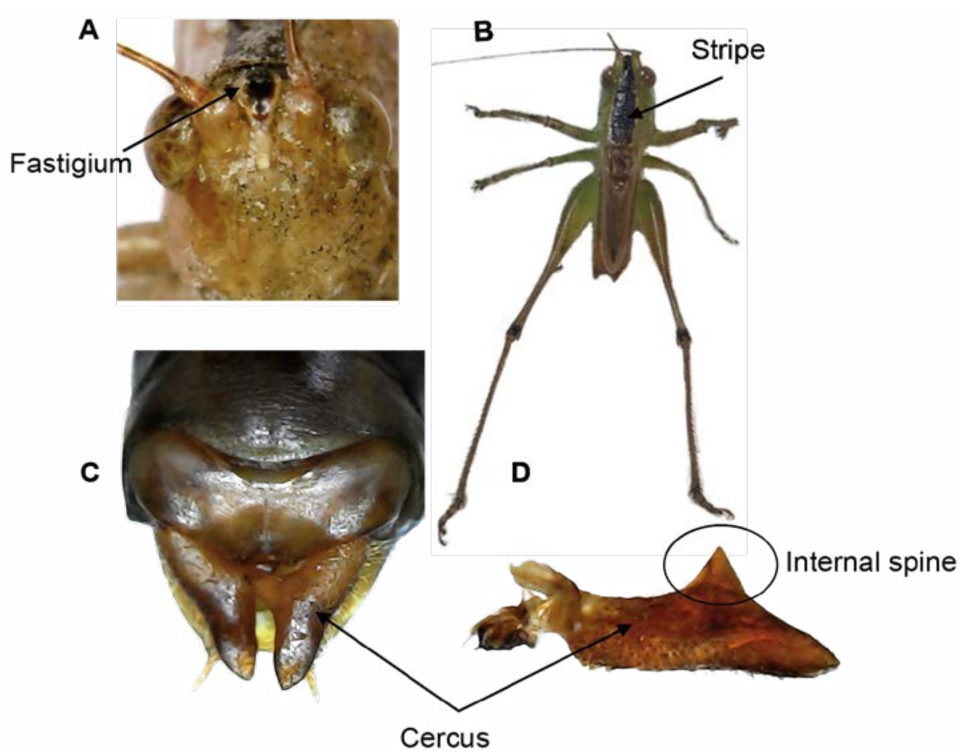

Figure 6. A-D. *Conocephalus cinereus*. Male (♂): A. The apical portion of the fastigium of the vertex tends to be slightly expanded laterally and measures from 2/3 to 3/4 of the scape width, B. Complete body dorsal view, dark brown stripe on the superior part of the head and the pronotum, C and D. Cercus with internal spine, dorsal view.

Conocephalus cinereus

Figure 7. *Conocephalus cinereus*. Thunberg, 1815 Female (♀). Narrow ovipositor, straight to weakly curved; clearly shorter than the posterior femur.

Neoconocephalus triops

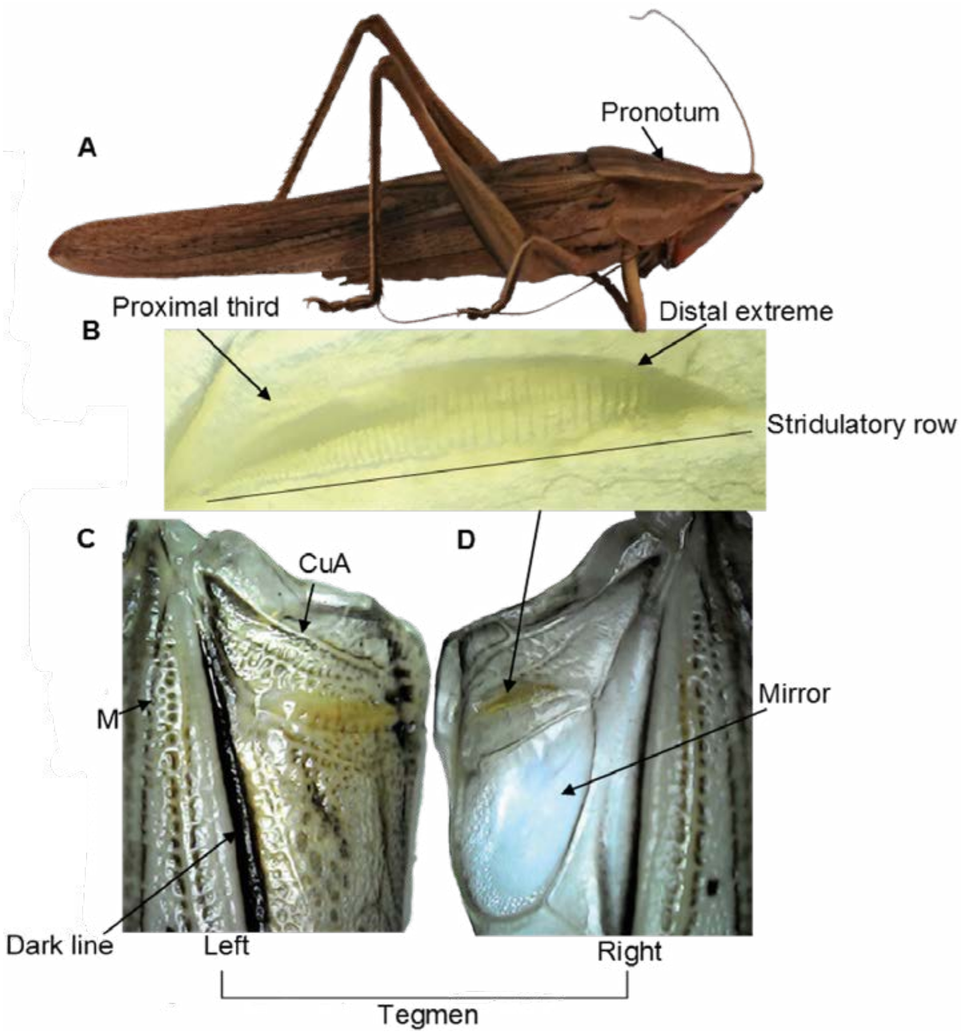

Figure 8. A-D: *Neoconocephalus triops*. Male (♂): A. Complete body lateral view. Pronotum with round appearance, B. Stridulation row with 65-86 teeth; thinner and more spaced teeth in the third proximal of the row, compared to the thickest and less spaced teeth of the distal extreme, C. Base of the left tegmen. Stridulatory area of the wing in most with short and black longitudinal line, parallel to the M+CuA veins, D. Mirror of the oval stridulator.

Neoconocephalus triops

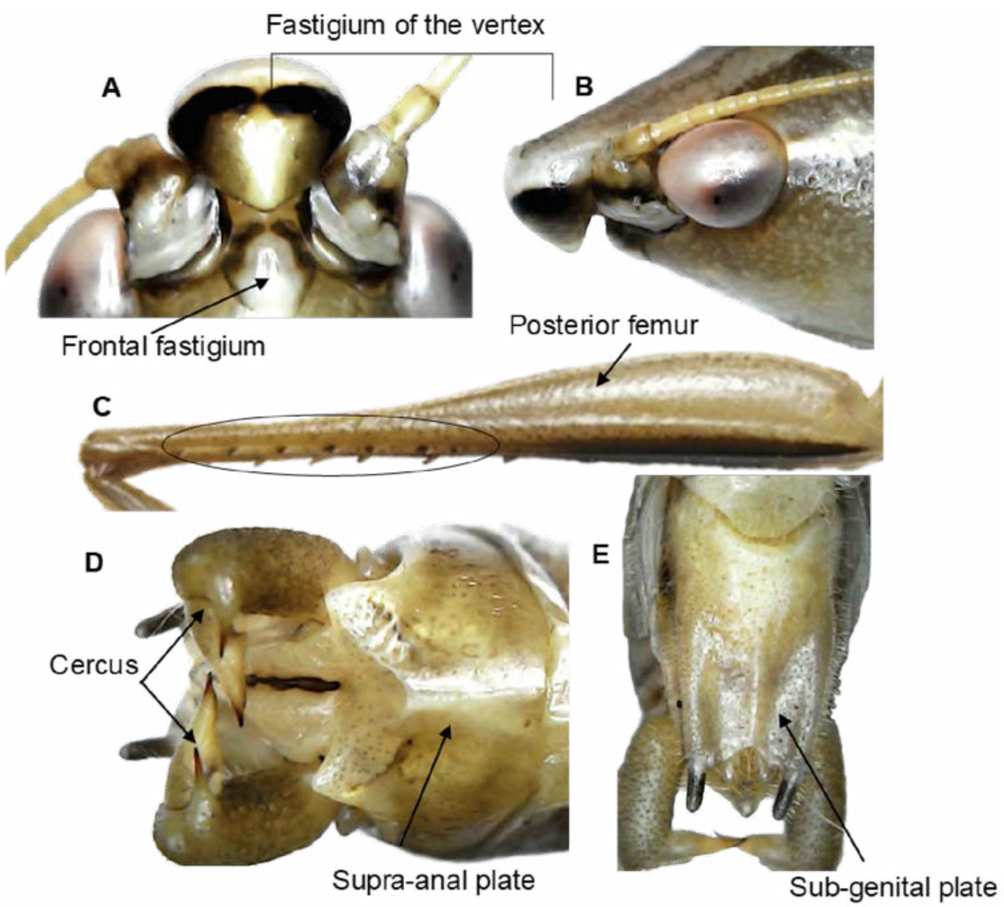

Figure 9. A-E: *Neoconocephalus triops*. Male (♂): A. Fastigium of the large vertex and generally wider rather than long, with round apex, on the frontal part with a weakly defined white band, generally accompanied by a dark thin line, fastigium of vertex separated from the frontal fastigium by a narrow space; blunt frontal fastigium, B. Fastigium lateral view, C. Caudal femur with small spines and dark marks in its base, D. Cercus dorsal view, E. Sub-genital plate.

Stilpnochlora azteca

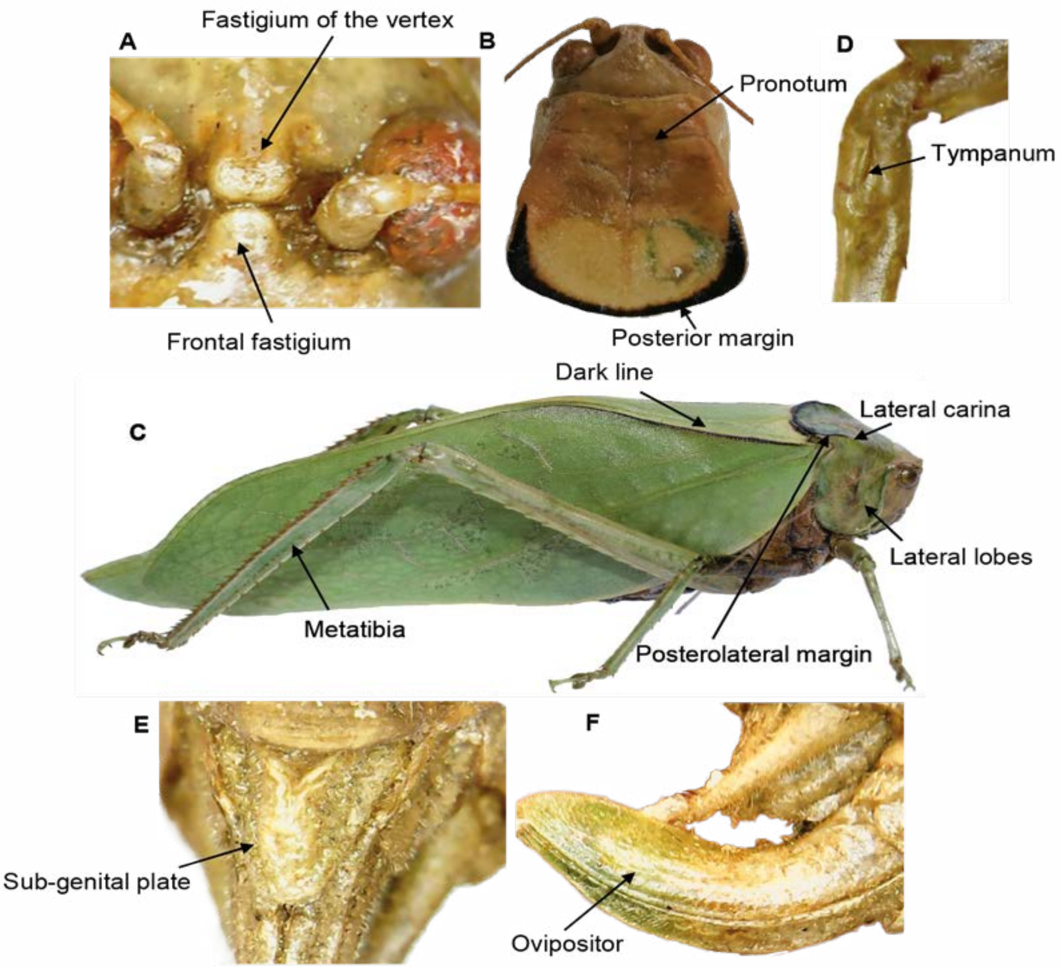

Figure 10. A-F: *Stilpnochlora azteca*. Female (♀), A. Fastigium of the strongly flexed vertex, apically swollen, furrowed in the middle dorsal part; robust and blunt frontal fastigium, B. Pronotum dorsal view, C. Complete body lateral view, approximate size of 95 mm. Lateral lobes that are much deeper rather than wide; anterior round lateral carina, giving the pronotum a curved appearance, D. Tympanum, E. Triangular subgenital plate, ventral view, F. Short ovipositor, raised at 65°, apically pointy, lateral view.

Stilpnochlora azteca

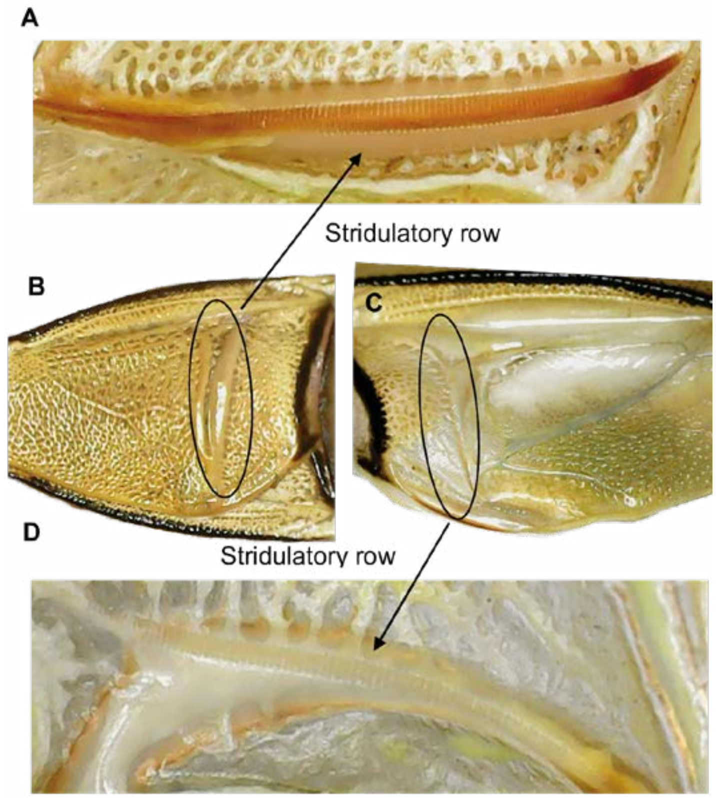

Figure 11. A-D: *Stilpnochlora azteca*. Male (♂): A. Internal face of the left tegmen; stridulation row approximately 61 mm long, with approximately 200 apparently functional teeth, B. Stridulatory apparatus dorsal view, C. Internal face of the stridulation row.

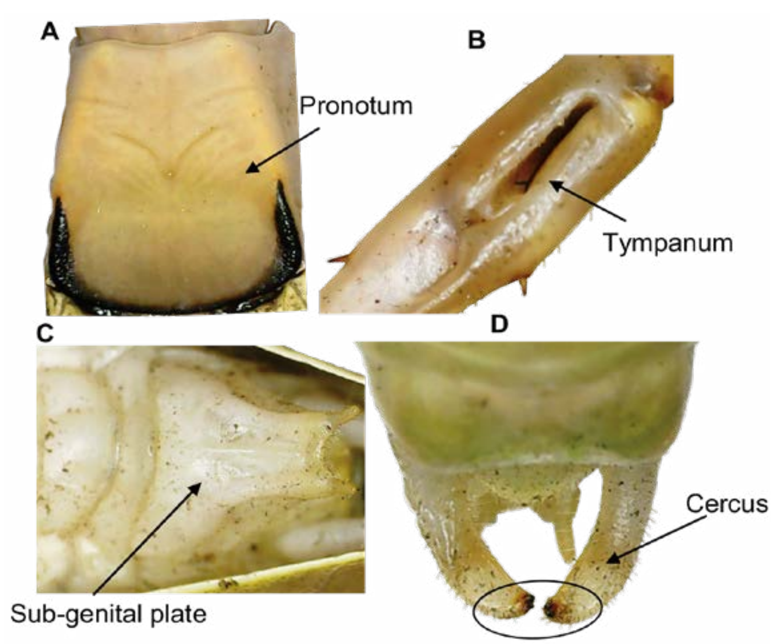

Figure 12. A-D: *Stilpnochlora azteca*. Male (♂): A. Pronotum dorsal view, B. Tympanum on the base of the anterior tibia, C. Articulated sub-genital plate, D. Cercus that end in a pair of similar blunt spines.

Pyrgocorypha **sp.**

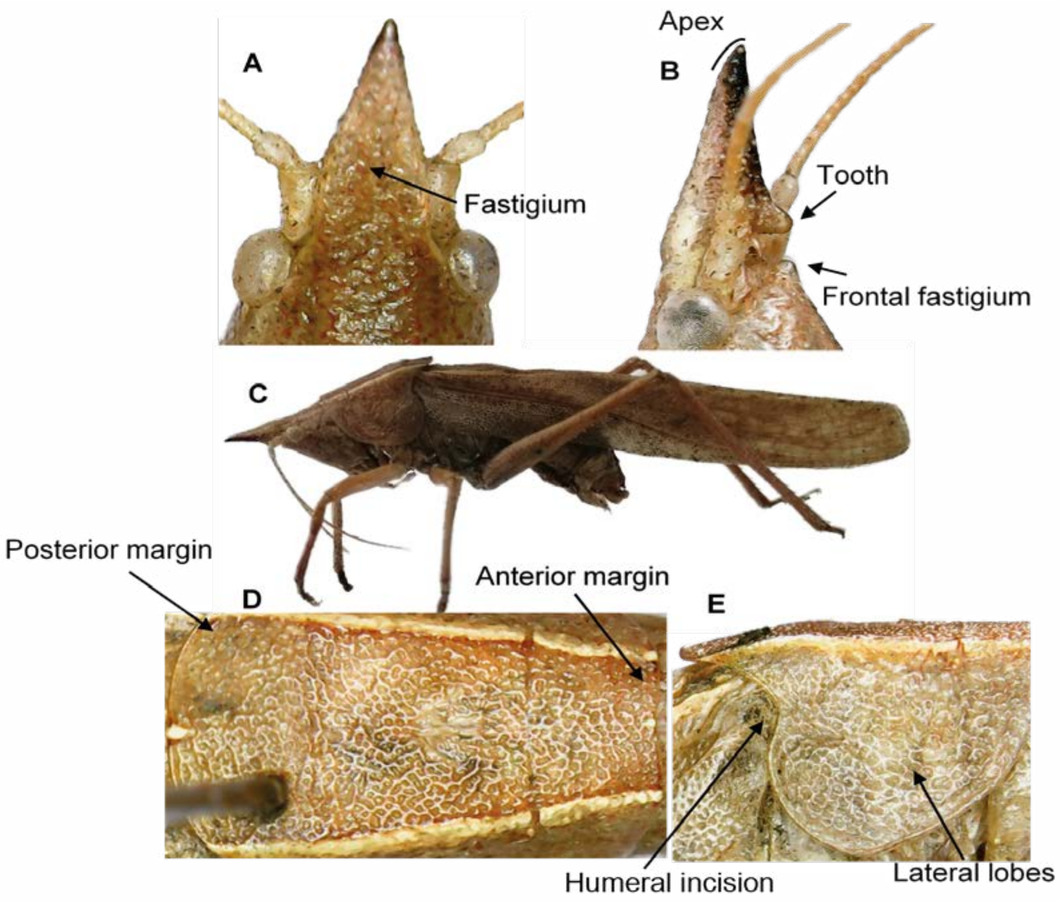

Figure 13. A-E: *Pyrgocorypha* sp. Male (♂): A. Fastigium of the strongly projected vertex, triangular in dorsal view, B. Apex of the fastigium often with a small hook and base with one tooth, separated from the front fastigium by a deep space, C. Complete body lateral view, D. Dorsal surface of the moderately granulated pronotum, flat or weakly convex; straight anterior dorsal margin, posterior straight to weakly convex, E. Lateral lobes with broadly rounded posterior angle and with well-developed humeral incision.

***Pyrgocorypha* sp.**

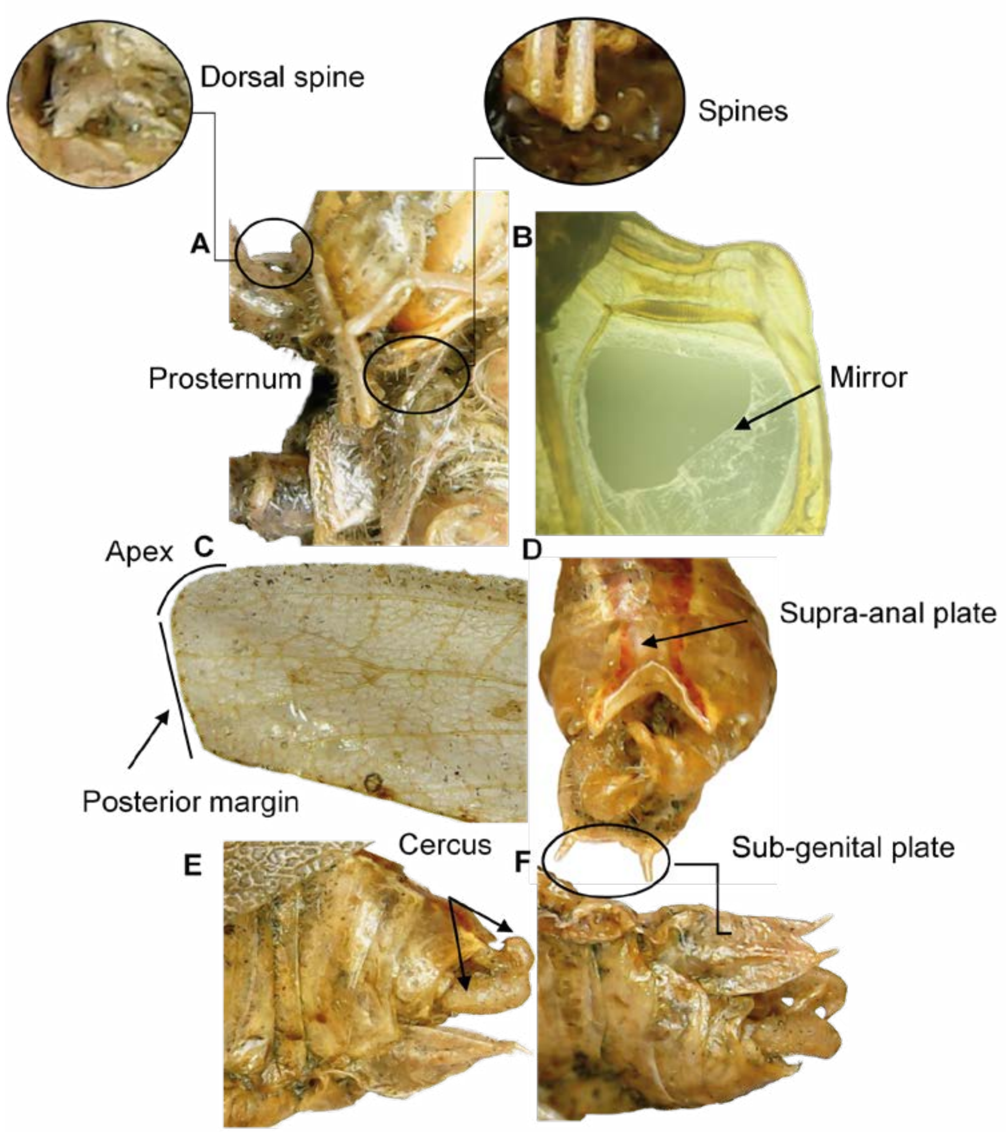

Figure 14. A-F: *Pyrgocorypha* sp. Male (♂): A. Anterior coxa with elongated spine projected forward, prosternum armed with two thin spines, B. Mirror of the right tegmen nearly circular, in ventral view, C. Posterior margin of the anterior straight wings with the rounded apex, D. Small, triangular supra-anal plate, E. Cercus lateral view, F. Sub-genital plate with a pair of styles, straight posterior margin, weakly emarginated or with a deep triangular incision.

Pyrgocorypha **sp.**

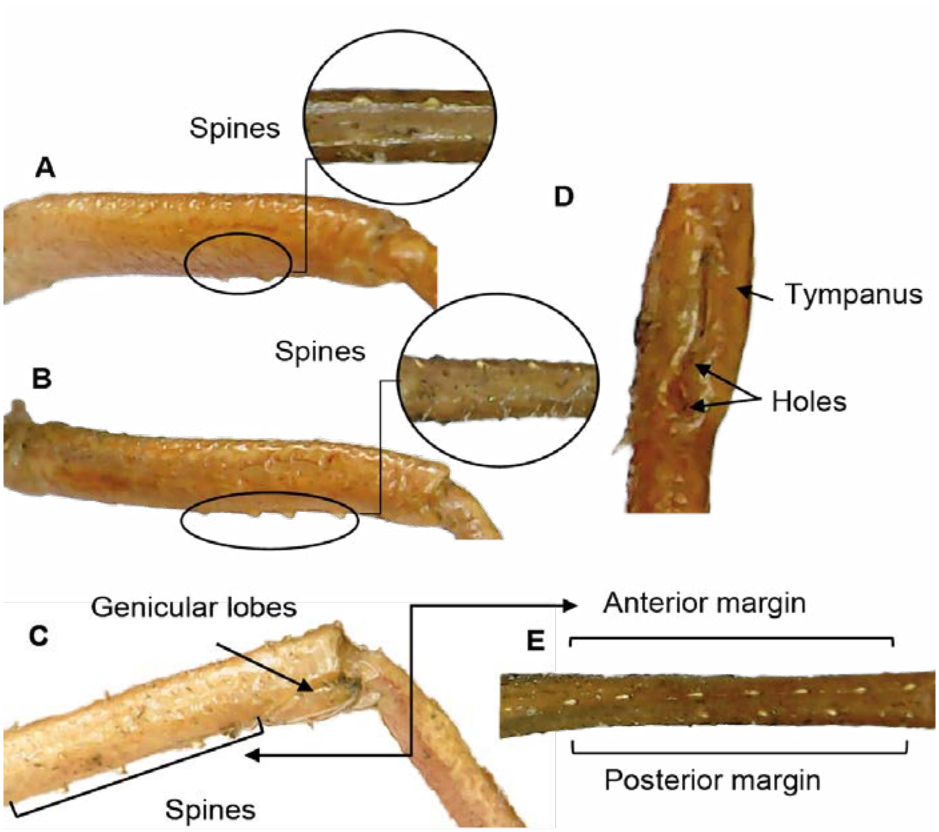

Figure 15. A-E: *Pyrgocorypha* sp. Male (♂): A and B. Anterior and middle femurs with ventral spines in anterior margins, C and E. Posterior femurs with spines in both ventral margins and genicular lobes, D. Tympanum of the anterior tibia closed bilaterally, slightly swollen tympanus area, with a pair of small holes under the tympanus fissures.

Achurum sumichrasti

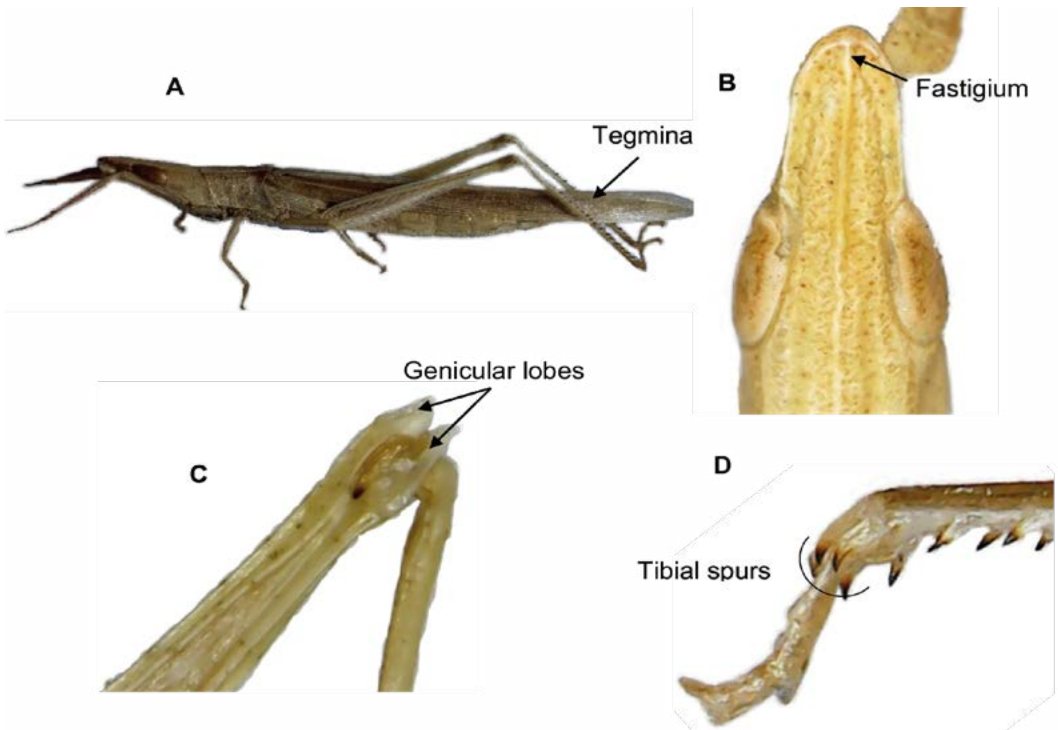

Figure 16. A-D: *Achurum sumichrasti*. Male (♂): A. Complete body lateral view, completely developed tegmina that extend beyond the tip of the abdomen, B. Long fastigium dorsal view, C. Superior and/or inferior elongated genicular lobes, D. Unequal posterior external and internal tibial spurs.

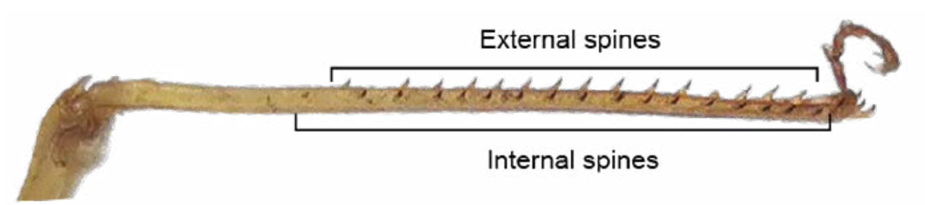

Figure 17. *Achurum sumichrasti*. Female (♀): Posterior tibia with 14-16 external, 16-17 internal spines.

Arphia conspersa

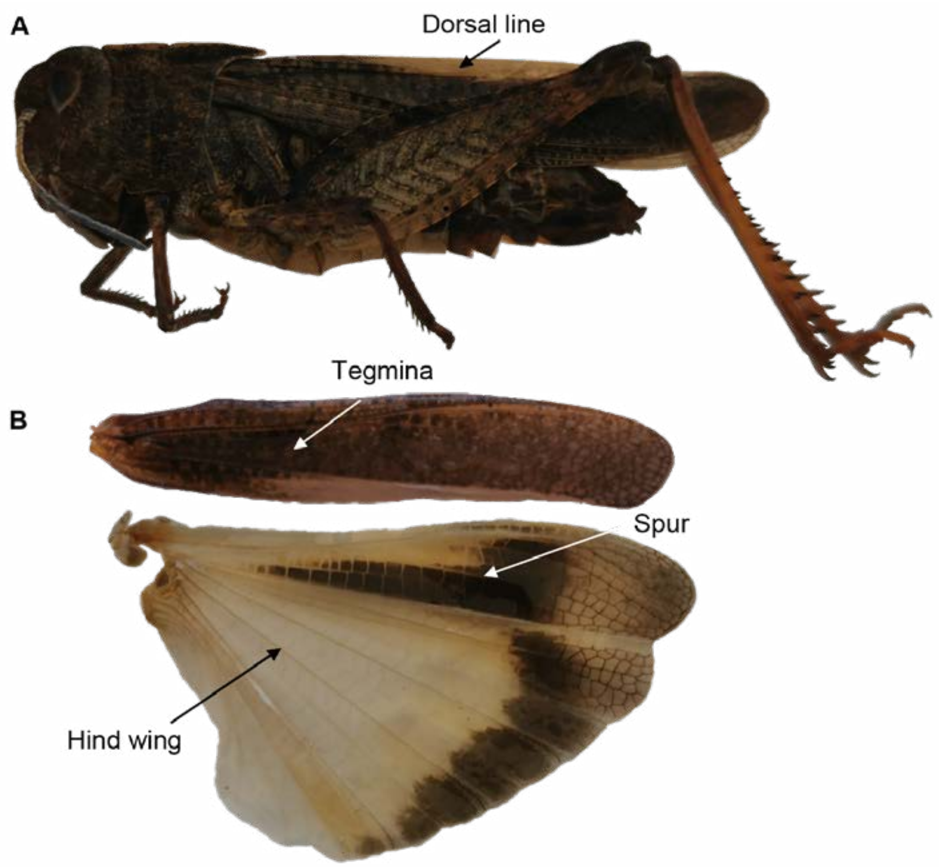

Figure 18. A and B: *Arphia conspersa*. Female (♀): A. Dorsal line of pale color, B. Disc of the posterior wings generally yellow, sometimes pink, with black spur that extends nearly to the base of the wing.

Arphia conspersa

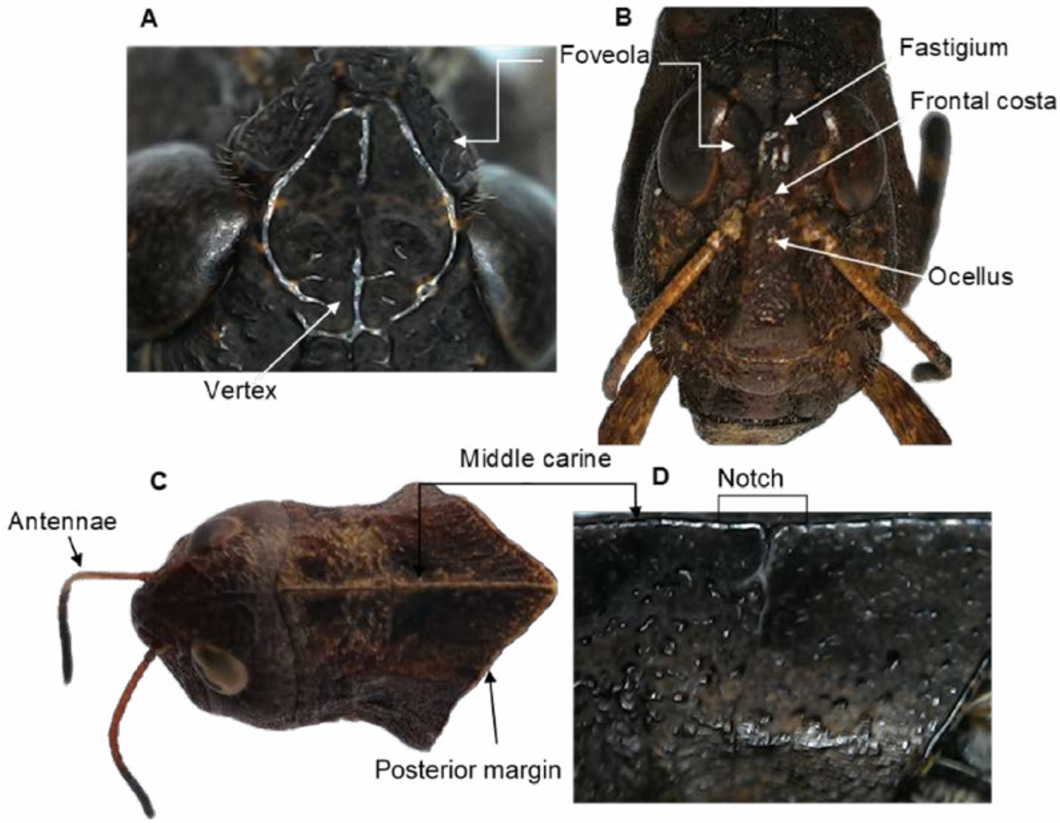

Figure 19. A-D: *Arphia conspersa*. Male (♂): A. Rounded vertex, B. The frontal costa is strongly held between the antennae, truncated fastigium, where it tends to be bifaveolated or with a middle carina. Thin antennae, gradually widening towards the tip, C. The dorsal posterior margin of the pronotum forms an acute angle, D. The middle carina of the pronotum slightly with a furrow near the middle.

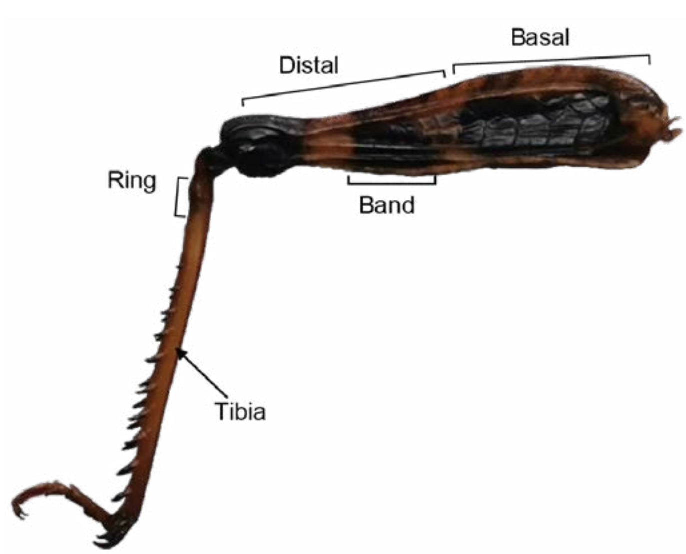

Figure 20. *Arphia conspersa*. Male (♂). Robust posterior femurs, internal face of black color in the basal half. In the yellow distal half with a black band. Light yellow posterior tibia, with dark ring in the third apical.

Aidemona azteca

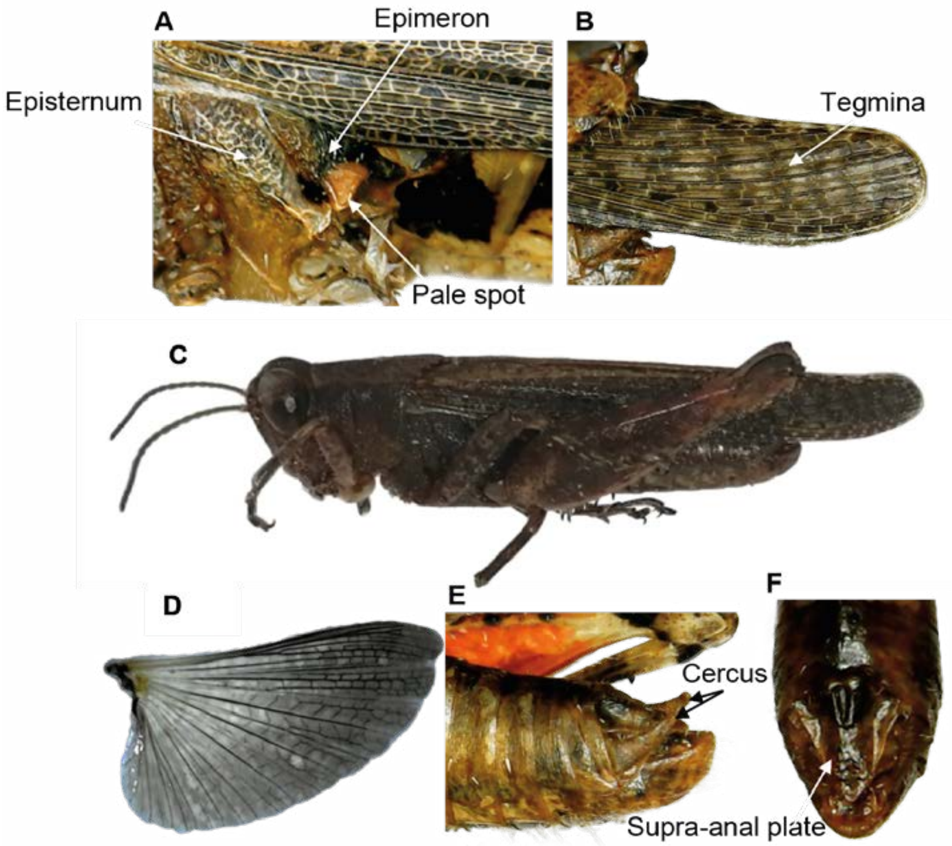

Figure 21. A-F: *Aidemona azteca*. Male (♂). A. Pale spot on the meta-thoracic epimeron on the base of the posterior leg, B. Tegmina larger than the length of the posterior femurs, C. Complete body lateral view, D. Clear posterior wing in its base and distal gray cloudy, E. Cercus of the male flattened on the base, with thin apices, directed towards the middle line, F. Sub-triangular supra-anal plate with round apex, furrowed in the middle basal line, but not divided transversally.

Aidemona azteca

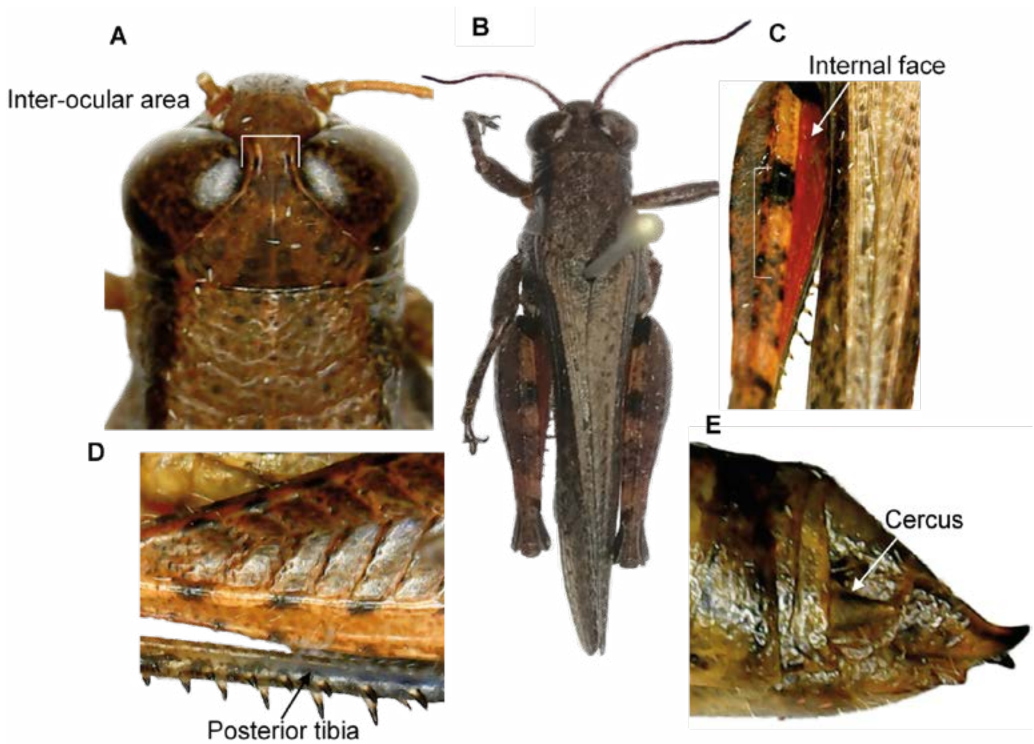

Figure 22. A-E: *Aidemona azteca*. Female (♀). A. Small head with prominent eyes, narrow inter-ocular area, B. Complete body dorsal view, C. Posterior femur with two darker transversal bands in the dorsal area, marginal ventral area, and internal red-orange face, D. Posterior blue tibia, E. Cercus lateral view.

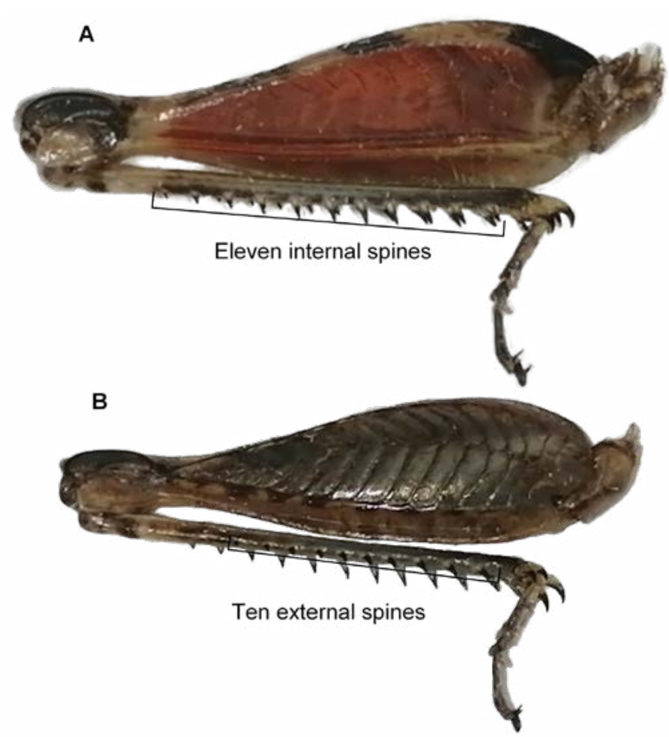

Figure 23. A and B: *Aidemona azteca*. Male (♂). A. Posterior tibia with eleven internal and ten external spines.

Dichromorpha prominula

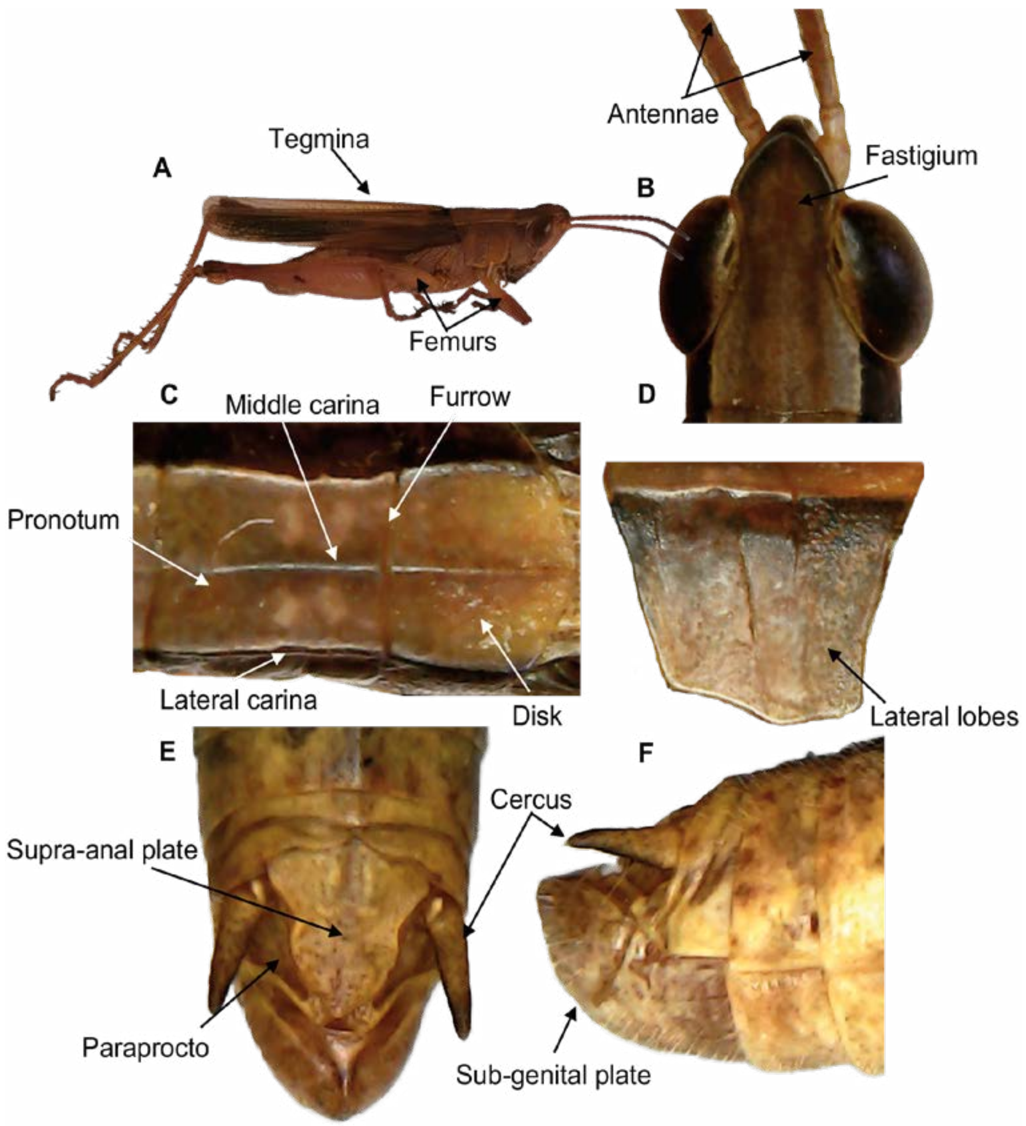

Figure 24. A-F: *Dichromorpha prominula*. Male (♂): A. Complete body lateral view, femurs of anterior and middle legs clearly engrossed. Long and narrow tegmina, exceeding towards the posterior femurs, B. Slightly ensiform antennae in the base. The anterior internal margin of the round fastigium, C. The pronotum disk is simple with three carinas, nearly straight and parallel, cut by one or two transversal furrows, D. The lateral lobes of the pronotum are perpendicular, E. Supra-anal sub-triangular plate, with round apex, slightly shorter than the paraproctos, F. Narrow sub-genital plate with round tip, with silks. Simple cercus, straight with blunt tips.

Melanoplus differentialis

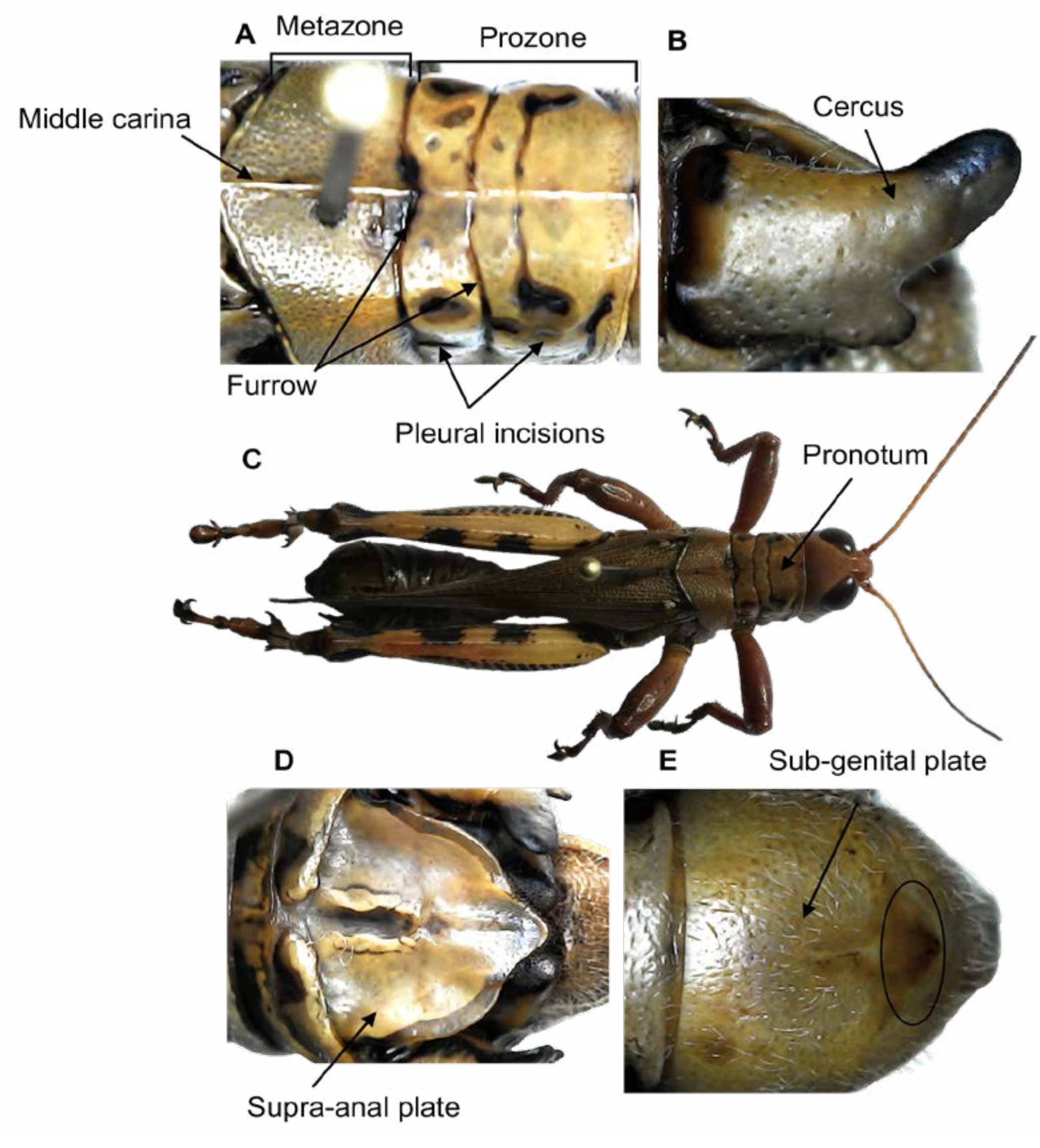

Figure 25. A-E: *Melanoplus differentialis*. Male (♂): A. Pronotum with transversal furrow and dark pleural incisions. Flat disk of the pronotum or nearly flat, distinct middle carina and sharp in metazone, less visible in the frontal half of the prozone, slightly longer prozone than the finely coarse metazone, B. Cercus, lateral view, C. Complete body dorsal view, D. Wide supra-anal plate, the sides of the rounded apical third and oblique on the back, E. Short sub-genital plate, wide, in the middle of the engrossed apical margin, slightly prolonged upwards, rarely with a weak notch.

Melanoplus differentialis

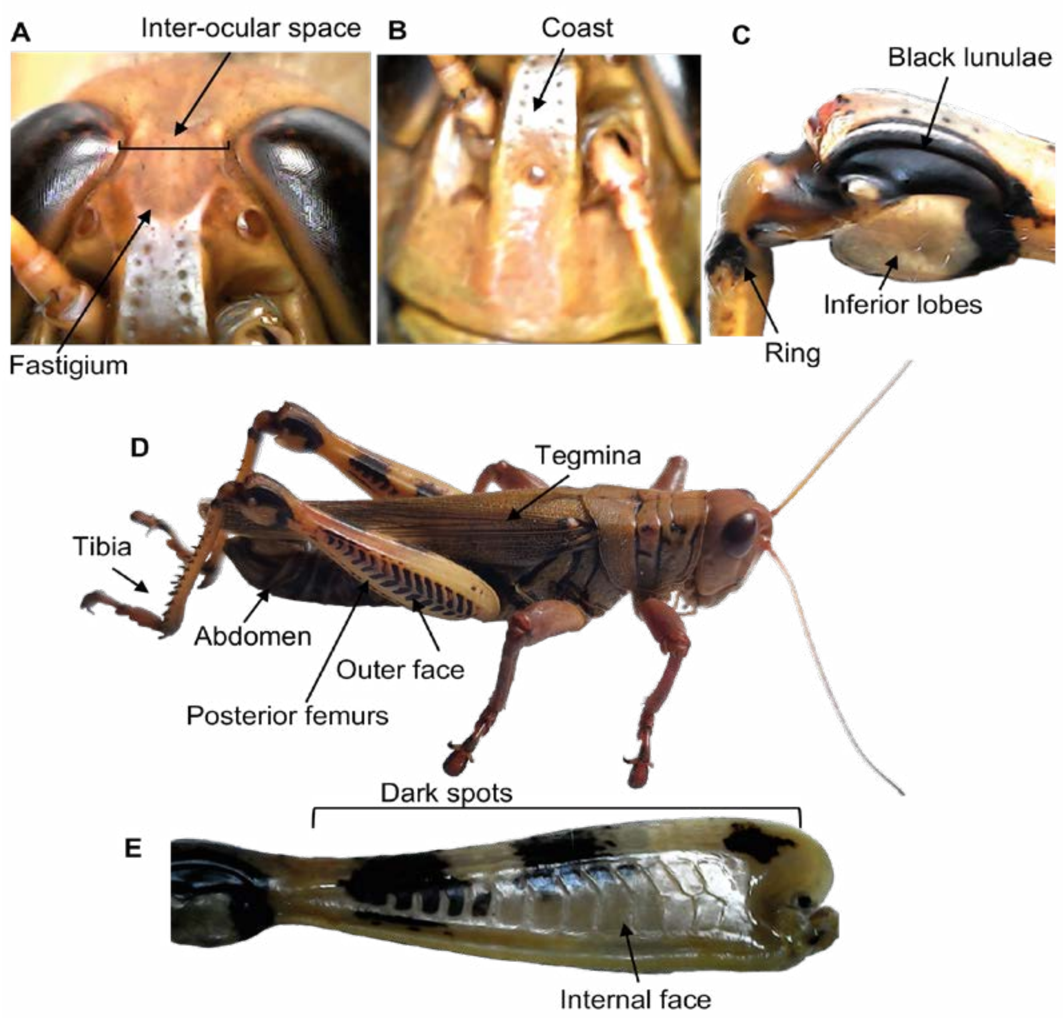

Figure 26. A-E: *Melanoplus differentialis*. Male (♂): A. Inter-ocular space twice or more times the length of the first antenna articulation; fastigium of soft, concave slope, broad but not deep, B. Wide frontal side, furrowed and broad under the ocellus, less distinctively in the female, C. Patella with black lunulae, their inferior exterior lobes of bright yellow color, D. Complete body lateral view. Tegmina exceeding to the tips of the posterior femurs in both sexes. Posterior yellow tibias, with narrow black basal ring and black spines, E. Posterior femurs of pale or bright yellow color, the superior margin with three dark oblique patches, F. Internal face of the posterior femurs, with inferior margin of yellow color.

Rhammatocerus viatorius

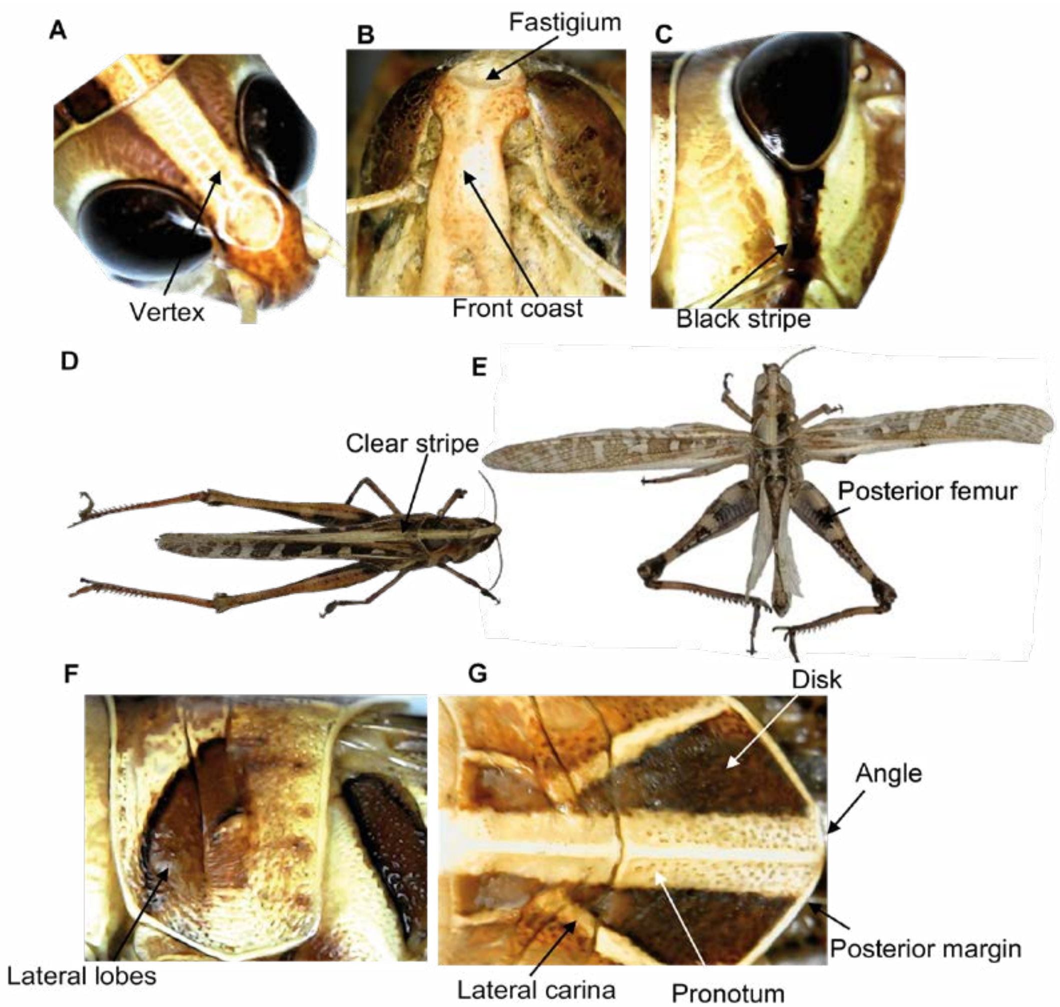

Figure 27. A-G: *Rhammatocerus viatorius*. Male (♂): A. Narrow head; horizontal vertex, B. Round fastigium, concave semi-circular fastigial depression. Strongly arched costa, prominent on the superior part, convex in the inferior part, C. Flexed, convex face and with a dark brown or black spot in the center of the lateral lobe, D. Female (♀) complete body dorsal view, with one light stripe that goes from the fastigium along the head, pronotum, dorsal field of the tegmina, and pro-thoracic lobes, E. Compressed body, F. Pronotum with dark lateral lobes, G. Dorsal view of the pronotum with constrictive lateral carinas of light color, forming a mark of hourglass shape. Disk of the dark pronotum, posterior angled margin.

Rhammatocerus viatorius

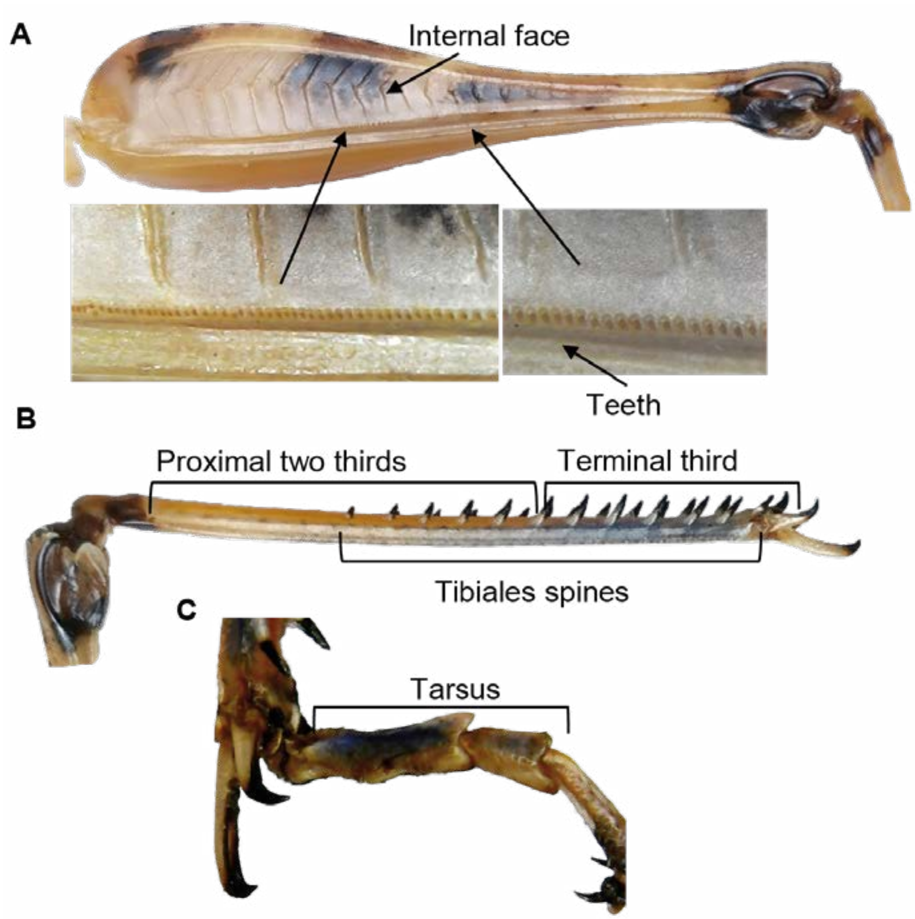

Figure 28. A-C: *Rhammatocerus viatorius*. Male (♂): A. The internal face of the posterior blue or yellow femurs, with three dark spots. Stridulated ridge with approximately 70 teeth, B. Posterior tibia with two thirds proximal yellow or orange color, with the terminal third slightly shaded in blue or gray, C. Blue tarsus dorsally, with the last light brown tarsomere.

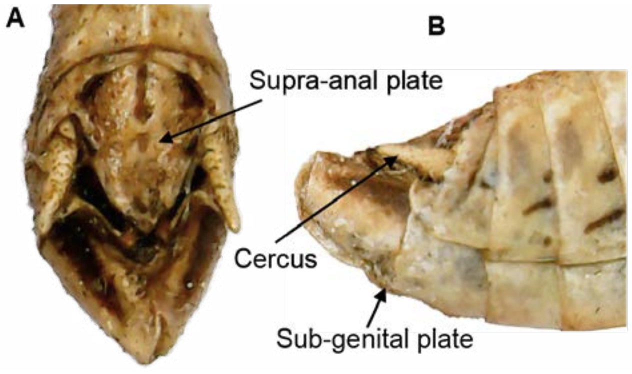

Figure 29. A and B: *Rhammatocerus viatorius*. Male (♂): A. Triangular supra-anal plate; longer cercus than the supra-anal plate, B. Whole sub-genital plate with a sharp tip, which is obliquely directed upwards in the lateral view.

Sphenarium purpurascens

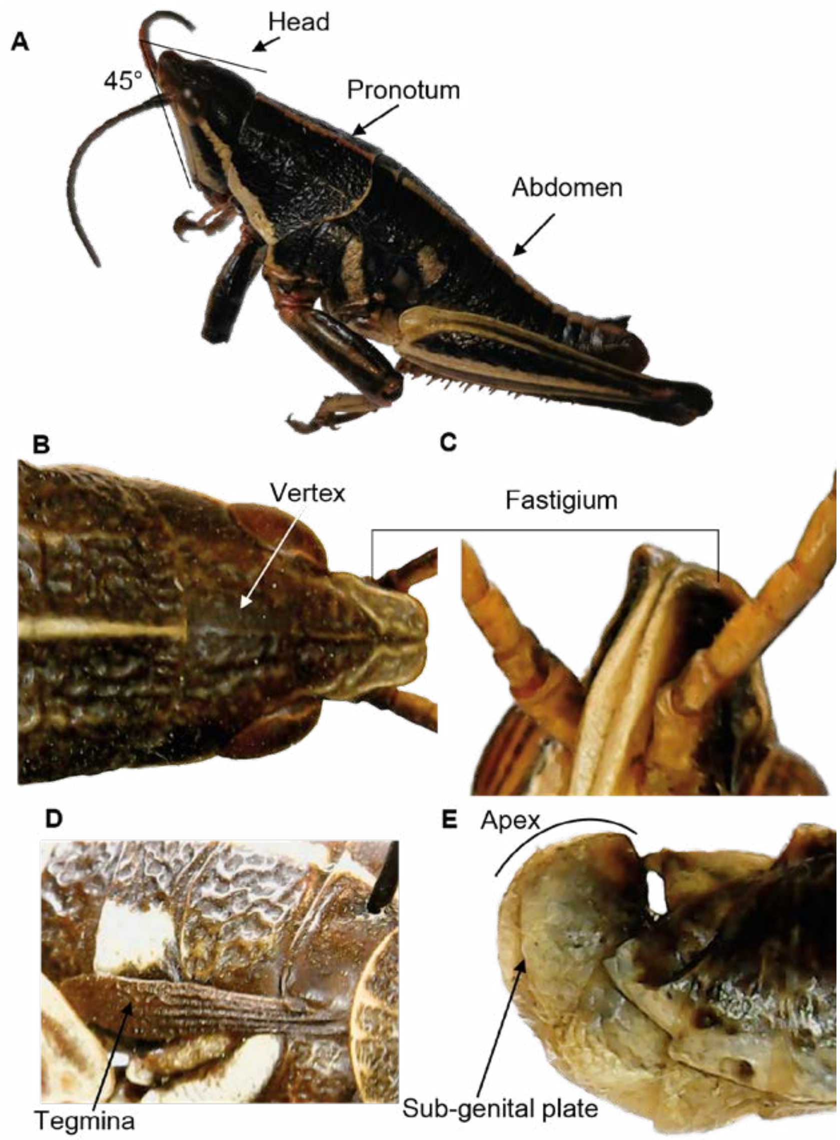

Figure 30. A-E: *Sphenarium purpurascens*. Male (♂): A. Complete body lateral view. Flexed face approximately at 45 degrees, wider in the inferior part than in the superior, sub-triangular compressed, B and C. Rounded vertex, wide and blunt fastigium, divided by an imprinted line, D. Tegmina in shape of spatula, narrow, reaching the second abdominal segment, E. Round sub-genital plate with apex curved upwards.

Sphenarium purpurascens

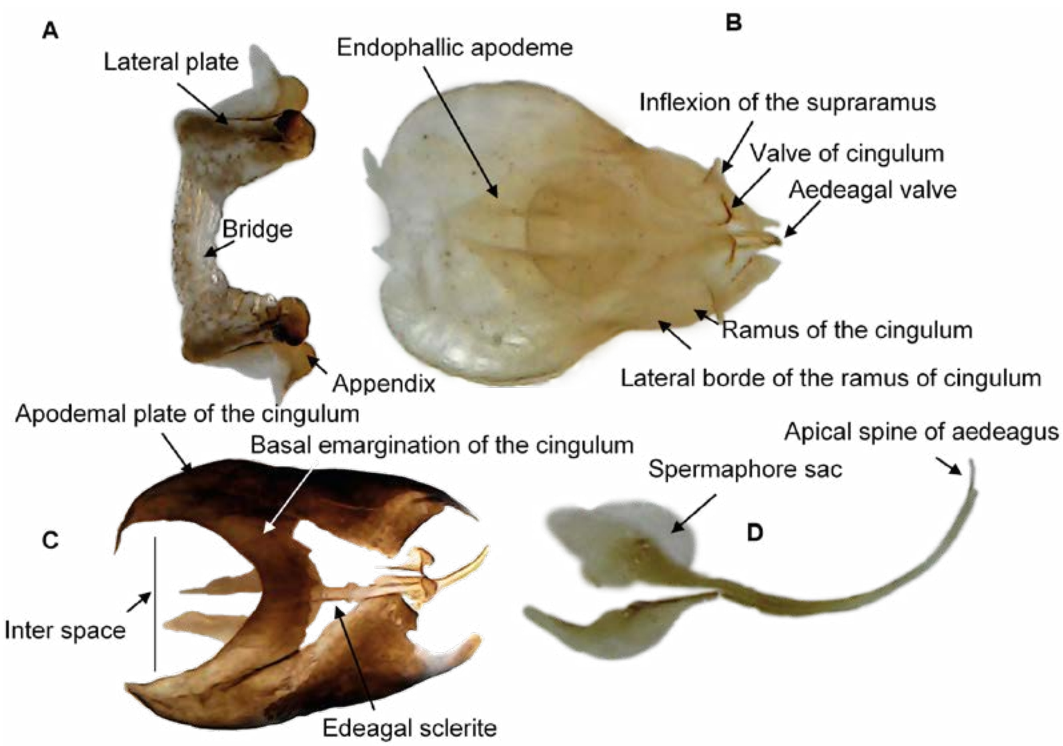

Figure 31. A-D: *Sphenarium purpurascens.* Genital structures of the Male (♂): A. Epiphallus with the bridge as long as the length of the lateral plates, B and C. Ectophallus and its main structures in dorsal view, D. Endophallus in lateral view.

Taeniopoda stali

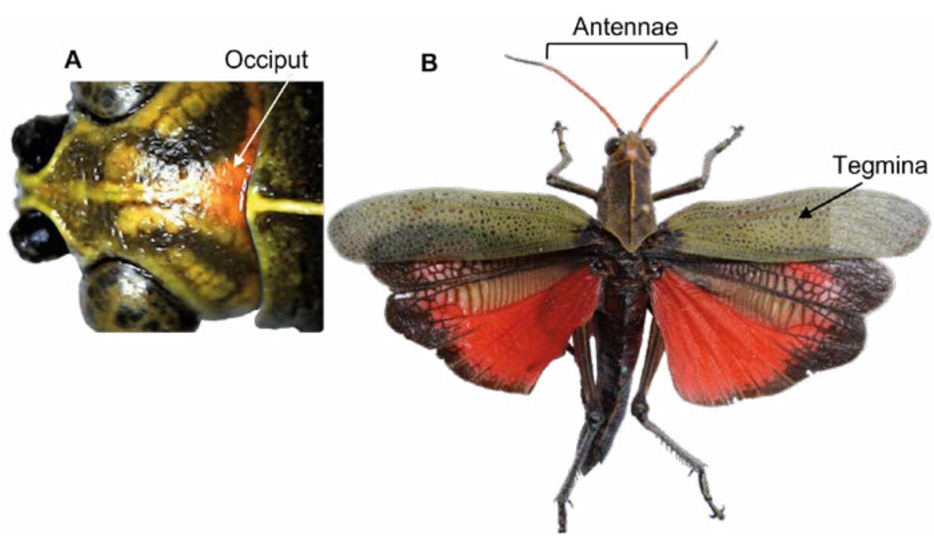

Figure 32. A and B: *Taeniopoda stali.* Female (♀): A. Posterior part of the occiput with a small red surface in the middle, B. The veins on the tegmina are deep green color, inter-vein separations with small areas of dark brown color. Antennae of intense red color.

Brachystola mexicana

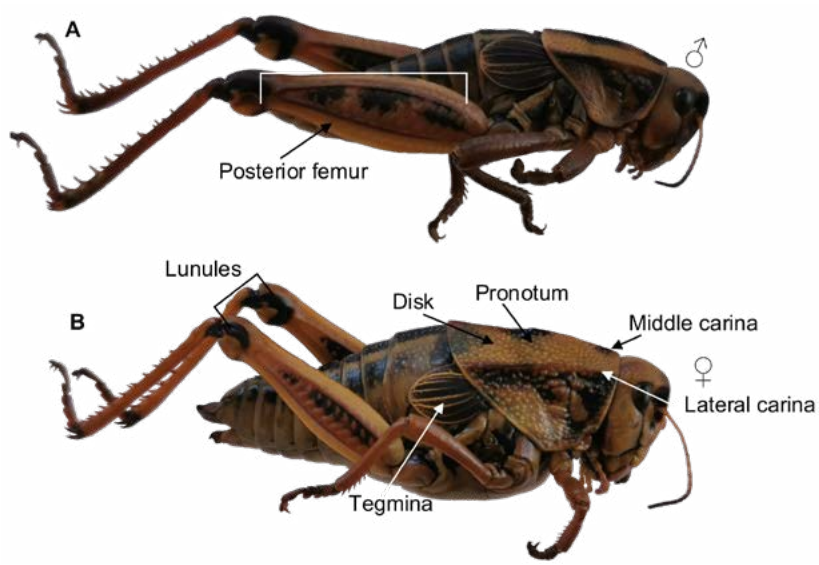

Figure 33. A and B: *Brachystola mexicana*. A. Male (♂), posterior femurs with three dark patches on the external face; one basal, one medium and another apical, dark internal face. Patella with black lunules, B. Female (♀): circular tegmina, small, of dark color with pale veins. Broad pronotum, lateral margins with very marked angle, anterior lobe with compressed disk, bulky on the posterior lobe. Prominent middle carina; prominent and strongly divergent lateral carina.

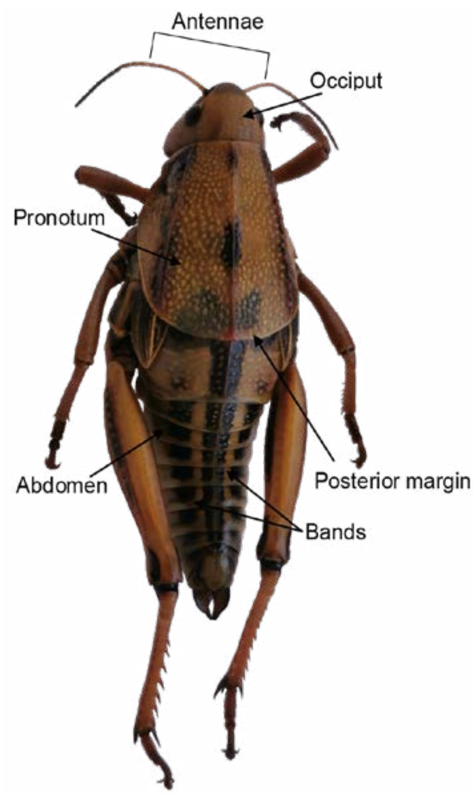

Figure 34. *Brachystola mexicana*. Female (♀): Short antennae, they reach the middle of the pronotum. Pronotum with broadly rounded posterior margin. Short abdomen on the base, thinning quickly in the middle and distal part. Dorsum of the abdomen with two pale bands, one on each side close to the middle of the body.

Brachystola mexicana

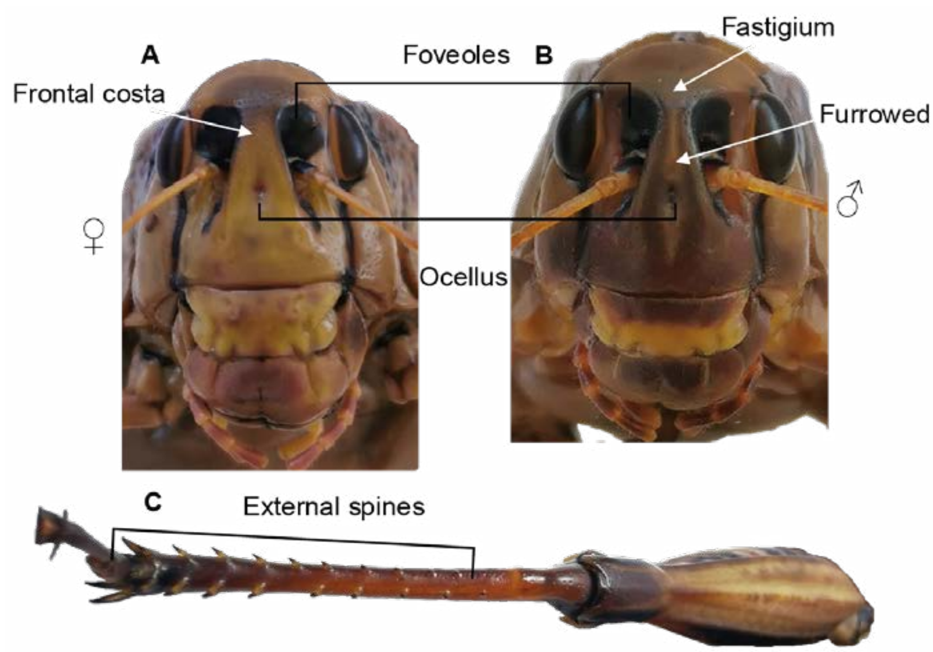

Figure 35. A-C: *Brachystola mexicana*. A, and B. Head of moderate size, wider in the inferior part than the superior, deep foveoles of dark color, the fastigium slightly furrowed, continually rounded with the prominent frontal costa; the costa expands uniformly above the ocellus, C. Posterior tibia with 10 small spines arranged irregularly on the exterior line.

***Stenopelmatus* sp.**

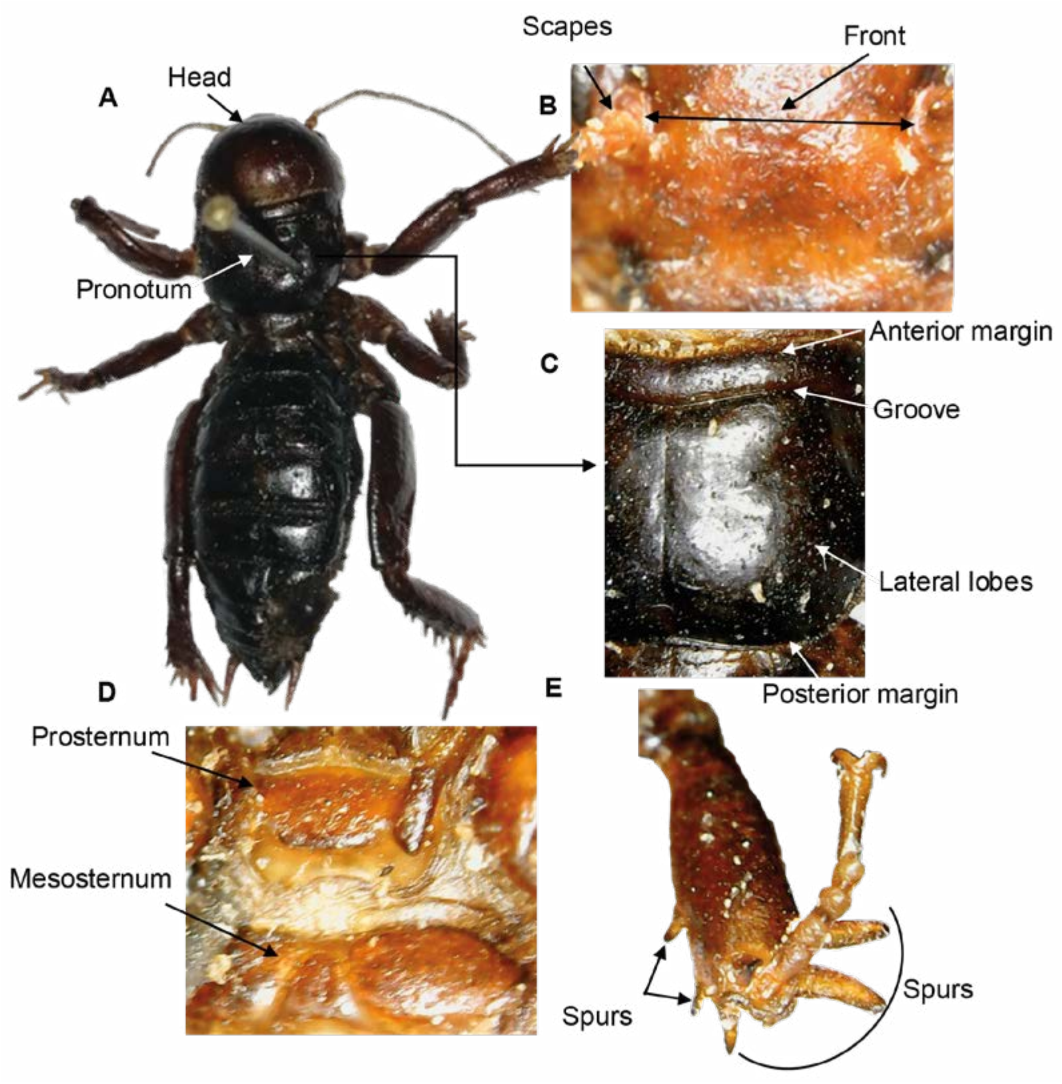

Figure 36. A-E. *Stenopelmatus* sp. Female (♀): A. Spherical head, B. Wide and flat front. The antenna cavities and the scapes are clearly smaller than the space between these cavities. Quite separate antennae, C. Narrow posterior pronotum and slightly bent upwards, except in the anterior margin. Short lateral lobes, slightly inclined, D. Prosternum approximately square, with bent margins, which end in a small blunt nodule on the posterior part, E. The anterior tibia distally wide with three pointy spurs on the interior of the apex and two shorter spurs in the inferior part.

Derived from the results of molecular biology studies carried out in the Laboratory of Integral Phytosanitary Diagnosis of Colegio de Postgraduados Campus Montecillo, in Texcoco, Estado de México, the following species were determined: *Ducetia japonica*, *Schistocerca nitens* and *Ceuthophilus pallidipes*.

The families Acrididae, Tettigoniidae and Pyrgomorphidae represented the highest number of genera determined taxonomically, with 41.40%, 23.32% and 21.73%, respectively. These results agree with those by Pocco *et al*. (2010), who mention these families as the most abundant in grasslands. For their part, Cornejo *et al*. (2006) point out that Tettigoniidae, Acrididae and Gryllidae represent the largest number of species in Mexico, perhaps because there is more bibliographic information for their determination.

From the Acrididae family, the genera with greatest dominance were *Schistocerca* and *Achurum* and, according to Fontana *et al*. (2017), they consider the genus *Schistocerca* as important because of the considerable damages it causes in the agricultural sector. For their part, Barrientos-Lozano *et al*. (2013) mention that the order Orthoptera includes pests of great economic and social impact, such as the various species of locust. From the Tettigoniidae family, the most abundant genera were: *Neoconocephalus*, *Pyrgocorypha*, and *Conocephalus*; Barrientos-Lozano *et al*. (2013) refer that these genera are found in humid meadows and are common in Mexico. On the contrary, Barranco and Pascual (1992) report that *Conocephalus* appears exclusively in farmed areas, and not in fallow land. The family Pyrgomorphidae was of great impact and the genus *Sphenarium* was collected, result that agrees with Ramírez-Méndez *et al*. (2019), who mentioned that out of 2806 orthopterans collected in the basin of the Pátzcuaro River, Michoacán, Mexico, the dominating species belonged to two families, Pyrgomorphidae and Acrididae, and the *Sphenarium* genus with 2746 specimens collected. Fontana *et al*. (2017) point out that some species of *Sphenarium* are used as food for humans because of their high density, although their potential as pest in monocrops has also been reported. Sanabria-Urbán *et al*. (2017) mention that *Sphenarium* is culturally and economically important for Mexican people since pre-Hispanic times. Barranco and Pascual (1993) refer that orthopterans are distributed according to the physiognomic type of vegetation, even when the plant species vary in time.

Population density and fluctuation

The highest density of orthopterans was found September, followed by October and November. The highest density of orthopterans was found in Sector I, possibly because in the nopal crop there were no agronomic disturbances. In second place, it was Sector IV, where corn used as forage for cattle was established, and therefore, the application of pesticides was not significant. Then, it was Sector III, with broccoli, bean and squash crops, where frequent disturbances were found, which hypothetically suggests instability of Arthropoda. Finally, the lowest density of orthopterans was found in Sector II, cultivated with avocado, where the agronomic management was *ad hoc* (Table 2) (Figure 37).

Table 2. Density of orthopterans collected by sector and crops during the year 2019.

Month	Collection date	Number of Orthoptera collected by sector				
		Sector I	Sector II	Sector III	Sector IV	Total
August	16-aug-19	28	4	51	25	108
	30-aug-19	45	3	37	38	123
September	13-sep-19	76	19	57	68	220
	27-sep-19	95	16	81	109	301
October	11-oct-19	86	29	98	73	286
	25-oct-19	68	15	65	68	216
November	08-nov-19	70	21	76	80	247
	22-nov-19	78	10	61	77	226
December	06-dec-19	57	1	58	48	164
	20-dec-19	47	5	30	51	133
Total	10	650	123	614	637	2024

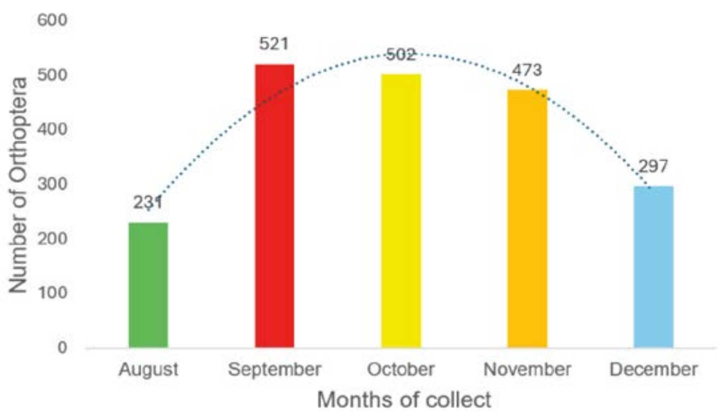

Figure 37. Population fluctuation and density of orthopterans from August to December 2019.

In quantitative terms, the results by genus and species are presented in Table 3, Figure 38. *Schistocerca nitens*, *Sphenarium purpurascens*, *Achurum sumichrasti*, *Neoconocephalus triops*, *Ceuthophilus pallidepes* and *Pyrgocorypha* spp., were the most abundant species, so it can be inferred that the sectors studied are dynamic and have an essential tendency to self-organize even by changing their composition, structure and function throughout time.

It is necessary to highlight that there are prominent differences in behavior between *Acanthorintes tauriformis*, *Conocephalus cinereus*, *Ducetia japónica*, *Neoconocephalus triops*, *Stilpnochlora azteca* and *Pyrgocorypha* spp., compared with *Achurum sumichrasti*, *Arphia conspersa*, *Aidemona azteca*, *Dichromorpha prominula*, *Melanoplus differentialis*, *Rhammatocerus viatorius* and *Schistocerca nitens*; the first have nocturnal habits, while the latter have daytime activity. However, the characteristic that both groups share is homochromy (Massa *et al.*, 2012). In the Analysis of Variance, for sectors and months of collection, whose dependent variable were the Orthopteroids (Table 4), highly significant differences were obtained, and this indicates that at least one sector was different. The coefficient of determination

Table 3. Abundance of genera by date of collection.

Genus	Abundance of Orthoptera genera collected from August to December 2019										
	16-aug-19	**30-aug-19**	**13-sep-19**	**27-sep-19**	**11-oct-19**	**25-oct-19**	**08-nov-19**	**22-nov-19**	**06-dec-19**	**20-dec-19**	**Total**
Acanthorintes tauriformis	2	0	3	6	4	2	3	6	3	1	30
Conocephalus cinereus	6	6	8	7	9	4	10	8	4	2	64
Neoconocephalus triops	**5**	**9**	**15**	**28**	**39**	**24**	**28**	**26**	**14**	**15**	**203**
Ducetia japónica	0	0	2	6	0	0	3	2	0	0	13
Stilpnochlora azteca	0	0	3	12	0	2	4	2	0	2	25
Brachystola mexicana	0	0	1	5	0	1	0	3	0	0	10
Pyrgocorypha spp.	**6**	**8**	**15**	**16**	**24**	**16**	**18**	**18**	**10**	**5**	**136**
Gryllus spp.	1	2	3	7	10	4	9	0	0	2	38
Ceuthophilus pallidipes	**12**	**8**	**10**	**18**	**27**	**17**	**26**	**19**	**13**	**11**	**161**
Achurum sumichrasti	**15**	**19**	**31**	**43**	**40**	**29**	**44**	**35**	**27**	**23**	**306**
Aidemona azteca	0	1	3	6	7	1	2	2	3	0	25
Arphia conspersa	2	0	0	8	2	3	4	5	0	0	24
Dichromorpha prominula	0	0	3	1	4	2	3	2	0	1	16
Melanoplus differentialis	1	2	4	7	10	4	5	7	5	5	50
Rhammatocerus viatorius	2	1	5	7	6	3	7	2	0	0	33
Schistocerca nitens	**30**	**35**	**60**	**63**	**59**	**51**	**38**	**45**	**40**	**32**	**453**
Taeniopoda stali	0	0	3	7	5	0	0	2	0	0	17
Sphenarium purpurascens	**25**	**30**	**51**	**53**	**38**	**52**	**41**	**41**	**45**	**34**	**410**
Stenopelmatus spp.	1	2	0	1	2	1	2	1	0	0	10
Total/Collection date	**108**	**123**	**220**	**301**	**286**	**216**	**247**	**226**	**164**	**133**	**2024**

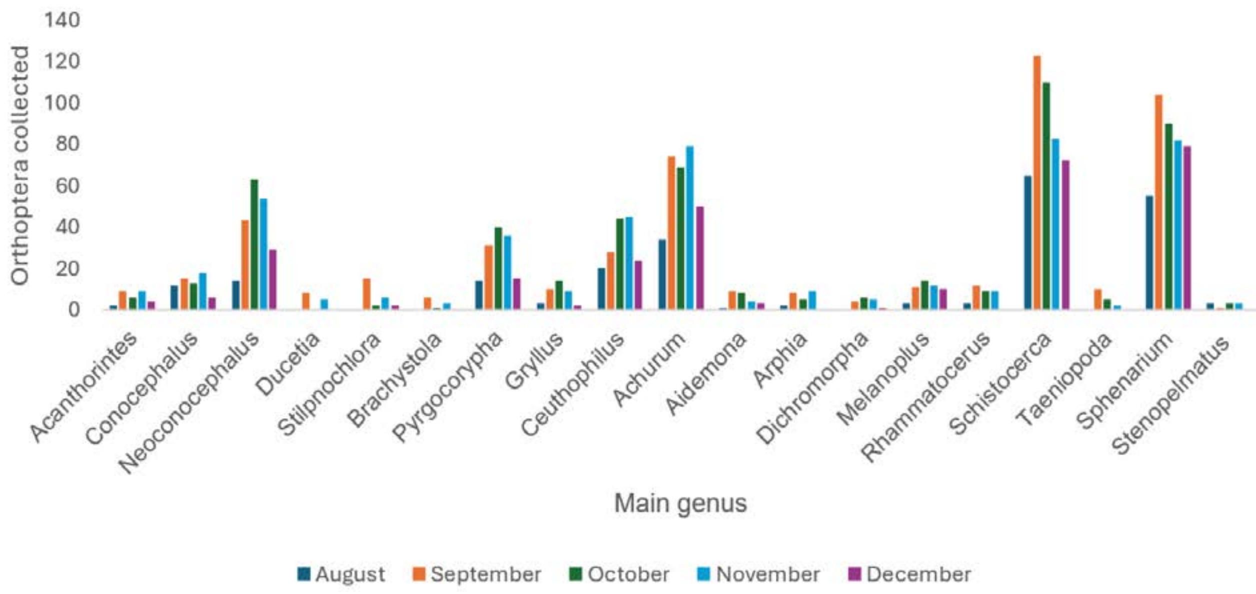

Figure 38. Population fluctuation of orthopterans during the months of August to December 2019.

(R^2) was 94.52% and this explains that the populations of orthopterans were dependent on the crops (sectors) and months of the year. The result from Tukey's means comparison test α=0.05, represents that the sectors farmed with nopal, broccoli, bean, squash and corn are statistically equal. In the sector with avocado the response variable was different.

What has been exposed up to this point can be attributed to the fact that in the sectors farmed with nopal, broccoli, bean, squash, and corn, orthopterans had greater availability of food and spaces to copulate, in contrast with the sector cultivated with avocado. Therefore, this condition generates the opportunity of reflecting on how the inputs are being used in the agroecosystems to satisfy the human needs and the impacts that can happen in the long term. It is a priority to propose the design of agricultural management models based on an approach that is more closely linked to the environment and socially more sensitive, centered not only on production, but also in the ecological stability of the production systems (Machado and Campos, 2008).

Statistically there was significance, and 93.78% of the species of Orthoptera depended on the months. In practice, this result has acceptability, since during the project there was rainfall, and this brought about conditions where orthopterans found food and refuge. The previous result agrees with Mysterud *et al.* (2003) and Hallett *et al.* (2004), who mentioned that during years with rainfall, the production of fresh biomass in plants is high and these factors favor the abundance and diversity of orthopterans. Meanwhile, when the drought period lasts for several years, the productivity of plants is low and, in the case of some grasslands, the accumulation of dry biomass affects negatively the populations of orthopterans because food is less digestible since it has high contents of fibers, and at the same time, it favors the recurrence of fires, which carbonize the eggs of these insects that are found in the soil in a state of diapause (Chambers and Samways 1998; Meyer *et al.*, 2002; Mysterud *et al.*, 2003; Hao and Kang 2004). When the means comparison test (Tukey=0.05) was carried out, whose results was indicated ´***´, *Schistocerca nitens* was the species with greatest significance, followed by *Sphenarium purpurascens* and *Achurum sumichrasti*.

Table 4. Analysis of Variance, for sectors and months of collection, where the dependent variable was the orthopterans.

Analysis of variance for the dependent variable orthoptera				
Source	**DF**	**Sum of squares**	**F Value**	**Pr>F**
Model	7	56607.200	29.62	0.0001
Error	12	3276.00		
Corrected total	19	59883.20		
R-Square		C.V		ORT Mean
0.945294		16.33		101.20
Analysis of variance for the dependent variable orthoptera				
Source	**DF**	**Type I SS**	**F Value**	**Pr>F**
Sector	3	39250.00	47.92	0.0001
Months	4	17357.20	15.89	0.0001

Table 5. Means comparison test of the dependent variable, orthopterans.

Means with the same letter are not significantly different			
Tukey Grouping	**Means**	**N**	**Sector**
A	130.00	5	I Prickly pear
A	127.00	5	III Polycultures
A	122.80	5	IV Corn
B	24.60	5	II Avocado

Table 6. Analysis of Variance, of the months and species, whose dependent variable was density.

Analysis of variance for the dependent variable density				
Source	**DF**	**Sum of squares**	**F Value**	**Pr>F**
Model	22	68413.29	41.88	0.0001
Error	61	4529.93		
Corrected total	83	72943.23		
R-Square		C.V		Den. Mean
0.937898		35.76		24.09
Analysis of variance for the dependent variable density				
Source	**DF**	**Type I SS**	**F Value**	**Pr>F**
Months	4	1405.18	4.73	0.0022
Species	18	67008.11	50.13	0.0001

For the time being, the genus *Schistocerca* can be considered among the most damaging pests in the world. Its different species devastate hundreds of thousands of farmed hectares year after year. The damage is caused by nymphs and adults when they feed on crops, fruit trees, grasses and wild species. In fruit trees, in addition to defoliating, eating fruits and stripping the bark, the swarms cause breaking of branches, when they settle on them (Garza, 2005). For their part, Cigliano *et al.* (2021) describe *Schistocerca nitens* as a solitary and non-migratory species, although under certain climatic conditions it can transform into gregarious and form swarms or invasive upsurges that cause damage to the crops and native flora, since it has a wide range of native geographic distribution, from the United States to Brazil. In contrast, Cano-Santana (1994) considers *S. purpurascens* among the most important Orthoptera herbivores, because of the large size that its populations reach. At the same time, Cano-Santana and Oyama (1992) state that the nymphs hatch at the end of May and beginning of June, while adults appear gradually starting in August and die between December and January. Márquez-Mayaudón (1968), in their observations, evidenced that *S. purpurascens* is very well represented throughout the year, even during the dry season which is the time when there is least availability of fresh food. This means that *S. purpurascens* is identified as a clearly dominating species in the structure of the Orthoptera community (Castellanos-Vargas *et al.*, 2015). Oyama *et al.* (1994) attribute that *S. purpurascens* is responsible for the high levels of floral and leaf damage that several plants experience as a result of their feeding activity. Camacho-Castillo (1999) state that adults prefer to feed in places with fresh vegetation and towards the end of the rainy season, approaching fall, they move to abrupt and closed sites where perennial plant species predominate, on which they feed. Briefly, the results from this study differ from those by Castellanos-Vargas and Cano-Santana (2009), who mention that *S. purpurascens* choose soils with high porosity (30 to 32%) to deposit their eggs, with predominance of sands (30 to 31%), low moisture (0 to 30%), and low compacting (0.44 ± 0.12 kg cm^{2-1}). In addition, they avoid the presence of high percentages of clay (>19.6%), as well as high levels of moisture (>25.5%), organic matter (>15.5%), and compacting ($>0.56 \pm 0.16$ kg cm^{2-1}). The type of soil in the sectors studied is characterized by being clayey.

CONCLUSIONS

The study determined taxonomically 16 species, and the prominent ones were: *Schistocerca nitens*, *Sphenarium purpurascens*, *Achurum sumichrasti*, *Neoconocephalus triops*, *Ceuthophilus pallidipes* and *Pyrgocorypha* spp. The highest number of orthopterans was collected in the sector cultivated with nopal. The lowest number of orthopterans was collected in the sector cultivated with avocado. The months of August to December were ideal to know the density of orthopterans, since they found the source of food, hosts and spaces to copulate in three sectors.

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Analysis of the agrifood situation in an elementary school community

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ABSTRACT
Objective: To analyze the agrifood situation (nutritional state) of elementary school children from the San Miguel El Piñón community, located in Españita, Tlaxcala, Mexico. The objective was to identify elements that could be used to design and implement strategies aimed to improve food and nutrition security in this rural area.
Design/Methodology/Approach: A participatory action research was conducted to analyze the information collected from mothers and children (n=44) from an elementary school located in San Miguel El Piñón, Tlaxcala.
Results: Based on their weight, size, and nutritional state, 69.6% of the participating children have a healthy weight. However, 26.1% of the participating children have symptoms of malnutrition, while no data was recorded for 4.3% of the children. Food insecurity was identified in the community as a consequence of which families cannot have a well-balanced diet.
Study Limitations/Implications: The results are only valid for the selected sample. Although the situation was studied in the field, the actual sample was small. Nevertheless, the national trend on this subject was confirmed.
Findings/Conclusions: This is a worrisome situation that can impact the physical, psychosocial, and cognitive growth and development of children. The sample evaluation revealed symptoms of malnutrition.

Keywords: school food, child nutrition, rural area.

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INTRODUCTION

All over the world, particularly in Latin America and the Caribbean, the food situation has undergone a critical transformation. This situation has caused a complex problem regarding inadequate child nutrition. Other factors —such as high malnutrition rates, hidden hunger (lack of vitamins and minerals), the rapid increase of overweight during the last three decades, and obesity, the most severe form of overweight— also impact children health (FAO, 2023). Overweight increased from 21.5% (2000) to 30.6% (2016) in children and teenagers (5-19 years old). According to UNICEF (2019), the triple burden of malnutrition threatens the survival, growth, and development of children, teenagers, economies, and nations.

In this context, the Food Security and Nutrition in the World 2020 report (FAO, 2020) indicated that food insecurity in Mexico increased from 8% (2014-2016) to 11.5% (2017-2019). Most of the Mexican population does not have access to enough nutritious and safe food and, consequently, one out of eight children suffers chronic malnutrition and one out of three children (6-19 years old) is overweight or obese (UNICEF, 2019). UNICEF (2023) points out that four out of ten children and teenagers in Mexico are obese and overweight, while five out of ten usually eat snacks, desserts, and candies as part of their daily diet. In contrast, only two out of ten children in elementary school include legumes and vegetables and only four out of ten include fruits in their daily diet (Secretaría de Salud, 2023).

Food is an essential factor of human development. However, the diet of Mexicans in the 21st century is very different from the diet of the recent past. Torres and Rojas (2018) have proved that the change of the traditional diet —based on grains, cereals, and legumes— for a fast food and industrial diet promoted by the industry —based on refined diets and sugary beverages— are key factors of the obesity problem in the country.

Overweight is an excessive fat accumulation in the body, while obesity is a chronic complex disease defined by excessive fat deposits for a person of a given size, weight, and gender (OMS, 2024). These metabolic disorders can be determined by genetic factors; however, they are mainly related to environmental factors such as unhealthy lifestyles, a combination of poor diets, sedentarism, and physical inactivity (Torres and Rojas, 2018, p. 149).

According to UNICEF (2023), overweight and obesity impact the health of children and teenagers, putting them at a premature risk of developing several chronic degenerative diseases, including type 2 diabetes, heart diseases, hypertension, metabolic syndrome, and certain types of cancer. In addition, overweight and obese children can suffer negative psychosocial effects, such as anxiety, depression, and low self-esteem, as a consequence of the stigmatization, discrimination, and rejection that impact their school performance and quality of life.

Meanwhile, the 2018 Encuesta Nacional en Salud y Nutrición (ENSANUT) evaluated the nutritional state of children between 5 and 11 years old in Mexican elementary schools. The results of the survey indicated a higher overweight prevalence in girls (18%) than in boys (17%). Nevertheless, the obesity percentage was higher (5.1% difference) in boys than in girls. Additionally, 22.2% of the boys and 16.9% of the girls that live in urban areas are obese; meanwhile, 14.6% of the boys and 10.2% of the girls that live in rural areas are obese (Shamah-Levy *et al.*, 2020).

The overweight and obesity levels begin to increase when children start to attend elementary school (at 6 years old). The average overweight and obesity rate is 24.3%. In addition, 12-year old children have an overweight and obesity index of 32.5% when they finish elementary school, accounting for a 12.2% increase (Barquera *et al.*, 2010).

Childhood obesity is a chronic and multifactorial disease, characterized by a fat excess. Children are considered obese when their weight surpasses their ideal weight by more than 20% (Castro *et al.*, 2012). In this regard, several researches carried out in Mexican elementary schools showed a high availability of processed food, such as potato chips and sugary beverages and candies. In addition, children eat more of this type of products during their school hours, consuming a greater volume than the average recommended, in shorter periods (Rivera *et al.*, 2015). In this sense, 92% of children between 5 and 11 years old consume sugary beverages and more than 50% consume snacks, candies, and processed cereals (Ensanut, 2021).

Consequently, the scientific evidence has proved that elementary schools contribute to overweight and obesity of children. However, they can also be the right place to implement effective interventions aimed to reduce both situations (Procter *et al.*, 2008). Elementary schools are an ideal milieu for the promotion of a healthy diet and physical activities that can be adopted for life (Coleman *et al.*, 2012).

In addition, elementary schools can improve the quality of the diets of children and, at the same time, they can reduce food and child health inequality. The findings support a comprehensive approach that consist of food quality consistency, food culture, and food education (Bryant *et al.*, 2023). Therefore, the objective of this research was to analyze the agrifood situation of the San Miguel El Piñón community, in Españita, Tlaxcala, based on the nutritional state of children in elementary school. The aim was to identify elements that could be used for the design and implementation of strategies to improve the food and nutrition security of this rural area, particularly for children in elementary school. The first hypothesis was that children from families with higher incomes have a higher probability of becoming overweight or obese, because they can buy more unhealthy industrialized products. These types of products prevail in obesogenic environments and are already established in rural areas. The second hypothesis was that children from families that produce their own food have a more balanced diet and, consequently, have a better nutritional state.

MATERIALS AND METHODS

Study area

San Miguel El Piñón is located at −98.403611 longitude and 19.419444 latitude, at 2,640 meters, in the municipality of Españita, Región Poniente-Calpulalpan, state of Tlaxcala, Mexico. The area has a 14 °C mean annual temperature, with a 25 °C maximum mean temperature, during April and May, and a minimum average temperature of 1.5 °C in January. The mean annual precipitation in the state reaches 720 mm, with summer rains from June to September (INEGI, 2024). The locality has 325 inhabitants, divided into 118 men and 132 women. The total inhabitant distribution by age was: 35.6% (under 18 years old), 57.1% (18-64 years old), and 7.3% (older than 65) (Censo local, 2023).

Participatory action research and information sources

Participatory action research (PAR) is a qualitative trend that arose in the 1970s. PAR was a response to the traditional social science crisis in face of social reality (Rahman and Fals Borda, 1989). A group of Latin American thinkers, headed by Orlando Fals Borda, defined the theoretical and conceptual basis and the practices of PAR. They were influenced by Dewey's pragmatic philosophy, Lewin's social change and knowledge generation theories, Marxism, Rousseau's liberal theories, Freire's political proposal about popular education, Bacon's philosophy, and even Ghandi and other authors from various disciplines (Zapata and Rondán, 2016).

Therefore, they established three main challenges that made up the main epistemic principles of PAR: 1) considering science as a social construct that, consequently, should be subordinated to reinterpretation, evaluation, adjustment, and improvement; 2) dialectical praxis and theory, which involves an active intervention in a given reality (praxis) and the construction of a theory or knowledge; and 3) rupture of the connection between the subject and the object of the research, positivist inheritance from natural sciences, adopting a horizontal subject-subject relationship which, from their collective and creative conscience, generates knowledge for social transformation (Zapata and Rondán, 2016; Gallego, 2007).

Delgado (2021) pointed out that PAR seeks a comprehensive understanding about the phenomenon: the qualitative vision of contextual comprehension, based on the incidence of subjects that interacts with the said phenomenon. Therefore, this study focused on determining the nutritional state of children from the Lázaro Cárdenas elementary school and dismissing anemia between them. In addition, a rural participatory analysis (RPA) of the agrifood area was carried out with the mothers and three teachers. This is a federal elementary school with multigrade teaching and one P.E. teacher, who teaches two hours per week.

The nutritional evaluation was carried out on October 2023, with the support of a nutritionist. A hemoglobin test was carried out and the anthropometric data (weight and size) of 42 children (out of the 46 that attend the school) were taken. Meanwhile, the RPA complemented the study and helped to develop a more comprehensive analysis of the students' agrifood context. In addition, workshops and in-depth interviews were carried out with the school principal, teachers, and 36 mothers. The workshops and interviews were focused on food production, availability, access, and consumption, both in and outside the school. A brief questionnaire was also applied to the 36 mothers; the questions included family data, such as income, family members, and education.

Data analysis

The hemoglobin test detects anemia, analyzing a 10 μl of blood sample with a HemoCue® hemoglobin photometer. The sample is collected in a microcuvette covered with dry reagents that reacts with the sample, disintegrating the erythrocyte membrane and allowing the release of hemoglobin. The results are shown in g/dL. The normal value of reference of hemoglobin for children between 6 and 12 years old is 11.5 to 15.5 g/dL (García and Sánchez *et al*., 2002).

The body mass index (BMI) was calculated to evaluate the children nutritional state. This is the most frequently used indicator for the identification of overweight and obesity in children older than 5 years and 6 months. BMI is the ratio between the body weight (kg) and the size and height (m) and is calculated by dividing a person's weight in kilograms by the square of their height in meters. According to the World Health Organization (WHO), a $\geq$25 BMI determines overweight, while a $\geq$30 BMI indicates obesity (Salud-Tlaxcala, 2014). The Instituto Mexicano del Seguro Social (IMSS) uses BMI tables to monitor and evaluate the nutritional state of children. These tables include reference values based on age, which were taken into account in this study. The results were analyzed based on the information from the RPA of the agrifood area.

RESULTS AND DISCUSSION

School context

This school has a canteen that resumed its activity on January 2023, after the Coronavirus SARS-Cov-2 pandemic. Although the school receives the support of the

Desayunos Escolares del Sistema Nacional para el Desarrollo Integral de la Familia (SNDIF), families still pay a small fee for warm meals at the school canteen, because the said program only provides cold breakfasts. In addition, the school has an educational-productive school garden, where they grow vegetables, prickly-pears, fruits, and medicinal plants for self-consumption. This project is supported by Unidad de Servicios Educativos del Estado de Tlaxcala (USET) and El Colegio de Tlaxcala A.C. The latter also offers food and nutrition workshops to children, teachers, and mothers.

The school has a good environment; the relationships among children are based on companionship, mutual respect, solidarity, and unity. Stable social, empathic, and friendly relationships predominate in the different activities of the school. However, in fifth and sixth year some students start to express their ideas in an individualistic and controversial way, which creates problems in school and community processes.

Family context

The school community is currently made up by 36 families, 10 out of which send two of their children to school. In average, each family had 4 to 5 members, out of which 2 children in average are younger than 11 years old. Likewise, 80% of the families in this student collective are nuclear families and 20% are single-parent families. Nuclear families are composed of mother, father, and their children; this is the most traditional family model. Single-parent families are currently understood as families with only one parent, whether as a result of death or separation or because the children were born outside of marriage (Puello *et al.*, 2014).

Children spend most of their time with their mothers, who feed and take care of them; likewise, mothers support the school activities of children and attend meetings, among other activities. Only in 10% of the cases grandparents act as guardians of the children and are therefore in charge of these activities. Consequently, determining the education of mothers and guardians was important for this study: 11% attended primary school, 44% junior high school, 27.8% technical certificate school, 11.1% high school, 2.8% technical degree college, and 2.8% undergraduate studies.

Agrifood diagnosis of the school community

Few families in the community of San Miguel El Piñón currently work on agriculture. This activity has undergone a progressive and significant decrease, mainly as a result of the fragmentation and the diminishing of cultivation plots. Water scarcity and adverse climate changes, along with the constant increase in the cost of agricultural inputs and the lack of governmental support, have hindered local agriculture.

However, 16 families (44.4% of the 36 families that make up the school community) carry out some kind of food production. Annual crops are grown in small spaces and, given the erratic and reduced rainy weather, families mostly grow corn, squash, beans, and prickly pears and, to a lesser extent, broad beans, barley, wheat, and some vegetables and fruits. Meanwhile, some families have a few animals, including backyard poultry (laying hens and chickens), pigs, and sheep. The families have a low agricultural and/or livestock production, which they consequently use for self-consumption. Only seven families reported that they sell the production excess within the locality. Therefore, local food production and availability are much lower than the demand of the community.

In this regard, most of the products consumed in the community are bought by the mothers in the San Martín Texmelucan open-air market (Puebla). In addition, mothers pointed out that food prices have significantly increased, particularly after the COVID pandemic. This situation, along with the low income of most families, hinders the constant access to a diverse variety of good quality food. Lemos *et al.* (2018) pointed out that the worldwide increase of food prices has been obviously reflected in Mexico, causing a significant increase in the cost of the basic food basket. Along with low incomes, this phenomenon means that millions of Mexicans lack economic access to the said basket.

Consequently, the purchase and consumption of protein-rich food, such as meat (mainly chicken) and dairy products, is limited: families consume up to 50% more eggs than meat and dairy products. They consume the former thrice a week, while the latter is consumed only once or twice a week. As a result of the high prices, the consumption of legumes (*e.g.*, beans, peas, and lentils) also decreased. Overall, the families consume seasonal fruits and vegetables, because they are less expensive. The most consumed local produce include banana, guava, pineapple, apple, melon, cucumber, jícama, chili, tomato, and prickly pear. Meanwhile, the purchase and consumption of cereals and tubers as source of carbohydrates is limited to rice, pasta, potato, tortillas, and bread. Families consume potatoes twice a week, while tortillas and bread are consumed at least four days a week.

Consequently, families cannot achieve a permanent balanced and good quality diet through the purchase of food. This situation can cause malnutrition problems in both children and adults. These results match the findings of the Informe de Pobreza y Evaluación 2022 Tlaxcala of the Consejo Nacional de Evaluación de la Política de Desarrollo Social (CONEVAL): the percentage of food insecurity in Tlaxcala increased from 24.3% to 34.9% (8.6%).

Diagnosis of the nutritional state of elementary school children

The hemoglobin test recorded a negative amenia result for all the evaluated children. The evaluation of the current nutritional state recorded that 69.6% (n=32) of the children have a healthy weight and 26.1% (n=12) have some malnutrition problem, while no data was recorded for 4.3% (n=2) (Table 1).

Table 1. Nutritional variables of children between 5 and 11 years old (mean±s.d.).

Nutritional status	Age (years)	Weight (kilograms)	Size (meters)	Members/family (number)
Healthy weight (n=32)	7.6±1.8	27.9±7.1	1.3±0.12	4.6±1.14
Underweight* (n=5)	7.8±2.1	23.0±4.2	1.3±0.11	4.6±2.51
Overweight (n=6)	8.0±1.7	40.6±12.1	1.4±0.13	5.0±0.63
Obesity (n=1)	7.0±0	41.5±0	1.33±0	4.0±0

Source: table developed by the authors. *Global malnutrition: children with a lower weight than the weight they should have based on their age.

Figure 1 shows that, out of the children with some malnutrition condition, five were underweight (10.9%), six were overweight (13%), and one was obese (2.2%). No data was recorded for the other 2 kids (4.3%).

According to Ensanut (2021), the overweight and obesity results are under the national average, because children between 5 and 11 years old recorded an overweight of 18.8% in 2020, while obesity reached 18.5%, showing that the prevalences increase along with the age of the children. In this study, only 2.2% of the children were obese. This result is different to the findings of Vaquero-Álvarez *et al.* (2019), who studied overweight and obesity in a rural area of Spain, recording 26.2% overweight and 22.3% obesity. Peña and Bacallao (2003) identified that, along with child malnutrition, poverty in the rural environment is a social problem associated with obesity. In 2022, moderate or serious food insecurity worldwide impacted 33.3% adults that lived in rural areas and 26% adults in urban areas. However, the purchase and consumption of ultra processed food is increasing in rural areas (FAO, 2023).

Relationship between the nutritional state of children and other socioeconomic family variables

Other variables obtained from the RPA results were analyzed to determine a wider view about the nutritional state of children. The sample consisted of 22 boys and 22 girls (50% each). Out of this total, 16 boys have a healthy weight, 3 are underweight, 3 are overweight, and 1 is obese. Meanwhile, 17 girls have a healthy weight, 2 are underweight, and 3 are overweight (Table 2).

Consequently, more boys (46.7%) than girls (29.4%) showed malnutrition problems in this study. These results are different from the national overweight trend. The results of the national study recorded a 16.6% (boys) and 21.2% (girls) prevalence. Nevertheless, the results of this study match the national obesity rates, which recorded a 23.8% and a 13.1% prevalence for boys and girls, respectively (Ensanut, 2021). Meanwhile, the underweight,

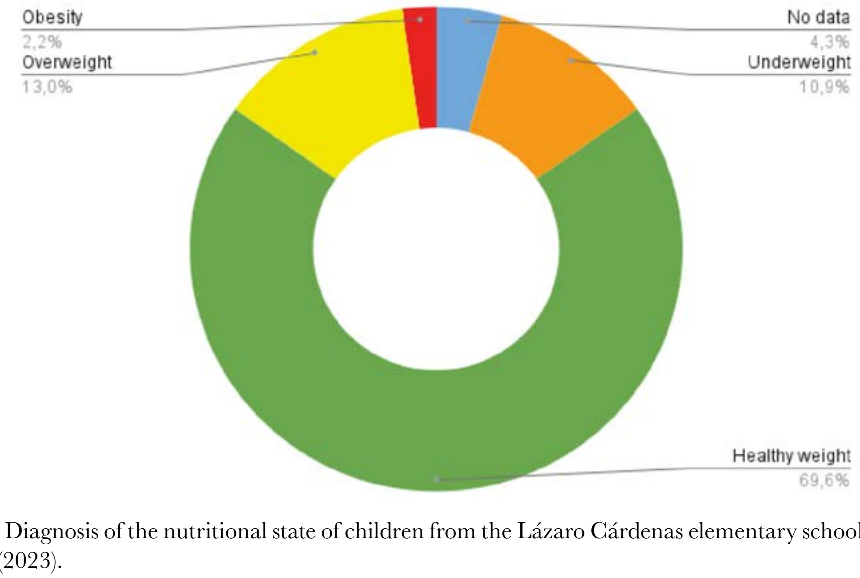

Figure 1. Diagnosis of the nutritional state of children from the Lázaro Cárdenas elementary school, Españita, Tlaxcala (2023).

Table 2. Family socioeconomic variables (%).

Nutritional status	economic income		Sex	
	1*	2*	boy	girl
Healthy weight	6	16	15	17
Underweight	1	4	3	2
Overweight	3	3	3	3
Obesity	0	1	1	0

Source: table developed by the authors. * A≤$2,375.00 and B=$2,376.00 to $6,999.00 monthly incomes.

overweight, and obesity rates did not record a significant difference regarding the age of the students. However, overweight and obesity are focused on children between 6 and 8 years old; in the case of girls, this problem starts at 10.

Although some families with higher incomes (between MXN$2,376 and MXN$6,999) have more children with some type of malnutrition than families with lower income (≤MXN$2,375) (8 and 4 children, respectively), the hypotheses of this study did not only consider overweight and obesity, but also underweight. For their part, the 16 families that grow food for self-consumption have children with healthy weights and others with malnutrition problems.

Consequently, the family income and food production for self-consumption variables are related with factors associated with the nutritional condition of the evaluated students. Nevertheless, it was no possible to identify how and to which degree these variables impacted the study area. Since the analysis of the nutritional evaluation of the family (education of the mothers or guardians, number of members, and number of children less than 11 years old) did not identify significant differences, longitudinal studies should be carried out to achieve a deeper understanding.

Therefore, malnutrition in children is related with a family diet that lacks enough quality nutritional elements. Malnutrition is consequently a multifactorial phenomenon which includes: low income, lower production of food for self-consumption, high price of food, little knowledge about food and nutrition among the family, and little physical activity. The school in question has a lower malnutrition level than the national averages, possibly as a result of its canteen, where children have access to a balanced breakfast. Likewise, the absence of a cooperative-run school store diminishes the consumption of junk or ultra-processed food, which is already available in this rural area, both in stores and during various community festivities.

Finally, the double burden of malnutrition —hereby demonstrated in a specific rural territory— has been primarily the consequence of the globalized agrifood system that has modified habits and food culture. This system creates obesogenic environments characterized by a high supply of ultra-processed foods and beverages, which have an extremely low or null nutritional quality. Given their apparent low cost and the influence of mass advertising in various media, a great part of the Mexican population chooses them. Structural factors are also involved, including public policies and changes in agricultural production.

CONCLUSIONS

The findings of this research confirm that several factors resulting from a globalized agrifood system have altered the agri-food situation of the rural community of San Miguel El Piñón, which consequently faces availability, access, and food consumption issues. This system has also negatively modified the traditional diet and continues to limit the access of many families to the basic food basket. As a consequence of this situation, the students suffer malnutrition problems. Consequently, their growth and physical, psychosocial, and cognitive development are hampered and they are also at higher risk of contracting various diseases. Therefore, this situation violates the rights of children and unleashes a series of socioeconomic and cultural problems.

The school milieu is ideal for the encouragement of healthy life styles (*e.g.*, a nutritious diet). Consequently, as the PAR advances, the issue of interest for this research changes for the benefit of the participants, since the aim of this research is to transform the social environment. Therefore, this research contributes to the improvement of the food intake and school nutrition of children, through pedagogical processes in which children, parents, teachers, and the persons in change of the school canteen participate. These processes include: a) agroecological food production; b) consumption of healthy and nutritious food; c) more information about the consequences that junk food has on human health; and d) revaluation of unprocessed local food. These four basic components of the design of regional strategies and public policy guidelines for school food will help to counteract the double burden of malnutrition in children that persists in the rural areas of Mexico.

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Colegio de Postgraduados

Pitahaya (*Hylocereus undatus* [Haworth] Britton & Rose) marketing margins for its sustainable development in Belize

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ABSTRACT
Objective: To determine the marketing margins and direct participation of producers in Belize aimed to support the sustainable development of pitahaya.
Design/Methodology/Approach: The information was collected between October and December 2022, implementing participatory workshops with producers and members of the pitahaya production chain in the Cayo and Orange Walk districts, Belize.
Results: The producer and the consumer (90%) are the main participating agents of the pitahaya marketing process. The gross marketing margin amounted to 40%, which indicated that, for every dollar paid by pitahaya consumers, 40 cents went to the intermediaries, while 60 cents went to the producers.
Study Limitations/Implications: An in-depth market analysis should be carried out.
Findings/Conclusions: Pitahaya cultivation is just starting in Belize. Nevertheless, the country's climate provides it with a high potential for success. The main marketing channel is made up of the producer and the final consumer. Belizean producers obtained 60% of the total price paid by the final consumer.

Keywords: Commercialization, potential, new crops.

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INTRODUCTION

The primary sector of the Belizean economy accounts for 12.8% of the GDP and it is mainly focused on sugarcane, banana, citrus, and grain production, for both the domestic and international markets. In 2021, the agricultural sector contributed US$312,062,652 to the Belizean economy and banana was the most valuable fruit, providing US$46 million of the total GDP (Canto, 2021). Nevertheless, the Belizean market demands other fruits, such as pitahaya —a fruit that is mainly used by the restaurant sector.

Pitahaya (*Hylocereus undatus* [Haworth] Britton & Rose) or dragon fruit is an exotic fruit that —as a result of its physicochemical and nutritional characteristics and bioactive compounds— is currently expanding to different areas of the world (Attar *et al*., 2022; Mordorintelligence, 2024).

According to the Ministry of Agriculture, Food Security, and Enterprise of Belize, 38,000 pounds of pitahaya were produced in 26 acres of two districts. Although pitahaya cultivation is just starting in Belize, it has drawn the attention of the Ministry as an ideal crop for small producers, due to its economic and nutritional potential (Martínez *et al*., 2024). In addition, this crop can obtain significant yields (Osuna *et al*., 2016) and Belize has the right weather conditions for its development. However, the analysis of the market components of non-traditional crops with development potential (*e.g*., pitahaya), as a consequence of its different uses, has become a fundamental task in the agricultural sector for the sustainable development of such crops (Mendoza, 2022).

Pitahaya production in Belize is carried out in backyard farming and it is commercialized in local markets and roadside stands. Although there are several pitahaya varieties, fruits with both red skin and pulp are visually more attractive and are more appreciated by the markets than those with red skin and white pulp. In response to the demand for this fruit, the Belizean government and the Mexican Instituto de Investigaciones Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) are working together to develop promotion production programs. These programs include the use of a technological package that will help to solve seasonality and post-harvest problems, improving pitahaya crops and strengthening technical capacities. Nevertheless, the lack of commercialization plans can result in overproduction and waste. Consequently, determining production costs and commercialization channels will provide key data for the improvement of the decision-making process, establishing productive and commercial strategies (Durán and Zolano, 2019). Marketing margins seek to cover costs and market risks, as well as to generate a profit for the agents involved in the process. In addition, the producer influence on the final price of the product covers seed costs, labor, supplies, and agricultural risks (Iregui, 2003). There is scarce information about the production and commercialization systems involved in the pitahaya crops of Belize. Therefore, the objective of this research was to determine the influence of the marketing margins and the direct participation of pitahaya producers in Belize on the sustainable development of this crop. The hypothesis was that the intermediaries obtain most of the marketing margins.

MATERIALS AND METHODS

Study area

Belize has a border with the Mexican states of Quintana Roo and Campeche and with Guatemala. It has 22,800 km^2 and is divided into six districts: Belize, Cayo, Corozal, Orange Walk, Stann Creek, and Toledo (Figure 1).

The study was carried out in the two district of Belize that, according to the Ministry of Agriculture, Food Security, and Enterprise, have the highest pitahaya production potential: Orange Walk and Cayo, with a pitahaya sowing area of 87.2% and 12.8%, respectively.

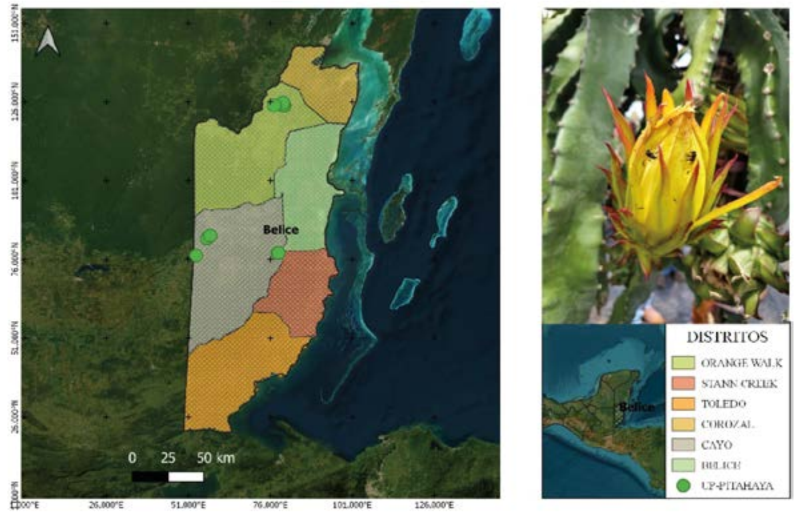

Figure 1. Geographical distribution of pitahaya production in Belize (2023). Source: Figure developed by the authors with data from the Ministry of Agriculture, Food Security, and Enterprise of Belize.

Data collection

Data were collected between October and December 2022, through the implementation of three participative workshops, with 99 participants. These workshops included members with key information and members of the productive pitahaya productive chain (producers, researchers, and traders). Pitahaya crop production costs, marketing margins, and commercial strategies and actions were identified (González *et al.*, 2014).

Marketing margin

The direct method was used to identify the commercialization channels: the pitahaya commercialization channel was monitored from the moment the fresh fruit left the plot to moment when it reached the final consumer. Based on the methodology proposed by Mendoza (1991), the average sell and buy prices were taken into account to determine the absolute (a) and relative (r) marketing margins. The calculus was carried out by price difference between the different market stages and levels; in all the cases, it was connected to the final price paid by the final consumer. The marketing margins and producer direct participation were estimated as follows:

$$GCM(a) = CP - PP \quad \text{and} \quad GCM(r) = \left(GCM(a)/CP\right) \times 100$$

Where: *GCM* is the gross commercial margin, *CP* is the price paid by the consumer, *PP* is the producer price.

The producer direct participation (PDP) was determined as follows:

$$PDP(a) = CP - GCM \quad \text{and} \quad PDP(r) = \left(PDP(a)/CP\right) \times 100$$

The data were processed and analyzed through descriptive statistics, using the IBM SPSS v11.0 statistical package for social sciences.

RESULTS AND DISCUSSION

Technological package proposal

The training was directed to Belizean technicians and producers, who were introduced to the pitahaya technological package developed at the Campo Experimental Cotaxtla (INIFAP). The establishment of live plant tutors was fundamental. The *Bursera simaruba* species was chosen for this purpose. The participants were told that the transplant should be carried out when the tutors had sprouts and showed an optimal apprehension (approximately three months). The recommended sowing density was 4×2 m, in a planting frame or square, which resulted in a 1,250-tutor density. An additional recommendation was to sow two plants per tutor, resulting in 2,500 pitahaya plants per hectare (Del Ángel *et al.*, 2012). This training provided an in-depth analysis about the supplies required for the transplant: 5 kg of compost or vermicompost per stock. In addition, participants were trained in the following cultural labors: formative and maintenance pruning, thinning, tutor pruning, pest and disease control, weed control, and harvesting. During the training, all the producers (80.80% men and 19.19% women) showed great interest in the technological package proposed. Field evaluations were carried out to measure the advancement of the producers after the training. The results showed that 80% of them were applying the technological package.

Analysis of the production costs

There is no information about the production cost of dragon fruit per hectare in Belize, because production is mainly carried out in family farming or small plots. According to the technological package, the costs are divided into establishment and maintenance costs. Plantation (73.9%) is the highest costs for the producer during the pitahaya plot establishment, followed by solid fertilization (16%). The main costs during the maintenance years are fertilization and weed control, reaching 50.6% and 13%, respectively (Figure 2).

The total production costs for 2023 —which account for crop establishment and maintenance during the first year— reached $9,491.18 BZD/ha (US$4,709.38/ha), while the average yield from the second year amounted to 11.25 t h^{-1}. Consequently, the gross income was $43,074.08 BZD/h (US$21,372.69/ha). The yield in Belize was more competitive than in Mexico. The Mexican states of Puebla, Yucatan, Quintana Roo, Campeche, Chiapas, Tabasco, and Veracruz recorded a yield that fluctuated between 3.5 and 16 t h^{-1} (Del Ángel *et al.*, 2022).

Meanwhile, the production cost proposed matched the reports of Diéguez-Santana *et al.* (2022), who pointed out that pitahaya production costs in Ecuador fluctuated between US$5,015 and US$6,935 per hectare.

Commercialization agents

In Belize, fresh pitahaya is locally commercialized by 90% of the producers. All (100%) the producers emphasized that the main problem they face is oversupply, which in turn

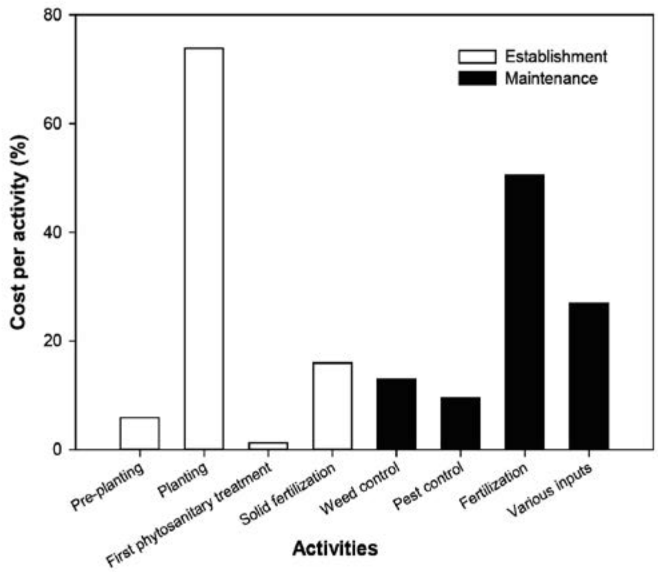

Figure 2. Pitahaya production costs during its establishment and maintenance.

creates competition between the small producers of the district. In addition, consumers are not aware of the product or its health benefits. A similar situation takes place in countries such as Costa Rica, where the use of pitahaya is not spread among the local population and it is mainly consumed by foreign residents in the country (García and Quirós, 2010).

Pitahaya production is aimed at domestic consumption, but it is not equally distributed in the country. Pitahaya fruits are mainly consumed within the neighboring localities of the production units. The price of pitahaya in roadside family stands is relatively low: an average of $3 BZD per pound (US$1.49).

Meanwhile, consumers have a diverse profile regarding income, schooling, age, purchase motive, and place of residence. Tourists are a potential market, because they are attracted by the exotic color of this tropical fruit. They have different purchase patterns regarding food souvenirs (García *et al.*, 2022). Lin (2017) mentioned that, in Asia, food is one of the favorite gift options for tourists. Consequently, developing strategies to manage, plan, commercialize, and position pitahaya fruits as a tourism resource within the destination is fundamental. In addition, the market should be divided into subgroups, in order to offer appropriate products and services. According to Sánchez-Sánchez (2022), segmentation is the division of the market into smaller portions, depending on its characteristics.

Nevertheless, 10% of the producers sell fresh fruit to wholesalers and plant cuttings to other producers. They also manufacture ice-cream, aguas frescas, marmalade, and other products. Nowadays, markets are highly competitive and, therefore, generating added value and obtaining strategic advantages are key factors to guarantee consumer satisfaction (Torres and Guerra, 2022).

Consequently, the gross marketing margin of pitahaya was 40%, indicating that intermediaries keep 40 cents of each Belize dollar paid by consumers, while pitahaya producers earned 60 cents. In other words, intermediaries obtain 40% of the final price paid by the consumer: $2 BZD per pound (US$0.99) (Table 1).

Table 1. Absolute and relative marketing margins and producer participation in the final price of pitahaya.

Item	Value (bzd/libra)	
Producer price	3.0	
Wholeseller price	4.0	
Consumer price	5.0	
	Absolute (bzd/pieza)	**Relative (%)**
Gross sales margin	2.0	40.0
Producer share	3.0	60.0

Source: Table developed by the authors (2022).

Kohls and Joseph (1990) reported similar results: processed products automatically have a higher marketing margin than fresh or non-processed products. When the product is sold outside the production unit, the marketing margin is lower. In this case, the margin can even be null, because the producer obtains 100% of the product price and only one transaction takes place (producer-consumer). In Germany, approximately 11% of all the fruits and vegetables are directly sold by the producer to the consumer (Dent and Macharia, 2017). The percentage in Mexico is lower, as a consequence of the long distances between the consumption and the production areas, as well as the land route infrastructure. Reducing the intermediaries is not always possible, as a result of the role they play in the commercialization, despite the costs they generate. In addition, markets have become increasingly demanding; consequently, a greater number of highly specialized intermediaries is sometimes required to achieve a higher efficiency (Álvarez *et al.*, 2021).

Commercial strategy

The workshops established the commercial strategies and actions to be followed for the improvement of the crop commercialization (Table 2).

Table 2. Commercial strategies and actions for pitahaya cultivation.

Strategy	Actions
Knowledge and capacity building	• Training modules and workshops on marketing, by-product processing, post-harvest handling, agribusiness management.
Stakeholder organization models	• Formation of producers' organizations in associative figures. • Integration of chain actors into legal organizations.
Follow-up and evaluation of basic actions in the commercial system	• Implementation of a commercial technical assistance program.
Establishment of infrastructure and equipment for marketing and industrialization	• Creation of collection, packing and preservation centers for fresh fruit. • Construction and equipping of pitahaya industrialization centers.
Market diagnosis and planning	• Preparation of a market study at the CARICOM level.
Financing schemes for stakeholders	• Formation of a financial fund for members of the production chain.
Legal and regulatory framework	• Dissemination of national and international quality standards for pitahaya.

Pitahaya is an agricultural alternative in regions with scarce water resources, because it thrives under limiting weather conditions. In addition, pitahaya has a competitive price in local, regional, domestic, and international markets (Montesinos-Cruz *et al.*, 2015).

CONCLUSIONS

Pitahaya cultivation is just starting in Belize, and it has a high potential due to the climate of the country and the nutraceutical properties of the fruit. The main commercialization channel is mainly made up by the producer and the final consumer. Belizean producers obtained 60% of the final price paid by the consumer. Nevertheless, training producers regarding technical and commercial activities is fundamental to expand the market and to offer quality fruit. The marketing margin analysis does not show profits or losses for the producers or the intermediaries. In truth, the market and the product itself define the extension of the commercialization channel, the time and storing type, the way in which a product is processed, the transportation, and the means of transport.

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Colegio de Postgraduados

Spatio-temporal analysis of scientific research on models to estimate water balance in hydrographic basins

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ABSTRACT

Objective: To identify in the world the main models or tools used to evaluate changes in water regulation, through a bibliometric review in the especialized in scientific information, Scopus database, in order to know the main variables evaluated that affect the hydrological regime of the basins.

Design/methodology/approach: With the help of bibliometric techniques, scientific articles available until December 2022 in the Scopus database were collected using the SUBJAREA (envi) TITLE-ABS-KEY ("water balance" AND "land use") AND (LIMIT-TO (OA, "all")) AND (LIMIT-TO (DOCTYPE, "ar")).

Results: 407 scientific articles were collected from 1965 to 2022, this scientific production shows an exponential growing trend that was concentrated above all in countries with agricultural traditions such as the United States (89 publications), China (33), Germany (28), the United Kingdom (25) and Australia (21).

Limitations on study/implications: Most of the research was developed on topics associated with land use change (82 texts), climate change (48), hydrological processes (44), subsoil water (34) and evapotranspiration calculation (33). The most recurrent models for calculating the water balance were the Direct Water Balance (230 texts) and the Soil and Water Assessment Tool (83).

Findings/conclusions: No research development was found in Latin American and African countries, so these results can help redirect efforts in the research centers of these countries, with the aim of generating new knowledge that helps make decisions about how to improve the efficient use of water in the face of climate change scenarios and dynamics of land use change.

Keywords: content analysis, bibliometrics, hydrological model, SCOPUS, SWAT.

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INTRODUCTION

The Water Balance (WB) measures the amount of water available in an ecosystem (Ruíz-Álvarez *et al.*, 2012). Through WB, it is possible to estimate the potential evapotranspiration, humidity index, and water surplus or deficit in a given area for a specific period (Sentelhas *et al.*, 2008). However, studies on WB have mainly focused on the analysis of potential

evapotranspiration, opening up opportunities for research on the effects of climate change and land use change dynamics on the hydrology of ecosystems with some utility for anthropogenic activities (González-Pérez *et al.*, 2023).

Climate change and land use changes are altering water cycles and regimes within basins. The increase in temperatures and changes in extreme weather conditions could reduce the availability of natural water resources in many parts of the world (Allan *et al.*, 2020). Therefore, understanding the potential impacts of climate change and land use changes on water resources is crucial for planning (Wang *et al.*, 2019).

In this context, the water balance has become one of the most useful tools for measuring the effects of climate change and land use changes in basins that supply human settlements (Ruíz-Álvarez *et al.*, 2012). Among these models, the Soil and Water Assessment Tool (SWAT) is one of the most widely used models for water quality in river basins and basins around the world. This is due to its supportive software application and open-source code, which can be adapted by model users to meet specific application needs (Akoko *et al.*, 2021).

However, Malamos *et al.* (2015) found that the models proposed by Thornthwaite (1948) and Thornthwaite and Mather (1957) for estimating the water balance are the most commonly used due to the simplicity of their calculations, as they require only temperature and precipitation data. Nevertheless, despite the importance of the water balance in water regulation and land use change, there is little research evaluating the relevance of publications on this topic (González-Pérez *et al.*, 2023).

To assess the importance of publications on a specific topic of interest, bibliometric studies are often a valuable tool. They allow for an objective evaluation of scientific interest topics, observe the trends they follow, and generate useful information to improve their management, quality, and performance (Peng, 2017). In this context, the objective of this study is to identify the main models or tools used worldwide to evaluate changes in water regulation through a bibliometric review in the specialized scientific information database, Scopus, in order to understand the main evaluated variables that affect the hydrological regime of basins.

MATERIALS AND METHODS

Source of Information

In this study, scientific articles that calculated the flow of basins affected by land use change were considered. To collect the publications, the Scopus database of journal articles was reviewed due to its larger number of indexed scientific articles within the various bibliographic databases (Scopus, 2023).

The algorithm used for the search was: SUBJAREA (envi) TITLE-ABS-KEY ("water balance" AND "land use") AND (LIMIT-TO (OA, "all")) AND (LIMIT-TO (DOCTYPE, "ar")). Only articles in English were considered, as it is more likely that scientific articles in English are cited, allowing for the capture of the vast majority of relevant publications (Leipold, 2014).

Scientific articles were collected from January 1965 to December 2022. Publications with open access in the subfield of environmental sciences —ecology, geology, meteorology, hydrology, and agronomy— were selected. Only scientific articles were considered, and through a content analysis, those without data, with errors, or duplicates were excluded (Aguado-López *et al*., 2009).

Bibliometric Indicators

Following the methodology proposed by Santillán-Fernández *et al*. (2021), the variables analyzed from each of the collected scientific articles were: authors, year of publication, title, journal name, research topic according to Scopus classification (2023), and bibliographic citations. Through a content analysis, the institution and country of origin of the first author were identified for each article, along with the methodology applied for calculating the flow of basins affected by land use change. Data collection was conducted in a spreadsheet.

Text Mining Analysis

The methodology described by González-Pérez *et al*. (2023) was followed. A timeline graph of scientific production was constructed using the variables of year of publication and number of citations. For the variable of the frequency of scientific articles per year, an ordinary least squares regression model was estimated to determine the trend in the frequency of publications (Gujarati, 2007).

Using Excel® tools, the countries of origin of the first authors were spatially mapped to identify where research on methods for estimating the flow of basins affected by land use change has been developed. The topics with the highest number of publications by country were also determined, as well as the most common methods for estimating basin flows, and bibliometric indicators for the journals and authors with the highest publication frequency were generated.

RESULTS AND DISCUSSION

Spatio-Temporal Evolution

A total of 407 scientific articles were collected that presented some methodology for estimating the flow of basins affected by land use change. All articles corresponded to the area of environmental sciences in the open access Scopus database (2023). The 407 scientific publications generated 9,203 bibliographic citations (Figure 1).

The period from 2012 to 2022 accounted for 83% of the publications (338) and 62% of the bibliographic citations (5,698). According to Malamos *et al*. (2015), the exponential growth (R^2=0.9791) in the number of publications can be attributed to the importance of changes in the hydrological flow of a basin due to land use change. Additionally, Santillán-Fernández *et al*. (2021) found that when the temporal growth between scientific publications and their bibliographic citations is directly proportional, it indicates topics of high relevance for the scientific community.

According to the country of origin of the first author of the research, the 407 scientific publications originated from 64 countries (Figure 2). Of these, 48.16% (196 articles) were concentrated in five countries: the United States (89 publications, 21.87%), China (33, 8.11%), Germany (28, 6.88%), the United Kingdom (25, 6.14%), and Australia (21, 5.16%).

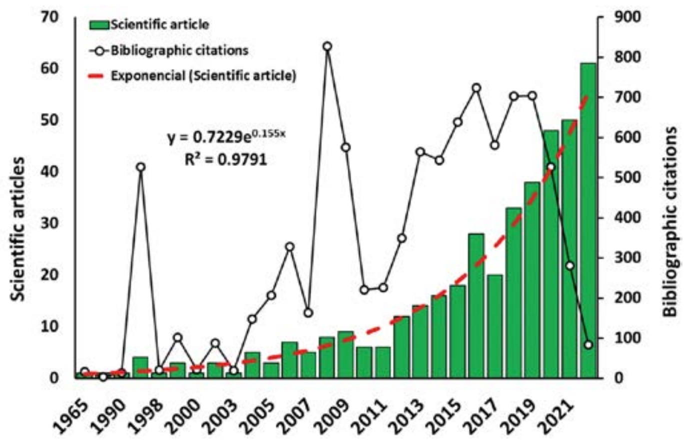

Figure 1. Temporal evolution of scientific production and bibliographic citations on methodologies for estimating the water balance affected by land use change, from 1965 to 2022.

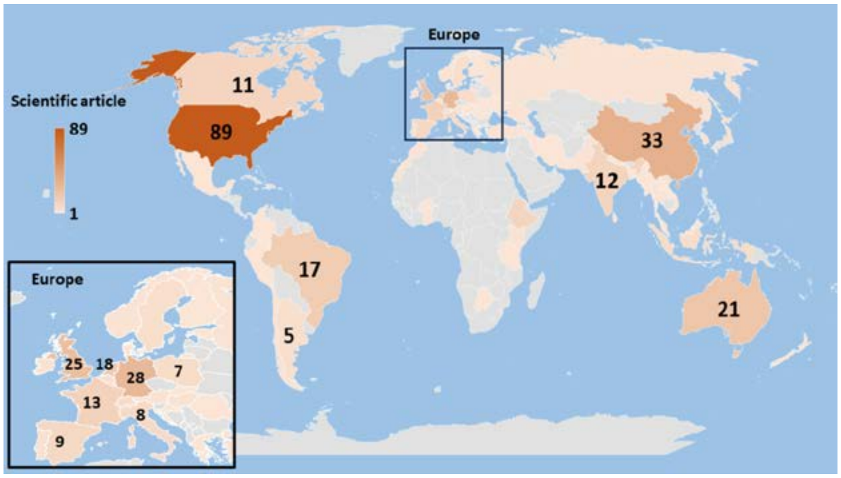

Figure 2. Spatial relationship of scientific production on methodologies for estimating the water balance affected by land use change, from 1965 to 2022.

In America, Brazil (17 articles, 4.18%) and Canada (11, 2.70%) were also notable. In Asia, India contributed 12 articles (2.95%), while in Europe, the Netherlands (18, 4.42%) and France (13, 3.19%) were significant contributors.

The countries with the most publications on methodologies for estimating the water balance affected by land use change were found to be the countries with the highest agricultural production in the world (FAOSTAT, 2024). This is explained, according to González-Pérez *et al.* (2023), by the fact that these countries have turned to methods to make water use more efficient in agricultural activities in the face of the sector's threats from the effects of climate change (Sandoz, 2016).

Bibliometric Indicators

In terms of the main research topics on methodologies for estimating the water balance affected by land use change, the most recurrent topic was land use change (82 articles, 20.15%), followed by climate change (48, 11.79%), hydrological processes (44, 10.81%), groundwater (34, 8.35%), and evapotranspiration calculations (33, 8.11%) (Table 1). A total of 63 topics were recorded; however, these five topics accounted for 241 articles, representing 59.21% of the total articles analyzed. A total of 63 topics were recorded; however, these five topics accounted for 241 articles, representing 59.21% of the total articles analyzed.

Santillán-Fernández *et al.* (2023) found that topics associated with climate change, land use change dynamics, and efficient water use in environmental processes are trending among the international scientific community due to concerns about generating new sustainable modes of production. However, González-Pérez *et al.* (2023) highlight that despite these advancements, there are still research gaps and propose the use of algorithms based on current technology as a helpful tool.

The 407 analyzed articles were published in 107 scientific journals. Eighty percent of the publications were published by high-impact journals belonging to the first quartile (Q1) of the Journal Citation Reports (WoS, 2021). However, 51.85% (211) of the total articles were concentrated in 10 journals, which contributed to 63.95% (5,886) of the bibliographic citations, in specific topics of hydrology (6) and environmental sciences (3) (Table 2).

These journals belong to the most prestigious publishers at the international level: MDPI (Multidisciplinary Digital Publishing Institute) with four journals, Elsevier (3), and John Wiley & Sons Ltd (2). According to Santillán-Fernández *et al.* (2021), this helps improve the impact of publications by increasing the likelihood of reaching a larger number of users.

Table 1. Main Research Topics by Country of Scientific Publications on Methodologies for Estimating the Water Balance Affected by Land Use Change, from 1965 to 2022.

Country	**Topics**						**Total (%)**	
	Land use change	**Climate change**	**Hydrological process**	**Groundwater**	**ETP**	**Others**		
USA	11	16	9	7	9	37	89	(21.87)
China	9	5	3	0	5	11	33	(8.11)
Germany	10	3	1	2	1	11	28	(6.88)
UK	3	3	2	3	0	14	25	(6.14)
Australia	3	0	2	5	1	10	21	(5.16)
NL	2	0	1	1	4	10	18	(4.42)
Brazil	7	0	3	0	3	4	17	(4.18)
France	1	1	3	1	1	6	13	(3.19)
India	3	1	2	4	0	2	12	(2.95)
Canada	0	2	0	1	2	6	11	(2.70)
Others (53)	33	17	18	10	7	55	140	(34.40)
Total (63)	82	48	44	34	33	166	407	(100.00)
(%)	(20.15)	(11.79)	(10.81)	(8.35)	(8.11)	(40.79)	(100.00)	

ETP: Evapotranspiration; USA: United States of America; UK: United Kingdom; NL: Netherlands.

Table 2. Bibliometric indicators of the main journals that published scientific articles on methodologies for estimating the water balance affected by land use change, ordered by the number of published articles.

Journal	Country	Editorial	WoS (2021)			Topics	Article		Citations	
			IF	H	Q		Number	%	Number	%
Hydrology ESS	Germany	European GU	6.3	147	Q1	Hydrology	62	15.23	2066	22.45
Water	Switzerland	MDPI	3.4	69	Q1	Environmenal	51	12.53	628	6.82
Water RR	USA	Wiley	5.4	231	Q1	Hydrology	19	4.67	1669	18.14
Sustainability	Switzerland	MDPI	3.9	109	Q1	Geography	14	3.44	149	1.62
J Hydrology RS	Netherlands	Elsevier	4.7	43	Q1	Hydrology	13	3.19	325	3.53
Hydrological P	UK	Wiley	3.2	169	Q2	Waters	12	2.95	381	4.14
Hydrology	Switzerland	MDPI	3.2	21	Q2	Hydrology	12	2.95	47	0.51
J Hydrology	Netherlands	Elsevier	6.4	241	Q1	Hydrology	12	2.95	293	3.18
Land	Switzerland	MDPI	3.2	32	Q2	Environmenal	8	1.97	39	0.42
ST Environment	Netherlands	Elsevier	9.8	275	Q1	Environmenal	8	1.97	289	3.14
Others (97)							196	48.16	3317	36.04
Total							407	100.0	9203	100.0

IF: Factor Impact; H: H Index; Q: Quartile; Hydrology ESS: Hydrology and Earth System Sciences; European GU: European Geosciences Union; Water RR: Water Resources Research; USA: United States of America; UK: United Kingdom; J Hydrology RS: Journal of Hydrology - Regional Studies; Hydrological P: Hydrological Processes; J Hydrology: Journal of Hydrology; ST Environment: Science of the Total Environment; Wiley: John Wiley & Sons Ltd.

Regarding the methods used to estimate the water balance affected by land use change, 58 methodologies were documented. However, 76.90% of the articles (313) relied on two methods: Direct Water Balance (DWB, 230 articles, 56.51%) and the Soil and Water Assessment Tool (SWAT, 83, 20.39%) (Table 3). Del Toro-Guerrero *et al.* (2014) describe DWB as an empirical method that assumes that soil water is lost over time until its reserves are depleted, thereby meeting the water needs of the system.

While Akoko *et al.* (2021) consider the SWAT method to be more accurate for estimating the flow of basins affected by land use change because it is based on the water balance in a

Table 3. Main methodologies for estimating the water balance affected by land use change in a basin.

Method	Formula	Variables
DWB Del Toro-Guerrero *et al.* (2014)	$P = ETR + Infiltration + Surface\ runoff$	*P* is the hydrological balance of the basin. *ETR* is the reference evapotranspiration *Infiltration* is the excess water moving into the groundwater *Surface runoff* is the excess water that flows into natural watercourses (*e.g.*, rivers). *ETR*, *Infiltration* and *Surface runoff* are variables calculated using the Thornthwaite (1948) method.
SWAT Akoko *et al.* (2021) Gassman *et al.* (2014)	$SW_t = SW_0 + \sum_{i=1}^{t}\left(R_{day} - Q_{surf} - E_a + W_{seep} - Q_{gw}\right)$	SW_t is the final soil water content (mm H_2O). SW_0 is the initial soil water content on day *i* (mm H_2O). *t* is time (days). R_{day} is the precipitation on day *i* (mm H_2O). Q_{surf} is the surface runoff on day *i* (mm H_2O) E_a is the evapotranspiration on day *i* (mm H_2O). W_{seep} is the water percolating through the soil profile on day *i* (mm H_2O). Q_{gw} is the groundwater flow on day *i* (mm H_2O).

system, Malamos *et al.* (2015) argue that DWB is more frequently used due to the simplicity of its calculations, requiring only precipitation and temperature data.

Finally, regarding the authors who published scientific articles applying a method to estimate the water balance affected by land use change in a basin, a total of 1,772 different authors were counted in the 407 publications. 21.06% of the articles were written by four authors, 17.95% by three, 15.25% by five, 14.64% by two, and 10.76% by six. 90.13% of the authors were affiliated with a university. Six of the ten authors with the highest number of publications conducted their research activities in the USA (Table 4).

Gersbach and Schneider (2015) found that the USA tends to invest more resources in its research centers, which has allowed for greater technological development and, consequently, for it to be considered an international agricultural engine. In this regard, González-Pérez *et al.* (2023) found that in Latin America, most research on water balance in an ecosystem has focused on calculating potential and reference evapotranspiration, leaving an opportunity for research on the calculation of flows in basins affected by land use change.

Finally, it should be noted that this research only analyzed scientific articles published in English, where Mexico's participation was not significant. This can be explained, according to Santillán-Fernández *et al.* (2021), by the tendency of researchers in Mexico to publish in institutional journals edited in Spanish, which restricts the constructive critique of peer review and limits the impact of publications, as English is the officially adopted language by the international scientific community. This leaves an area of opportunity for Mexican researchers to publish in English and in higher-impact journals (Santillán-Fernández *et al.*, 2023).

Table 4. Bibliometric indicators of the main authors with publications on methodologies for estimating the water balance affected by land use change in a watershed.

Author	Article		Country	Afiliation	Google Scholar (2024)		
	WB	Others			H-Index	Citations	Coauthor
Sun, G.	10	272	USA	USDA_FS	61	12201	868
Chen, J.	6	424	USA	Michigan_U	77	25125	1712
Bernhofer, C.	4	233	Germany	TU_Dresden	62	29872	1127
van Griensven, A.	4	131	Belgium	VU_Brussel	36	6634	328
Zhang, Y.	4	87	China	Southern_UST	24	2588	99
Chen, X.	3	20	USA	U_Cincinnati	13	457	47
Diekkrüger, B.	3	159	Germany	Universität Bonn	38	3978	349
Driscoll, C.T.	3	515	USA	Syracuse University	88	31765	853
Dulai, H.	3	74	USA	U_Hawaii	30	3012	180
El-Kadi, A.I.	3	77	USA	U_Hawaii	22	1199	73

WB: Water Balance; USDA_FS: USDA Forest Service; Michigan_U: Michigan State University; TU_Dresden: Technische Universität Dresden; VU_Brussel: Vrije Universiteit Brussel; Southern_UST: Southern University of Science and Technology; U_Cincinnati: University of Cincinnati; U-Hawaii: University of Hawaiʻi at Mānoa.

CONCLUSIONS

From 1965 to 2022, 407 articles on methods for calculating changes in the flow of a watershed due to land use changes were compiled. This scientific production showed an exponential growth trend (R^2=0.9791) that was concentrated mainly in countries with agricultural traditions such as the United States (89 publications, 21.87%), China (33, 8.11%), Germany (28, 6.88%), the United Kingdom (25, 6.14%), Australia (21, 5.16%), the Netherlands (18, 4.42%), Brazil (17, 4.18%), France (13, 3.19%), India (12, 2.95%), and Canada (11, 2.70%). The research focused on topics related to land use change (82 articles, 20.15%), climate change (48, 11.79%), hydrological processes (44, 10.81%), subsurface water (34, 8.35%), and evapotranspiration calculations (33, 8.11%). The most commonly used models for calculating the water balance were BHD (Direct Water Balance, 230 articles, 56.51%) and SWAT (Soil and Water Assessment Tool, 83, 20.39%). However, a lack of research development was also found in countries in Latin America and Africa. Therefore, these results can help redirect efforts in research centers in these countries to generate new knowledge that aids in making decisions on how to improve the efficient use of water in the face of climate change scenarios and land use change dynamics.

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AGRO PRODUCTIVIDAD

Impact of Treated Wastewater Use on Heavy Metal Accumulation in Soils and Sudan Grass Crops in the Mexicali Valley

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ABSTRACT
This study investigated the impact of using treated wastewater on the accumulation of heavy metals in soils and sudan grass crops. The transfer rate of heavy metals from soil to Sudan grass was determined. The Sudan crop was established in two plots: one irrigated with fresh water from the Colorado River and the other with treated wastewater from the Las Arenitas treatment plant. Metals Pb, Cr, Cd, Ni, and Cu were evaluated at two soil depths. Results showed that the concentration of metals in the treated wastewater is higher than in freshwater but within permissible limits. At a depth of 0-30 cm, the concentrations (mg/kg) of Pb, Cr, Cd, Ni, and Cu in soils irrigated with fresh and treated wastewater were 24.59, 19.25, 0.61, 25.33, 26.70, and 31.78, 20.10, 28.31, 28.33, respectively. These values show significant differences in the concentration of Pb and Cd. Analysis of metals in the Sudan grass showed similar results for soils irrigated with both types of water, with Pb and Cd not detected. The transfer rate was less than one for all metals. These results indicate no contamination or toxicity risks when using treated wastewater in Sudan grass crops. However, continuous monitoring is necessary to prevent contamination risks. These findings provide a scientific basis for developing policies and strategies for sustainable water resource management in arid and semi-arid regions.

Keywords: Heavy metals, wastewater, bioaccumulation factor, sudan grass cultivation, irrigation water.

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INTRODUCTION

The rapid population growth has created the need to increase food production, intensifying agriculture and raising the demand for fresh water [1]. It has been reported that approximately 3 Mm^3 of water is allocated annually for agriculture, representing 70% of global freshwater use on the planet [2]. Additionally, the constant growth of the urban and industrial sectors exerts additional pressure on water resources. An alternative to alleviate the pressure on water resources is the use of treated wastewater for agricultural irrigation. This sustainable water supply practice is common in arid and semi-arid regions, where water deficits are more pronounced [3].

The agricultural use of treated wastewater promotes the conservation of water resources, reduces environmental impact on receiving bodies, and protects aquatic

ecosystems [4]. Additionally, treated wastewater contains nutrients and organic matter that improve soil fertility, support crop growth, and reduce the need for fertilizers. However, its use raises significant concerns due to the potential accumulation of heavy metals in the soil and crops [5]. Heavy metals, such as lead (Pb), nickel (Ni), chromium (Cr), and cadmium (Cd), are toxic elements that can have harmful effects on human health and the environment. These metals can be absorbed by plants and enter the food chain, increasing the risk of chronic toxicity in humans and animals [6].

The Mexicali Valley is one of the most active agricultural regions in Mexico; however, it is located in the desert region of Baja California state. This area is characterized by low annual precipitation, with arid and semi-arid soils. Currently, the Mexicali Valley faces significant water scarcity, and its groundwater and surface freshwater sources are overexploited. It is estimated that the annual deficit amounts to 265.12 hm^3 [7]. To address water scarcity issues, the use of wastewater for irrigating certain forage crops, such as Sudan grass, has been adopted. In the Mexicali Valley, the wastewater used comes from the Las Arenitas treatment plant, located at latitude 32.42516 and longitude −115.32085. This plant processes approximately 27.4 Mm^3 annually, of which 40% is allocated to agriculture [8].

Currently, there is limited information on the presence of heavy metals in soils or crops irrigated with wastewater in the Mexicali Valley. Although there is significant global research on this topic, it cannot be generalized due to differences in soil conditions and wastewater quality. Therefore, this study investigated the effect of using treated wastewater on the accumulation of heavy metals in agricultural soils and Sudan grass crops. Two plots were compared: one irrigated with wastewater from the Las Arenitas plant and the other with fresh water from the Colorado River. The metals evaluated were Pb, Ni, Cr, and Cd. The results of this investigation provide fundamental information for developing policies and strategies for resource management.

MATERIALS AND METHODS

Study Area

This research was conducted in the Mexicali Valley, Baja California, Mexico. This region is situated at an altitude between 5-28 meters above sea level and is characterized by low precipitation, a dry and extreme climate, and arid to semi-arid soils. For this study, Sudan grass was planted in two plots of the Irrigation Module Number 12, part of the Irrigation District Number 014, Colorado River (DR-014). Plot No. 2 was irrigated with treated wastewater from the treatment plant, while Plot No. 50 was used as a control and irrigated with fresh water from the Colorado River. The studied plots are located relatively close to each other and have a similar surface area; their geographical location is shown in Figure 1.

Soil Sampling and Treatment

Soil sampling and treatment were conducted following current Mexican standards. A total of 10 sampling points were selected for each plot [9]. Soil samples were collected before planting and after harvesting Sudan grass, at two depths (0-30 and 30-60 cm) using

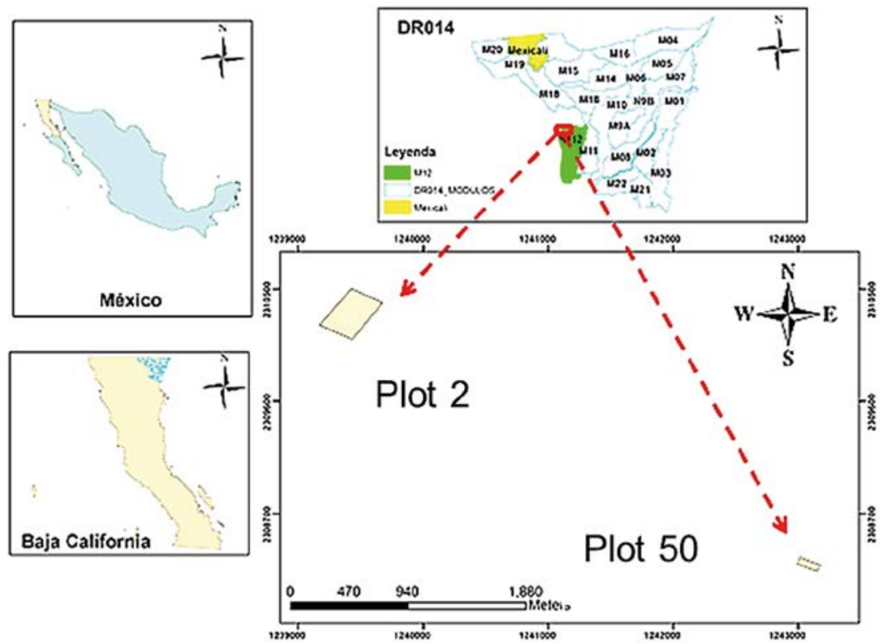

Figure 1. Plots used for the heavy metals study.

a soil auger. 1 kg of soil was extracted from each sampling point, which was then sieved to a particle size smaller than 0.25 mm. The ground and sieved samples were stored in airtight, sterile bags for subsequent analysis.

Water Sampling and Treatment

Fresh water and treated wastewater samples were collected from the irrigation channels of Module 12. Samples were collected from the gates allowing irrigation water to enter the plots, following the NOM-001-SEMARNAT-1996 standard [10]. Water samples were taken during each irrigation application, totaling five irrigations for each plot. Each sample contained 5 liters of water and was stored in polyethylene bottles that had been pre-washed with nitric acid (1%). The bottles containing the samples were then stored at 4 °C for subsequent analysis. For treated wastewater samples, a filtration process was applied to reduce turbidity [11].

Sampling and Treatment of Sudan Grass Plants

Plant samples were collected one day before the scheduled harvesting date for Sudan grass, following the NOM-021-SEMARNAT-200 standard [9]. The sampling points for plant collection coincided with the soil sampling points. Samples were collected by grouping 5 to 10 Sudan grass plants around the sampling site. Both aerial parts and roots were sampled, and the samples were washed with deionized water to remove soil or dust particles adhering to the surface. The samples were then dried at room temperature for 5 days. After drying, the samples were ground and sieved to reduce the particle size to 0.25 mm.

Physicochemical Analysis of Soil and Water

The physicochemical properties evaluated in soil and water were conducted according to the NOM-021-SEMARNAT-2000 and NOM-001-SEMARNAT-1997 standards, respectively [9,10]. The analyses performed included soil classification, pH determination, electrical conductivity (EC), organic carbon, and organic matter.

Total Metals Analysis

The total metal content in soil, water, and plant samples was determined. For solid samples, an acid digestion process was necessary. The digestion was performed on a heating plate using oxidizing acids such as HF, HNO_3, and HCl for 35 minutes. The digested samples were treated with a saturated H_3BO_3 solution to neutralize excess HF. The samples were then filtered and transferred to 50 mL volumetric flasks. The flasks were filled up to the mark with MilliQ water, and the contents were transferred to polyethylene flasks. Total metals were quantified using an atomic absorption spectrophotometer (Perkin Elmer 3100) with a hollow cathode lamp and an air-acetylene flame. The metals analyzed were Pb, Cd, Cr, and Ni, following the NMX-AA-051-SCFI-2016 standard [11]. Calibration curves were created from stock solutions, and the analyses were performed in triplicate to ensure the accuracy of the experimental data. The instrument's response was constantly verified during the analyses using certified standards of known concentration.

Transfer Factor

The transfer factor or bioaccumulation factor was studied to determine the fraction of trace elements that are transferred from the soil to the edible fraction of the Sudan plant. The transfer rate is determined by dividing the total metal concentration in the plant by the total metal concentration in the soil, as shown in Equation 1 [1, 3].

$$Tr = C_p / C_s \qquad \text{Equation 1}$$

Where Tr is the transfer rate (dimensionless), C_p is the total metal concentration in the plant sample (mg/kg), and C_s is the total metal concentration in the soil (mg/kg).

Statistical analysis

The analyses were conducted in triplicate, and the results are expressed as mean and standard deviation. The experimental data were analyzed using Minitab 17 statistical software. A one-way analysis of variance (ANOVA) was applied, and pairwise comparisons were performed using Tukey's test with a 95% confidence interval [12].

RESULTS AND DISCUSSION

Physicochemical analysis of soil and water

The results of the physical and chemical analyses of water and soil samples used for the cultivation of Sudan grass are presented in Table 1. Regarding water analysis, significant differences were found between the electrical conductivity (EC) of fresh water and treated wastewater. Additionally, it was found that the average pH of treated wastewater exceeded

Table 1. Physicochemical analysis of soil and irrigation water.

Parameter	Soil FW		Soil TW		FW	TW
	0 – 30 cm	30 – 60 cm	0 – 30 cm	30 – 60 cm		
pH	8.2±0.2	8.3±0.1	8.5±0.1	8.3±0.1	7.90±0.10	8.10±0.20
E.C. (dS/cm)	1.730	1.704	2.213a	2.445a	1.24±0.17a	2.12±3.17a
% Moisture	23.1±5.7	27.7±7.1	17.9±1.3	19.8±3.2	-	-
% OM	5.6±0.7	2.2±1.9	2.6±1.3	4.6±1.2	-	-
Texture	Clayey	Fragic clayey	Clayey	Clayey	-	-

[a] Indicates that the pairs of FW and TW values are significantly different ($p<0.05$).

the standards established for irrigation water by the WHO (2007) [13]. Regarding soil analysis, it was found that the pH of soil irrigated with fresh water (FW soil) is slightly lower than the pH of soil irrigated with treated wastewater (TWW soil). However, Tukey's test indicates that there are no significant differences in soil pH at different depths. Regarding EC, significant differences were found between FW soil and TWW soil at different depths. However, TWW soil exhibited higher conductivity compared to FW soil. Another parameter studied was moisture content. It was found that FW soil contains a higher moisture percentage compared to TWW soil. Additionally, the highest organic matter percentages were observed at depths of 0-30 cm and 30-60 cm for FW soil and TWW soil, respectively. Finally, the soil texture was determined, classifying them within the category of clay loam soils [9].

Total Metal Analysis in Irrigation Water

Table 2 presents the concentration of total metals in agricultural irrigation water from the Mexicali Valley. These values are compared with the permissible ranges reported by different national and international standards or organizations. The results indicate that the concentration of the studied metals in fresh water from the Colorado River and treated wastewater are within the permissible limits according to Mexican regulations and WHO standards [10, 13]. Specifically, Pb and Cd are below the detection limit (<0.025 mg L^{-1}), indicating a low presence of these metals. However, the results highlight the need to continuously monitor Cd and Cu metals, as they approached the limit values proposed

Table 2. Concentration of heavy metals in treated wastewater and fresh water.

Metal (mg L^{-1})	freshwater (Rio Colorado)	treated wastewater	Maximum permissible limits (mg L^{-1})	
			NOM-001-SEMARNAT-1996	WHO
Pb	<0.025	<0.025	5.00	0.50
Cr	0.004±0.001	0.007±0.002	0.50	0.10
Cd	<0.025	<0.025	0.05	0.01
Ni	0.035±0.006	0.039±0.010	2.00	0.20
Cu	0.010±0.001	0.020±0.001 [a]	4.00	0.20

[a] Indicates that the pairs of FW and TW values are significantly different ($p<0.05$).

by the WHO. Moreover, the statistical analysis showed significant differences between the concentration of copper in fresh water and treated wastewater.

Analysis of total metals in soil

The average concentration of metals present in FW soil and TWW soil at different depths was determined, as shown in Table 3. It was found that the average concentration of heavy metals in FW and TWW soil at different depths is below the limits established by the EPA (2012) [14], WHO (2007) [13], and the European Union (EU, 2001) [15]. However, it was observed that the concentration of heavy metals in TWW soil was higher compared to FW soil, except for copper at the 30-60 cm depth. Therefore, it is recommended to continuously monitor the concentration of heavy metals in soils irrigated with treated wastewater to prevent metal accumulation in agricultural soils and mitigate health risks.

A statistical analysis of the data obtained on the concentration of metals at different depths for each soil type was performed. It was found that there are no significant differences in the average metal concentration with respect to depth in FW and TWW soil, except for Cu in TWW soil. A second statistical analysis was carried out to compare the heavy metal concentration in FW soil with TWW soil. Significant differences were found in the average concentration of Pb and Cd between both soils at the two studied depths. Figure 2 shows the average total metal concentrations in FW Soil and TWW Soil for the depths of 0-30 cm and 30-60 cm, respectively.

Total metals in Sudan grass plants

The results of the total metal concentration in Sudan grass irrigated with fresh water (Sudan FW) and Sudan irrigated with treated wastewater (Sudan TWW) are presented in Table 4. The findings indicated that there is no risk of heavy metal contamination (Ni, Cr, Pb, Cu, and Cd) in Sudan grass when irrigated with fresh water or treated wastewater. Sudan TWW crops do not exceed the permissible limits reported by Lepp (1985) and MacLean *et al.* (1987) [13,16,17]. Previous studies have reported the use of wastewater in

Table 3. Concentration of heavy metals in agricultural soil according to depth and irrigation water.

Metals (mg kg^{-1})	Soil irrigated with freshwater (Soil FA)		Soil irrigated with treated wastewater (Soil TW)		Maximum permissible limits		
	Depth of sampling (cm)				EPA [14]	WHO [13]	EU [15]
	0-30	30-60	0-30	30-60			
Pb	24.59±5.79^{b}	25.40±8.42^{b}	31.78±2.77^{a}	32.80±3.67^{a}	250	250-500	300
Cr	19.25±2.24	18.04±4.77	20.10±1.61	20.55±1.58	100	-	180
Cd	0.61±0.14	0.60±0.18	0.82±0.08^{a}	0.74±0.09	3	3-6	3
Ni	25.33±4.82	25.55±7.78	28.31±4.81	32.39±10.68	100	75-150	75
Cu	26.70±7.07	27.71±10.09	28.33±2.27^{c}	25.65±3.01^{c}	170	135-270	140

% recovery: Pb=97.5, Cr=101.2, Cd=99.7, Ni=96.2, Cu=95.0.
a Indicates that pairs of Soil FW and TW values are significantly different ($p<0.05$).
b Indicates that the pairs of Soil FW values 0-30 and 30-60 cm are significantly different ($p\leq0.05$).
c Indicates that the pairs of Soil TW values of 0-30 and 30-60 cm are significantly different ($p\leq0.05$).

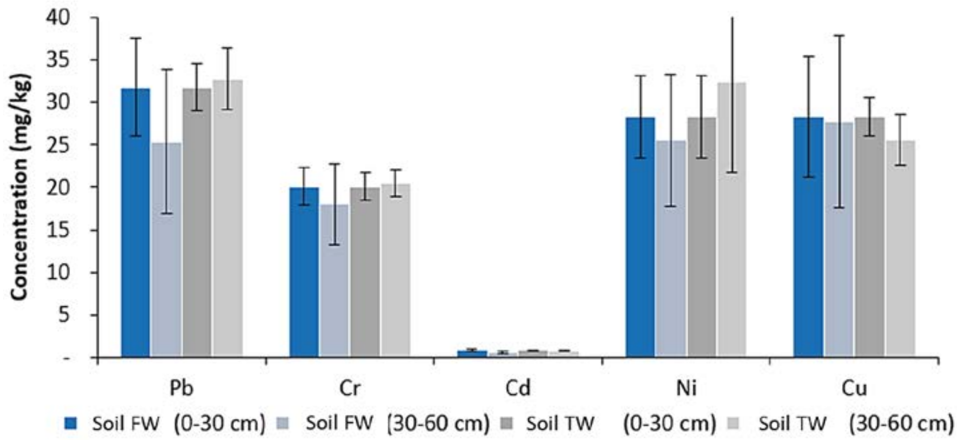

Figure 2. Total metal concentration in agricultural soil at different depths.

Table 4. Metal concentration in Sudan grass crops.

Metal (mg kg^{-1})	**sudan FW**	**sudan TW**	**Maximum permissible limits (mg kg^{-1}) [13, 16, 17]**
Pb	ND	ND	2.5
Cr	0.390±0.02	0.529±0.01	-
Cd	ND	ND	1.5
Ni	0.820±0.16	0.900±0.2	1.5
Cu	3.120±0.75	3.050±0.6	30

% recovery: Pb=98.5, Cr=97.1, Cd=96.9, Ni=99.0, Zn=89.1, Cu=93.3
[a] indicates that the pairs of values for Sudan FW and TW are significantly different ($p<0.05$).

forage crops for over 17 years without observing significant accumulation of heavy metals that pose toxicity risks [1]. The Tukey test indicated no significant differences in metal concentrations between Sudan FW and Sudan TWW plants.

Transfer Rate for the Agricultural Cycle of Sudan Grass

The transfer rate (Tr) of heavy metals (Pb, Cr, Cd, Ni, and Cu) from the soil to the Sudan grass plant was determined, considering different soil depths. The results are shown in Figure 3. It can be observed that Pb and Cd did not show any transfer rate (Tr) for either FW or TWW soils. In the case of Cr, low Tr values were observed, with no significant differences based on depth or water type. Meanwhile, Ni showed a consistent Tr under all studied conditions.

For Cu, lower Tr values than Ni were observed. However, when irrigated with treated wastewater and at a depth of 30-60 cm, the Tr increased significantly. This could be concerning from a toxicity and contamination perspective. In general, the Tr for most of the studied metals is less than one, indicating a low risk of metal accumulation in the sudangrass plant. The metals that showed the highest transfer for both water types, in ascending order, were Cu > Ni > Cr. The transfer values obtained are lower than those reported for other forage crops [1,4].

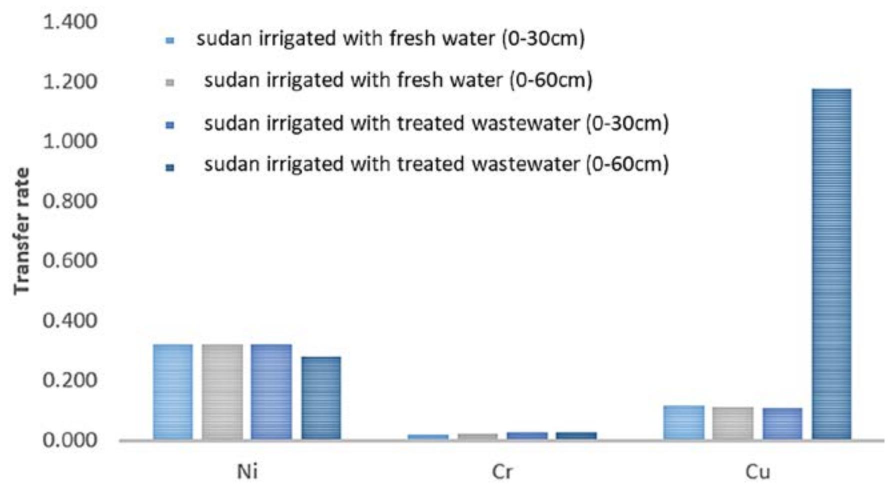

Figure 3. Heavy metal transfer rate from soil to plant.

CONCLUSIONS

This study investigated the accumulation of heavy metals in soils and Sudan grass crops irrigated with treated wastewater and fresh water in the Mexicali Valley. The results indicated that the use of treated wastewater could be a viable solution for agriculture in water-scarce areas like the Mexicali Valley. However, it is crucial to continuously monitor the concentration of heavy metals in both soil and crops to prevent toxicity risks. It was observed that the concentration of heavy metals in soil irrigated with treated wastewater was higher compared to soil irrigated with fresh water. Nonetheless, the values in both soils remained below the permissible limits established by the EPA, WHO, and the European Union. On the other hand, metal analyses in the Sudan grass tissue indicated no significant risk of contamination with heavy metals when irrigated with treated wastewater, as concentrations remained below permissible limits. Additionally, the transfer factors showed low risks of metal accumulation in plants, with values less than one.

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Colegio de Postgraduados

Rodents in Xerophilous Shrubland and Semi-Desert Grassland Communities of Southeastern Coahuila, Mexico

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ABSTRACT

Objective: To assess the diversity of rodent communities in semi-desert grasslands and xerophilous shrubland at the "Los Ángeles" Ranch in southeastern Coahuila.

Design/methodology/approach: Monthly samplings were carried out from May to November 2020 using Sherman traps. The capture-recapture method was used to estimate species richness and abundance. Non-parametric estimators, species rarefaction curves, and rank abundance plots were utilized to measure species diversity. The Whittaker index was employed to assess species turnover between sampled communities.

Results: A total of 205 individuals from three families and eight species of rodents were recorded, with the Cricetidae family being the best represented. The species rarefaction curve showed that the shrubland exhibited greater diversity compared to the grassland. The dominant species were *Onychomys arenicola* in the semi-desert grassland and *Peromyscus zamorae* in the xerophilous shrubland.

Limitations/implications: There remains a need to extend long-term monitoring efforts to detect how anthropogenic activities influence species composition.

Findings/conclusions: The study highlights the importance of grasslands for the conservation of rodents in the state. Semi-desert grasslands and shrublands provide natural resources that should be preserved to maintain biodiversity in semi-arid ecosystems.

Keywords: Rodentia, habitat, grassland, semi desertic ecosystem, diversity.

INTRODUCTION

Coahuila is an important part of the arid and semi-arid ecosystems of northern Mexico, as the majority of its surface is immersed in the Chihuahuan Desert (Villarreal-Quintanilla and Encina-Domínguez, 2005). However, some areas within this region experience notable anthropogenic impact due to livestock activities, which generate

modifications in the structure and vegetation cover, thus affecting the availability of habitats for various wildlife species (Hernández-Betancourt *et al.*, 2012). Therefore, it is essential to have information about the composition of the different faunistic groups that these ecosystems harbor. Among these, rodents are particularly interesting for understanding the functioning of ecosystems, as they are vulnerable to habitat disturbances and require specific physical and climatic conditions for establishment (Aragón *et al.*, 2012). Additionally, this faunistic group plays an important role as seed dispersers and consumers (Godó *et al.*, 2022) and constitutes a significant part of the biomass for predators (Hernández *et al.*, 2011). Although various studies on mammals have been conducted in Coahuila, most of them have focused on the central and northern regions of the state (Sierra Mojada and San Buenaventura), primarily addressing aspects of population dynamics and faunistic inventories (González-Uribe *et al.*, 2023; Valdés-Alarcón *et al.*, 2023). Meanwhile, in other areas of the state, studies have been conducted on distribution patterns (Ramírez-Pulido *et al.*, 2018; Pineda *et al.*, 2024). Therefore, it is essential to increase knowledge in other regions to understand the diversity and composition of mammal communities, such as rodents in the state (Ramírez-Pulido *et al.*, 2016). In this context, the objective of this study was to evaluate the diversity of rodent communities in semi-desert ecosystems, such as semi-desert grasslands and xerophilous shrubland at the "Los Ángeles" Ranch in southeastern Coahuila. This was done to generate basic information about the species that inhabit this region and to fill gaps in the knowledge of species richness and population abundance, there by contributing to the conservation of local biodiversity in the arid and semi-arid ecosystems of Coahuila.

MATERIALS AND METHODS

The study was conducted at the "Los Ángeles" Ranch located in the municipality of Saltillo, Coahuila (25° 04' 12" to 25° 08' 51" N, 100° 58' 07" to 101° 03' 12" W) (Figure 1), at an average altitude of 2,150 m (Heredia-Pineda *et al.*, 2017). This area covers 7,000 hectares and features elevated zones and valleys. The climate is dry, arid-semi-warm, with a cool winter, and the average annual temperature varies between 18 and 22 °C (García, 2004). The average annual precipitation ranges from 450 to 550 mm, with rainfall primarily occurring during summer and winter (López-Santos *et al.*, 2008). The main activity in the area is extensive beef cattle production through rotational grazing.

The dominant types of vegetation are xerophilous shrubland and semi-desert grassland (Encina-Domínguez *et al.*, 2018).

Fieldwork: Sampling was conducted monthly from May to November 2020, where four sampling sites were established (two sites in semi-desert grassland and two in xerophilous shrubland). At each site, a quadrant of 4,000 m^2 was established with 40 trapping stations distributed at equal distances of 10 m. At each station, a Sherman trap was placed (González-Romero, 2011). Capturing was conducted over three consecutive nights at each site. The capture and recapture method was used (Krebs, 1985), where each individual was marked by ectomizing phalanges (Pacheco *et al.*, 2000; Romero-Almaraz *et al.*, 2007). For species identification, the somatic measurements of the

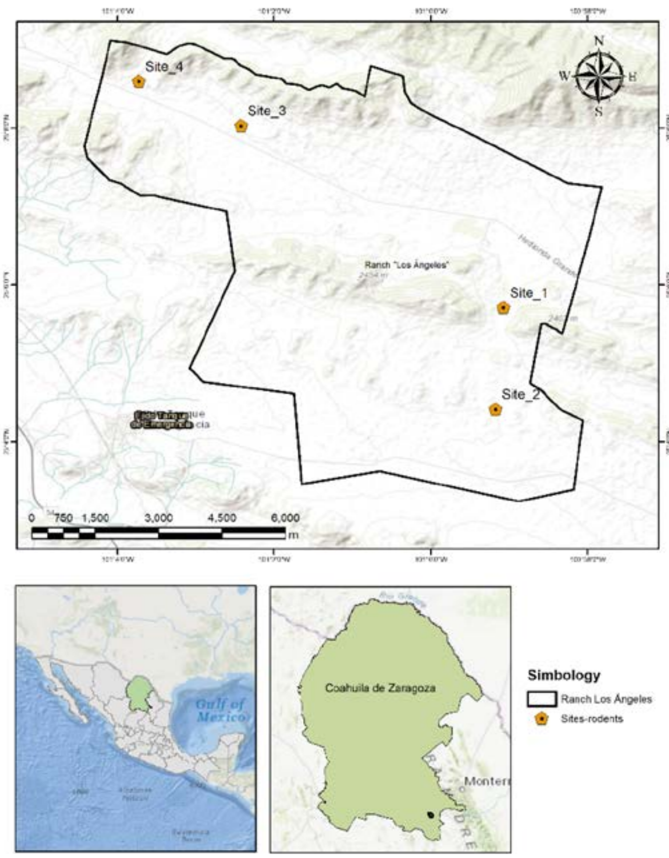

Figure 1. Location of the Los Ángeles Ranch in southeastern Coahuila.

captured individuals were recorded. Subsequently, guides with specialized taxonomic keys were utilized (Ceballos, 2014; Álvarez-Castañeda *et al.*, 2015). Once identified, the individuals were released *in situ*.

Statistical analysis: To estimate species richness, non-parametric estimators ICE and Jackknife 1 were employed (Gotelli & Colwell, 2011), calculated using the EstimateS 9.10 program (Colwell, R. K. 2013). For the analyses, true diversity was used at three levels, where q=0 corresponds to species richness, q=1 is the exponential of Shannon, and q=2 is the inverse of Simpson's index (Jost, 2006). The rarefaction curve was calculated using RStudio 4.3.0 (RStudio Team, 2023) with the iNEXT package (Hsieh *et al.*, 2016), with 95% confidence intervals (Moreno, 2001; Jiménez-Valverde and Hortal *et al.*, 2003). True diversity at each site was measured using the exponential of the first-order Shannon index (1D) (Jost, 2006; García-Morales *et al.*, 2011; Moreno *et al.*, 2011). Rank-abundance graphs were generated to observe the structure of species within the different sites. Finally, to evaluate species turnover among the sampled plant communities, the Whittaker index (Beta diversity) was used, which is defined as the ratio between gamma diversity (at the regional level) and alpha diversity (at the local level), expressed as gamma divided by alpha ($\beta=\gamma/\alpha$). This index measures the degree of differentiation among biological communities (Baselga and Gómez-Rodríguez, 2019).

RESULTS AND DISCUSSION

The recorded rodent species in the study area constitute 15.4% of what has been reported for Coahuila, 9.7% for the Chihuahuan Desert (Ramírez-Pulido *et al.*, 2018), and 3.3% at the national level (Ceballos, 2014; Ramírez-Pulido *et al.*, 2014). A total of 205 individuals were recorded, belonging to three families (Cricetidae, Heteromyidae, and Sciuridae) and eight species (Table 1). The dominance of the Cricetidae family was observed in the "Los Ángeles" Ranch, reflecting their fundamental role in the ecosystem; they have developed physiological and behavioral adaptations, such as fat and water storage in their bodies, allowing them to thrive in environments with limited resources (Harris & Pritchard, 2012) (Table 1).

The Mexican prairie dog (*Cynomys mexicanus*), the spotted ground squirrel (*Xerospermophilus spilosoma*), and the yellow harvest mouse (*Reithrodontomys fulvescens*) were recorded outside the sampling period; however, they were included in the species richness list (Figure 2).

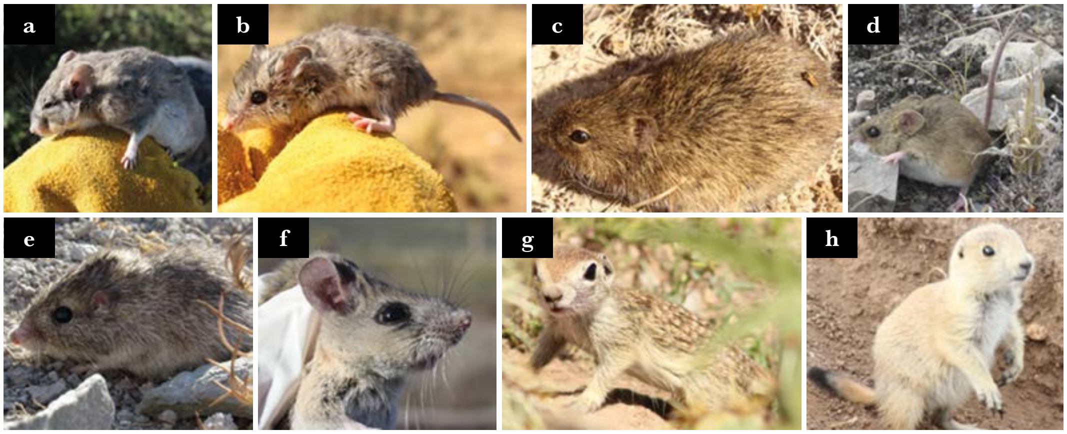

Figure 2. Rodents recorded at the "Los Ángeles" Ranch: a=*Neotoma leucodon*; b=*Onychomys arenicola*; c=*Sigmodon hispidus*; d=*Reithrodontomys fulvescens*; e=*Chaetodipus nelsoni*; f=*Peromyscus zamorae*; g=*Xerospermophilus spilosoma*; h=*Cynomys mexicanus*.

Table 1. Recorded species in each studied plant community.

Species	Name	Distribution	UICN/NOM-59-SEMARNAT-2010	Types of vegetation	
				Grassland	Scrub
Neotoma leucodon	White-toothed woodrat				✓
Onychomys arenicola	Chihuahuan grasshopper mouse			✓	✓
Peromyscus zamorae	Zamora deer mouse	Endemic		✓	✓
Sigmodon hispidus	Hispid cotton rat				✓
Chaetodipus nelsoni	Nelson's pocket mouse			✓	✓
*Reithrodontomys fulvescens**	Fulvous harvest mouse				✓
*Cynomys mexicanus**	Mexican prairie dog	Endemic	EN, P	✓	
*Xerospermophilus spilosoma**	Spotted ground squirrel			✓	

* Species recorded outside the sampling period; IUCN=International Union for Conservation of Nature (species included in the Red List of Threatened Species); NOM-059-SEMARNAT-2010=Species with some risk category under the Official Mexican Standard-059; Types of vegetation: ZAC=Semidesert Grassland (Species recorded in grassland); MAT=Xerophytic Shrubland (Species recorded in shrubland).

In relation to the species classified as at risk under the Official Mexican Standard 059-2010 (NOM-059-SEMARNAT-2010), the Mexican prairie dog was recorded in the endangered category (P) and also holds the same status (EN) within the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. This species plays a fundamental role in the structure of grassland communities as an umbrella species, as it modifies the landscape by creating habitats for other species through its burrowing activities. The decline in population, attributed to habitat fragmentation, can have negative repercussions on the structure and functionality of the ecosystem, affecting species that depend on the habitats created by this rodent (Castellanos-Morales *et al.*, 2016; O'Brien & Kinnaird, 2016). The species rarefaction curve showed the highest richness for the xerophilous scrub community with five species, while the semidesertic grassland recorded the highest number of individuals (93). Although the rarefaction curve reached the asymptote, there is a probability of recording greater species richness if sampling continues. Regarding the number of effective species, differences in species richness (q=0) were observed: five species in the xerophilous scrub and three in the semidesertic grassland. The exponential of the Shannon index (q=1) indicated that the xerophilous scrub vegetation exhibited the highest diversity (2.62) compared to the semidesertic grassland (1.74). For the inverse Simpson value (q=2), it was higher in the semidesertic grassland (0.69) compared to the scrub (0.54). The wider confidence intervals for Shannon and Simpson diversity in the scrub indicate greater variability in species evenness compared to the grassland (Figure 3).

Regarding the abundance-rank curve in the two plant communities (Figure 4), the semidesert grassland was dominated by the sandy chapuliner mouse (*Onychomys arenicola*), as it is a carnivorous species with fossorial habits that requires open areas for its burrows.

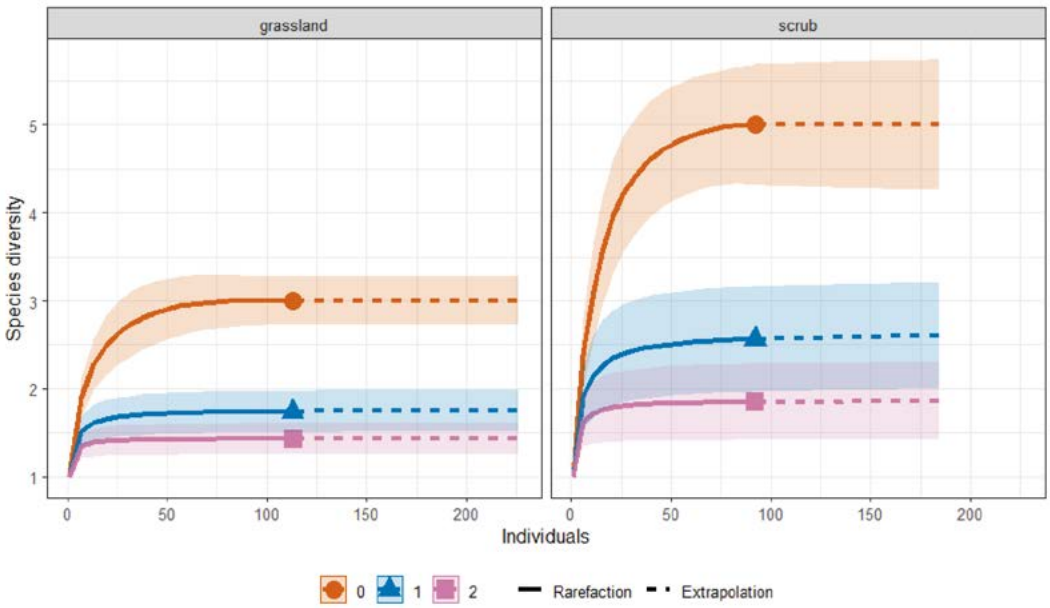

Figure 3. Species rarefaction curve in the two studied plant communities: 0=richness, 1=exponential of Shannon, 2=inverse of Simpson.

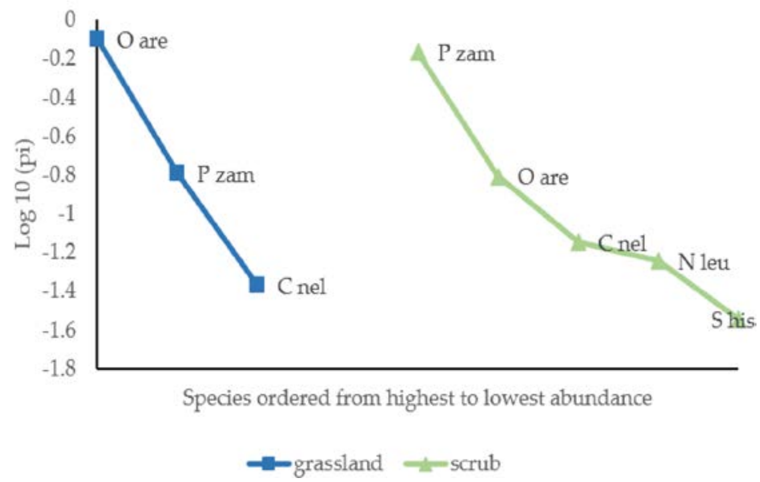

Figure 4. Species abundance rank curve recorded in each type of vegetation. O are=*Onychomys arenicola*; P zam=*Peromyscus zamorae*; C nel=*Chaetodipus nelsoni*; N leu=*Neotoma leucodon*; S his=*Sigmodon hispidus*.

This allows for soil aeration and visibility of potential predators, as well as foraging opportunities (Martín-Regalado *et al*., 2019; Langley, 2021). In the xerophytic scrub, the dominant species was the Zamora deer mouse (*Peromyscus zamorae*), which is due to its populations being associated with the productivity of resources provided by the plant community (vegetation cover that serves as refuge and raw material for food) (Whitford & Steinberger, 2010, Ceballos, 2014). Among the species with low dominance in the grassland, the Nelson's pocket mouse (*Chaetodipus nelsoni*) was recorded, possibly due to the type of habitat where it is distributed, which is associated with areas featuring rocky slopes and grasslands with shrubs such as governadora and hojasén, which serve as protection against predators and as food sources (Neiswenter *et al*., 2019; Martínez-Calderas *et al*., 2023). The record of the cotton rat (*Sigmodon hispidus*) may be attributed to its ability to adapt to conditions with anthropogenic impact, as its populations establish themselves in agricultural areas and regions with livestock activities (Wright & Russell, 2010; Tomé *et al*., 2020). Regarding species turnover (Beta Diversity), it resulted in 1.25, indicating low differentiation between the vegetation communities, as they share most of their species with low turnover values.

CONCLUSIONS

The information generated in this research provided a baseline on knowledge about the richness and abundance of rodents in the "Los Ángeles" Ranch, highlighting the importance of grasslands as key areas for the protection and maintenance of rodent populations, as well as the conservation potential that both vegetation communities offer for protection in southeastern Coahuila. It is recommended that future studies evaluate other environmental variables such as soil (texture, nutrient content, porosity, and permeability), altitude, diversity of grasses and shrub species, as well as the establishment of a greater number of sampling sites to broaden conservation efforts, particularly for species with conservation status, thereby ensuring the continuity of natural resources.

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Colegio de Postgraduados

Dynamic stochastic model of allometric equations and cumulative distribution for biomass-carbon in *Pinus hartwegii* Lindl., facing climate change

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ABSTRACT

Objective: to construct a dynamic stochastic model with validated biospheric-interaction, estimating allometric equations for the total volumetric increase and cumulative distribution of biomass for *Pinus hartwegii* Lindl in the states of Mexico and Puebla, considering climate change.

Design/Methodology/Approach: the methodology included the use of SiBiFor numerical databases, NASA Power data, Ordinary Least Squares mathematical models, the Random-Forest software, Ridge model with regression, R algorithms and Newton volumetric estimation equations.

Results: estimated allometric equations were obtained for the total volume of trees in 2023, highlighting the importance of linear regression models and the validity of the variables used. Newton's mathematical equations and theoretical models for excurrent tree-form types were found to have the best accuracy in estimating the total volume of the barked tree.

Limitations of the study/Implications: this study has limitations in terms of generalization to other forest types and the availability of data in Mexico. However, it highlights the importance of understanding forest responses to environmental changes and the need for validated dynamic stochastic models to estimate allometric equations and assess carbon sequestration.

Findings/Conclusions: this study highlights the importance of understanding and assessing the carbon storage capacity of forests, especially in the context of climate change. In addition, it underlines the usefulness of linear regression models and variable validation to estimate carbon sequestration in *Pinus hartwegii* forests.

Keywords: stochastic modeling, biomass-carbon, *Pinus hartwegii*, climate change, Random Forest.

INTRODUCTION

Developing the theoretical-practical mathematical modeling capacity based on the dialectical relationship between the logics of mathematics and engineering responds to the

interest of solving situations specific to the engineering profession (Torrecilla Díaz *et al.*, 2015). A concrete example is a stochastic mathematical model, defined as a mathematical abstraction of an empirical process governed by probabilistic laws and set as a quantitative model in which more than one state of nature exists. This model is created to calculate the conditional outcomes of decision alternatives in each state (Vega, 2022). An example of the practical application of these models is the reduction of natural vegetation and the importance of forest masses as CO_2 reservoirs based on their sensitivity to climate change at a global scale (Sandoval, 2020).

Each species or plant population requires specific conditions of temperature, humidity and light for its physiological processes. If it exceeds its degree of tolerance this will be reflected in the alteration of species distribution, which is more severe in individuals of mountainous ecosystems. Even though there are extensive investigations with criteria of climate change, wood traits and molecular variations, quantification of organic carbon stored in soil per ha^{-1}, physical-chemical properties of wood, dendro-epidometric measurements, development, validation, simulation with mathematical modeling, they are included in few forestry investigations. Due to this; the theory of stochastic processes includes random vectors of infinite dimension or arbitrary infinite collections of arbitrary variables for dynamic model analysis, which account for the evolution of those variables over time (Valderrama, 2009), yet, they are scarce in Mexico.

The cumulative biomass is conditioned simultaneously by the biocoenotic and abiocoenotic components in situ at any instant; thus describing temporal evolutionary displacement of *P. hartwegii* Lindl forests in those Mexican highland states. In the face of climate change, mitigation is of great relevance. Static and dynamic biometric models, deterministic as well as stochastic, are essential to understand the dynamics and to evaluate the carbon storage capacity of forests (Santiago *et al.*, 2020), which underlines the need to contribute to this knowledge (Martínez-Luna *et al.*, 2020; Ortega Rivera, 2021).

Based on this, the objective of this research was to construct a dynamic stochastic model with a validated biosphere-interaction modulus that would estimate allometric equations of the total volumetric increase, and the cumulative distribution of biomass for *Pinus hartwegii* Lindl, to evaluate carbon sequestration in the states of Mexico and Puebla.

MATERIALS AND METHODS

The study was implemented in *Pinus hartwegii* Lindl forests in the states of Mexico and Puebla. The numerical databases of the SiBiFor Project were used (Vargas-Larreta *et al.*, 2017Guerrero, Jalisco, Oaxaca, Michoacán, Puebla, State of Mexico, Hidalgo, Tlaxcala, Veracruz and Quintana Roo. Field data collection was done by using destructive and non-destructive sampling in each Regional Forest Management Unit (RFMU) considering the 2013-2015 time series and the NASA methodology based on the POWER Project (Al-Kilani *et al.* 2021; NASA, 2021; POWER 2021) to analyze significant climatic variables that influence the growth of *Pinus hartwegii* forests. Measurements of normal diameter (DN), total height, and commercial height of the trees were analyzed.

The numerical databases in each time (2013-2015) were taken in the field by the technical staff of SiBiFor for the timber species *Pinus hartwegii* Lindl. in the states of

Mexico and Puebla. Then, they were divided into the categories "commercial volumetric dimension", with dasometric measurements of dominant and codominant trees and "non-commercial volumetric dimension", with dasometric measurements of suppressed trees, with the aim of estimating a model for each category separately, since it would not justify its use only with the statistical criterion of R^2 and, consequently, the predictions would be biased. Based on this, with the use of Newton's mathematical equation to estimate timber volumes; theoretical models for excurrent dendrometric types (volume per timber unit, *i.e*. logs); allometric equations used in the field by the governmental institutions of the Mexican states; the Ordinary Least Squares method (OLS) and algorithms in R; the allometric equations for "commercial volumetric dimension" of *Pinus hartwegii* Lindl were estimated using mathematical models in the states of Mexico and Puebla.

A dynamic stochastic model of allometric equations and cumulative distribution was used to estimate carbon sequestration in *Pinus hartwegii* forests. Different statistical methods and algorithms were applied to validate the model, including regression analysis, and the Ordinary Least Squares method (OLS) (Briggs & MacCallum, 2003; Pretzsch, 2009).

In this research, five exploratory techniques of the Graphical Method were used; the scatter plots of the squared residuals of the model against the values of the variables, both endogenous and exogenous were analized, searching for systematic patterns that could reveal heteroskedasticity. In case the residues are homoscedastic, the scatter plot would take the form of an almost constant horizontal band. If patterns are observed in the graphs, there would be evidence of dependence between the squared residuals and the variables, moreover, it would be known which variable is the cause of this dependence. The graphic methods used were residual graph, residuals *vs*. fitted graph, Q-Q Normal Chart, Scale Location Model Chart, and Residuals *vs*. Laverage Chart.

Since the Ridge regression is a biased estimate that starts from the solution of the Least Squares (LS) regression, it is vital to establish the conditions for which the central Student's t-distribution used in the hypothesis test in CM, is also applicable to the regression. The proof of this important result is presented in this research. The method allows detecting multicollinearity within a regression model of the type $Y = X^t \hat{\beta} + \epsilon$, that is used to operate such biased models.

Random Forest is an assembly-based machine learning algorithm that combines multiple decision-trees to improve accuracy and to reduce variance in predictions (Breiman, 2001) cited in Alaminos (2023). Along recent years, Random Forest has been used in many practical applications and has proven effective in a wide variety of problems.

Data for DN, total height, and commercial tree height were used to develop allometric equations and cumulative distributions to estimate carbon sequestration in *Pinus hartwegii* forests. In addition, calculations of timber volume were applied by OLS criterion and Ridge regression (Hoerl & Kennard ,1970; Piña-Monarrez *et al*., 2007) to assess the carbon storage capacity of forests.

Statistical tests were performed and graphical methods were used to validate the proposed model. Statistical software R was used through the Random Forest procedure (Mohamed *et al*., 2024) to run data analysis and output generation.

RESULTS AND DISCUSSION

The use of climate data obtained through NASA POWER project (NASA, 2021; POWER 2021), including solar radiation, temperature and humidity, were significant variables for the growth of *Pinus hartwegii* forests. These data were fundamental for the estimation of allometric equations of total volumetric increase and cumulative distribution of biomass, as well as for assessing carbon sequestration in the forests studied.

Table 1 presents the allometric equations estimated by OLS (R software) and SiBiFor methodology (Vargas-Larreta *et al.*, 2017) for the *Pinus hartwegii* forests in the states of Mexico and Puebla.

Table 2 includes the allometric equations estimated by OLS (Ridge Model) for *Pinus hartwegii* considering those climatic variables that are relevant. These equations were developed using climate data provided by the POWER project. It was found that the linear allometric equations estimated by OLS with the Ridge Model provide a better volumetric approximation to climate change.

Table 3 shows the predictions of the numerical values representing the direct relationship between the apparent and specific climate conditions and their effects on *Pinus hartwegii* Lindl during the time span between 2022 and 2024 in the territories of the states of Mexico and Puebla.

According to the graphic validations, some models met the evaluation criteria. The estimated allometric equations "non-commercial volumetric dimension" of *Pinus hartwegii* Lindl using mathematical models in the states of Mexico and Puebla showed low statistics under analysis of variance criteria. This occurred because dasometric measurements of suppressed trees differ markedly. According to the validation charts, not all of the models met the evaluation criteria; but, based on field cross-validation, the traditional mathematical equation with the best accuracy in estimating the total volume of barked trees for *Pinus hartwegii* Lindl in the states of Mexico and Puebla is Newton with an evaluation of 95% and 98%, respectively. Since it uses the largest, middle and smallest diameters of logs while the allometric equation estimated by mathematical models showed the best estimate of the total tree volume with bark for this timber forest species. The allometric equation estimated by mathematical models with better estimation of the TAV cc was the theoretical model for excurrent tree form types (volume per logs) in the states of Mexico and Puebla with an evaluation of 90.5% and 95%, respectively.

Based on the use of the SiBiFor numerical databases from a given time (2013-2015) for the timber species *Pinus hartwegii* Lindl., the application of the 'Random Forest' for R package to interpolate and estimate the non-existent information of the variable "Edad_no_anillos" in the UMAFOR Num.1508 in the state of Mexico and Num. 2105 in the state of Puebla; the use of Power data, in monthly time series format of (1980-2021) from NASA methodology; and the discernment of which climatic variables are relevant in the volumetric growth of a tree, allometric equations of "commercial volumetric dimension" with significant climatic variables for *Pinus hartwegii* Lindl. were estimated in the states of Mexico and Puebla.

According to Hoerl & Kennard (1970), this occurs because the advantage of Ridge regression over least squares has its origin in the bias-variance compensation. As α increases,

Table 1. Summary of allometric equations estimated by Ordinary Least Squares (OLS) with R and SiBiFor for *Pinus hartwegii* for the state of Mexico and Puebla.

State	Volumetric	Method of fitting							
		OLS					SiBiFor		
		Equation	**Multiple Square R**	**Square R Fitted**	**F- statistic**	**p-value**	**UMAFOR**	**Equation**	**R^2**
México	Newton	log(Vol)=−0.12 + 0 log_h + 0.33 log_diam	0.9827	0.9826	8037	<2.2e-16	1508	0.00005*(Diam^1.97258)*(Alt^0.92797)	0.9618
	Theoretical (logs)	log(Vol)=0.11 + 0.12 log_h + 0.25 log_diam	0.9748	0.9746	5463	<2.2e-16	1510	0.00004*(Diam^2.15224)*(Alt^0.78917)	0.9585
	State	log(Vol)=−9.94 + 0.85 log_h + 2.05 log_diam	0.9971	0.997	43730	<2.2e-16			
Puebla	Newton	log(Vol)=−0.07 + 0.05 log_h + 0.27 log_diam	0.9627	0.9624	3831	<2.2e-16	2101	0.00006*(Diam^1.99376)*(Alt^0.90054)	0.9721
	Theoretical (logs)	log(Vol)=0.12 + 0.15 log_h + 0.22 log_diam	0.9642	0.964	4000	<2.2e-16	2105	0.00004*(Diam^2.20234)*(Alt^0.66536)	0.9772
	State	log(Vol)=−8.18 + 0.61 log_h + 1.79 log_diam	0.9709	0.9707	4889	<2.2e-16	2108	EXP(-9.63495649+1.86670523*LN(diam)+0.99551381*LN(alt))	0.9939

*Method of fitting: it indicates the method used to fit the allometric equations, either OLS or SiBiFor; State/State: it shows the geographic location for which the allometric equations were estimated; Volumetric method: it describes the method used to calculate the volume of trees (Newton; Theoretical by log [as unit of timber] and the one commonly used by State); Equation: it presents the allometric equation resulting from the fit done; R^2: explained variance percentage; Multiple Square R: it is a measure of the proportion of variability in the dependent variable that is explained by the model, it indicates how well the model fits the data; Square R fitted: This is similar to multiple square R, but it takes into account the number of predictors in the model; it helps to compare models with different numbers of predictors; F-statistic: it is the value that test the global significance of the regression model; p-value: this is the value of p associated with the F-statistic, it indicates whether the model is significant.

Table 2. Summary of allometric equations estimated by MCO with Ridge Model for *Pinus hartwegii* with significant climatic variables.

Concept	Estado de México			Puebla		
	Method for Commercial volumetric dimension			Method for Commercial volumetric dimension		
	Newton	Theoretical (logs)	State	Newton	Theoretical (logs)	State
Explained Variance percentage (R^2)	0.951499	0.939338	0.928746	0.93038	0.934537	0.93575
(Intercept)	39.859663	50.64726	−64.30451	105.415	154.6166	−131.9211
Altura total (h)	0.01572	0.045025	0.031397	0.00809	0.027984	0.013225
Diámetro normal (diam)	0.025014	0.026166	0.028203	0.01493	0.018439	0.047649
Earth Skin Temperature (°C) (TS)	−0.010908	−0.012784	0.001895	−0.003	−0.005506	0.007905
Temperature at 2 meters (°C) (T2M)	−0.010975	−0.013165	0.007274	−0.0123	−0.020297	0.021197
Top−of−Atmosphere Shortwave Downward Irradiance (TOA_SW_DWN)	−1.924237	−2.505947	2.253705	−3.4611	−4.817098	3.986277
All Sky Surface PAR Total (ALLSKY_SFC_PAR_TOT)	0.002949	0.004038	−0.003183	0.00437	0.00509	−0.003492
Clear Sky Surface PAR Total (CLRSKY_SFC_PAR_TOT)	0.006476	0.008882	−0.004877	0.00597	0.005833	−0.003405
Surface Pressure (PS)	0.338027	0.454903	−0.163521	0.17873	0.146928	−0.074175
Specific Humidity at 2 meters (QV2M)	0.045728	0.059961	−0.013913	0.0136	0.009449	−0.004173
Relative Humidity at 2 meters (RH2M)	0.004126	0.005259	−0.000788	0.00069	0.000407	−0.000158
Root Zone Soil Wetness (GWETROOT)	0.229238	0.283766	−0.027523	0.02171	0.010888	−0.003766
Precipitation Corrected (PRECTOTCORR)	−0.029698	−0.035689	0.002254	−0.0016	−0.000687	0.000212
"Edad_no_anillos" variable	0.006924	0.006797	−0.000682	0.00566	0.007813	0.015973

*Concept: describing each term used by OLS with Ridge Model; State (Mexico or Puebla): it indicates the Mexican state in which the allometric equations were estimated; Method for Commercial Volumetric Dimension: it shows the method used to calculate the commercial volumetric dimension of trees (Newton; Theoretical –by logs– or by State); Explained variance percentage (R^2); Intercept: it refers to the intercept of the allometric equation, which is the value of the dependent variable when all independent variables are equal to zero; Total height (h), Normal diameter (DN) and other climatic variables: estimated coefficients for each of the independent variables included in the allometric model, indicating how each variable contributes to the calculation of tree volume.

the variance of the Ridge regression decreases, but the bias increases. In addition, in the least squares coefficient estimates, which correspond to the Ridge regression with $\lambda=0$, the variance is high but there is no bias. Also, the Paterson Productivity Index C.P.V. indicated that climate is one of the essential elements in forest production, tough Paterson limited his study to temperature, humidity, timing of the growth period and intensity of radiation, since climate is the main ecological factor on a regional scale and its influence is mainly expressed in changes in the physiognomy of vegetation and floristic composition (Walter, 1977; Petagna Del Río 1993; Gliessman *et al.*,1998).

Table 3. Estimates of the linear coefficients of significant climate variables in the period 2022-2024 for *Pinus hartwegii* Lindl in the states of Mexico and Puebla.

Climate variable	Linear coefficients estimated by Mexican state					
	México			Puebla		
	2022	2023	2024	2022	2023	2024
Earth Skin Temperature (C) (TS)	17.3631605	17.2562204	17.2075609	15.0913378	15.093867	15.09378
Temperature at 2 meters (C) (T2M)	16.0688180	15.9727000	15.9824568	13.9756634	13.9538095	13.9546881
Top-Of-Atmosphere Shortwave Downward Irradiance (TOA_SW_DWN)	34.0226381	34.0161901	34.0216112	34.2100035	34.2068739	34.2110193
All Sky Surface PAR Total (ALLSKY_SFC_PAR_TOT)	102.2113483	101.4544898	101.8056317	107.4957497	107.3481481	107.4148577
Clear Sky Surface PAR Total (CLRSKY_SFC_PAR_TOT)	122.7126980	122.6059464	122.5449116	125.1707133	125.4006844	125.3800238
Surface Pressure (PS)	77.2082785	77.2019272	77.2015360	76.6635899	76.6581183	76.6585532
Specific Humidity at 2 meters (QV2M)	8.4555264	8.5050894	8.4706968	9.2473988	9.2468102	9.2464988
Relative Humidity at 2 meters (RH2M)	61.9231341	62.2058595	62.3261776	72.2132697	72.4343616	72.074441
Root Zone Soil Wetness (GWETROOT)	0.4857650	0.4844374	0.4903292	0.4207275	0.4195527	0.4167194
Precipitation Corrected (PRECTOTCORR)	1.8141824	1.7827351	1.8955158	2.6239403	2.6892061	2.6296263

*Climate variable: name of the measured climate variable, such as temperature, solar radiation, atmospheric pressure, humidity, etc.; Linear coefficients estimated by Mexican state: numerical values represent the estimates of linear coefficients for each climate variable in the years 2022, 2023 and 2024, respectively, for the Mexican states of Mexico and Puebla.

Finally, regarding the estimation of timber volume for *Pinus hartwegii* Lindl based on linear allometric equations estimated by OLS with the Ridge Model for normal diameter, height and significant climatic variables, the simulations carried out with the linear diameter-height coefficients per tree located in the UMAFOR, and the significant climatic variables indicated a better volumetric approximation by Newton mathematical equations, and theoretical models (volume by timber unit, logs) for excurrent dendrometric types. According to Martínez-Luna *et al.* (2020) "if a coefficient of 50.81% is applied to the biomass calculated with the allometric equations for basal diameter or total height, it is sufficient to know the carbon content in a tree, a stand, or a plantation of *Pinus hartwegii* Lindl seedlings; therefore, its use is reliable." However, the adjusted model is of the where the dependent variable (Y) is biomass or carbon (in kilograms) and the independent variable is the normal diameter at 1.3 m height (DN) in centimeters determine that "the trees of this species concentrate most of the aerial carbon in the stem, followed by the branches and finally the foliage".

Based on the above, this research offers valuable information on the growth and yield of *Pinus hartwegii* forest in the states of Mexico and Puebla, in response to different environmental factors through the development of allometric equations. As well as the evaluation of carbon sequestration, which is key to understand the dynamics of the forest ecosystem facing climate change. The findings of this study are consistent with previous research that has highlighted the importance of understanding forest responses to environmental changes (Valderrama-Bonnet, 2009); the need for validated dynamic stochastic models to estimate allometric equations assessing carbon sequestration (Vargas-Larreta *et al.*, 2018; SEMARNAT, 2023); and the application of the principles of parsimony and simplicity in the selection of a model (Vanclay, 1994; Weiskittel *et al.*, 2011; Burkhart & Tomé, 2012; Barrera Pérez 2018; Vargas-Larreta *et al.* 2018).

However, this study has some limitations, because it was focused in the states of Mexico and Puebla; therefore, the results may not be generalized to other types of forests. In addition, the network of sampling sites in Mexico is not yet sufficient to draw complete conclusions about the growth of many species in stands under different treatments and at sites with different productivity (Corral *et al.*, 2014; Aguirre-Calderón, 2015; Vargas-Larreta *et al.* 2018). Finally, forestry research in Mexico still faces challenges in terms of data availability and development of models that are in fact widely used (Vargas-Larreta *et al.* 2018; SEMARNAT, 2023), thus providing a solid basis for future research to develop forest management strategies based on mathematical evidence.

CONCLUSIONS

The research achieved significant results using these statistical methods and data sources: SiBiFor databases and Power data from NASA methodology. Statistical models: Ordinary Least Squares (OLS), Ridge Model with regression, and Random Forest in R. Validation techniques: analysis of variance, F distribution theorem, residual validation graphs, statistical criterion R^2. Simulations: linear allometric equations estimated by OLS with Ridge Model, and incorporation of significant climatic variables.

It was accepted that linear regression models were significant and explained the variability of Y; and allometric equations of "commercial volumetric dimension" for *Pinus hartwegii* Lindl with significant climatic variables were estimated. The best precision in estimating the total volume of trees with bark was found with Newton traditional mathematical equations and estimated allometric equations in the states of Mexico and Puebla. The second better estimator were theoretical models (volume by timber unit, logs) for excurrent dendrometric types.

The allometric equation estimated by mathematical models with the best estimation of the TAV cc is theoretical models for excurrent dendrometric types (log volume) in the states of Mexico and Puebla with an evaluation of 90.5% and 95%, respectively. Thus, we obtained a better volumetric approach facing climate change with the Newton mathematical equations, and theoretical models for excurrent dendrometric types (volume per timber unit, logs).

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Colegio de Postgraduados

Traditional knowledge in an agroforestry system and perspectives about agroecological practices in Nahua communities

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ABSTRACT
Objective: To identify traditional knowledge associated to the traditional agroforestry system (TAFS) integrated by corn-coffee-allspice and to recognize perceptions and interests of Nahua communities towards the incorporation of agroecological practices.
Design/methodology/approach: Work was done in two Nahua communities of Cuetzalan, Puebla, Mexico, devoted to the production of corn (*Zea mays* L.), coffee (*Coffea arabica* L.) and allspice (*Pimenta dioica* Mill), in TAFS. A qualitative research approach was used, in which semi-structured interviews were conducted.
Results: A representative population sample was interviewed; 74% of the population were women, 38% was older than 65, 100% self-described as Nahua and recounted that maintenance of the TAFS is a family activity. Performing soil and organic matter conservation was manifested by 29%; 26% described promoting the diversity of species (cultivated and tolerated); 9% described establishing *Scaptotrigona mexicana* (endemic pollinator) in the plot; 65% knows an agroecological practice, and of this portion, 42% would like to use organic fertilizers, 41% considers that management of fertility and soil conservation are key to maintain the TAFS.
Limitations on study/implications: The findings reported are applicable to the context of Nahua communities in the Sierra Norte region of Puebla.
Findings/conclusions: There are traditional understandings associated to the TAFS. These have an approach of adaptation and emulation of local environmental characteristics to satisfy self-consumption. The approach of integral management of the agroecosystem tends to sustainability. The perception of the incorporation of agroecological practices is positive, and there is special interest in the management of soil fertility and conservation.

Keywords: traditional agroforestry system, sustainability, agricultural practices, perceptions.

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INTRODUCTION

Traditional knowledge is called popular wisdom, peasant knowledge, local understandings, among other; these understandings seek the recognition of unique cultures and identities to contribute solutions from a specific cultural approach (Argueta, 2016). Traditional knowledge is part of the immaterial cultural heritage and contributes to socioecological resistance, especially in small-scale agricultural systems. The participation of rural women especially contributes to the medicinal and nutritional sphere through daily practice (Ramírez-Santos *et al.*, 2019).

These understandings are related to the ways of thinking and to the territory of native peoples, which are constructed collectively. However, processes of acculturation and capitalist development threaten this knowledge, since the population adopts new cultural patterns, relegating their conservation and transmission (Argueta, 2016; Cervantes-Herrera *et al.*, 2016). Despite of this, the rural population shows interest in revaluing, recovering and disseminating agricultural traditional knowledge. In Latin America, in the 1990s, programs of agricultural extension work influenced by the Green Revolution could not adapt to the demands that emerged from globalization and the sustainability approach. Likewise, the interaction between agriculture and ecology decreased as a result of omitting and/or surpassing ecological principles (Arcilla-Moreno, 2020; Carnero-Avilés *et al.*, 2021). This disconnect between agriculture and ecology evidenced the importance of the transmission of these understandings, so channeling these efforts is required for the dissemination of traditional knowledge about agroforestry systems (Castillo-Arriaga and Jiménez-Osornio, 2020).

An expression of traditional agricultural technology corresponds to the traditional agroforestry system (TAFS), where biocultural diversity has developed; the practices associated to TAFS transcend history, even Pre-Hispanic, with multiple strategies for the use and management of diversity, associated to diverse social groups and ecosystems. In Mexico, various types of TAFS can be identified: low-intensity plot, medium-high intensity plot and garden, forest, medium-highly intensive plot, high-intensity plot and garden, intensive garden (Moreno-Calles *et al.*, 2013). Although agroforestry systems are identified as resilient and potentially appropriate systems for sustainable production, there remains a worry about their simplification and about technologies that are inadequately transferred to the environment and culture (Illescas *et al.*, 2020). In the Sierra Norte region of Puebla, Nahua peasants call the diversified TAFS that has followed the forest and native rainforest, "Kuojtakiloyan", which means "productive forest" in the Nahuat language. The species that make up the Kuojtakiloyan are not a product of the natural succession process (acahual), but the union of ideas and the work of the peasant in consonance with regional biological and ecological processes (Toledo, 2016). The promotion of technological and educational packages in native communities has failed because it does not consider integral development, from an environment of equality, dignity and respect for free determination (Hirose-López, 2018). Therefore, it is crucial to perform studies and proposals that involve traditional understandings, to improve them, to contribute to their rescue, and to fortify existing knowledge about them (Illescas *et al.*, 2020). The objective of this study was to identify traditional knowledge that contributes to the production of corn-coffee-allspice, as well as the perceptions, limitations and interests of Nahua communities towards the application of agroecological practices.

MATERIALS AND METHODS

The study was conducted in the Nahua communities of Yohualichan and Capola, Cuetzalan del Progreso, devoted to the production of corn (*Zea mays*), coffee (*Coffea arabiga*) and allspice (*Pimenta dioica*), through agroforestry systems (Figure 1). The community of Yohualichan has a total population of 602 inhabitants and in Capola there are 328 inhabitants (INEGI, 2020).

The quandary was addressed from a qualitative research approach, seeking to delve into information collection through interview as an instrument to approach the conceptions and meanings of people. The information was obtained through the application of semi-structured interviews, technique that allows responding with more flexibility and capturing

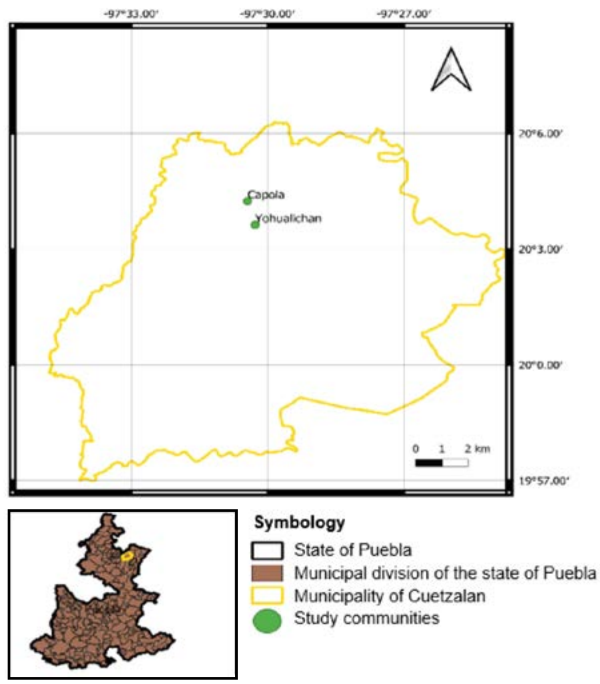

Figure 1. Study zone (Yohualichan and Capola, Cuetzalan del Progreso, Puebla). Prepared by the authors with vector information from INEGI (2020).

the detailed perception of the interview respondents (Velasco-Hernández *et al.*, 2016; Troncoso-Pantoja and Amaya-Placencia, 2017).

The semi-structured interview included the following sections: population profile, characteristics of the agroforestry system, traditional knowledge about the agroforestry system, perception about agroecological practices, and problems for the implementation of agroecological practices.

The target population was made up by men and women farmers with experience in corn, coffee and allspice farming for at least 10 years. The size of the population was 70 people, devoted to corn, coffee and allspice production in agroforestry systems in the communities of Yohualichan and Capola, Cuetzalan (Personal communication). A population sample of 34 people was estimated, with an accuracy of 10% and the normal distribution value of 1.65 and reliability of 90%, through the following formula (Castañeda-Guerrero *et al.*, 2020):

$$n=\frac{NZ^2Z^2_{a/2}P_nq_n}{Nd^2+Z^2_{a/2}P_nq_n}$$

N=total population of peasants involved in the corn-coffee-allspice agroecosystem; n=size of the sample population selected randomly; d=accuracy (10%); $Z^2_{a/2}$=reliability (90%)=1.65; P_n=0.5; q_n=0.5

The information collected was systematized in a database and analyzed through descriptive statistics.

RESULTS AND DISCUSSION

Profile of the population and characteristics of the agroforestry system

Of the people interviewed, 74% were women and 26% were men (Figure 2A). Within the age groups, the one that concentrated the greatest number of people is older than 65, followed by the group of 31 to 50 years of age (Figure 2B). The population interviewed has occupations in addition to farming, which are mostly domestic work and the elaboration of crafts (59% and 15%, respectively) (Figure 2C).

Of the population, 91% manifested they do not receive government backing to maintain the TAFS (Figure 2D); 94% responded that they had land of their own, although most have plots with a surface of <0.5 ha (Figure 2E and F).

All the population interviewed (100%) mentioned identifying themselves as Nahua and speaking Nahuat as native language, in addition to mentioning that two or more members of the family are involved in the maintenance tasks of the agroforestry system.

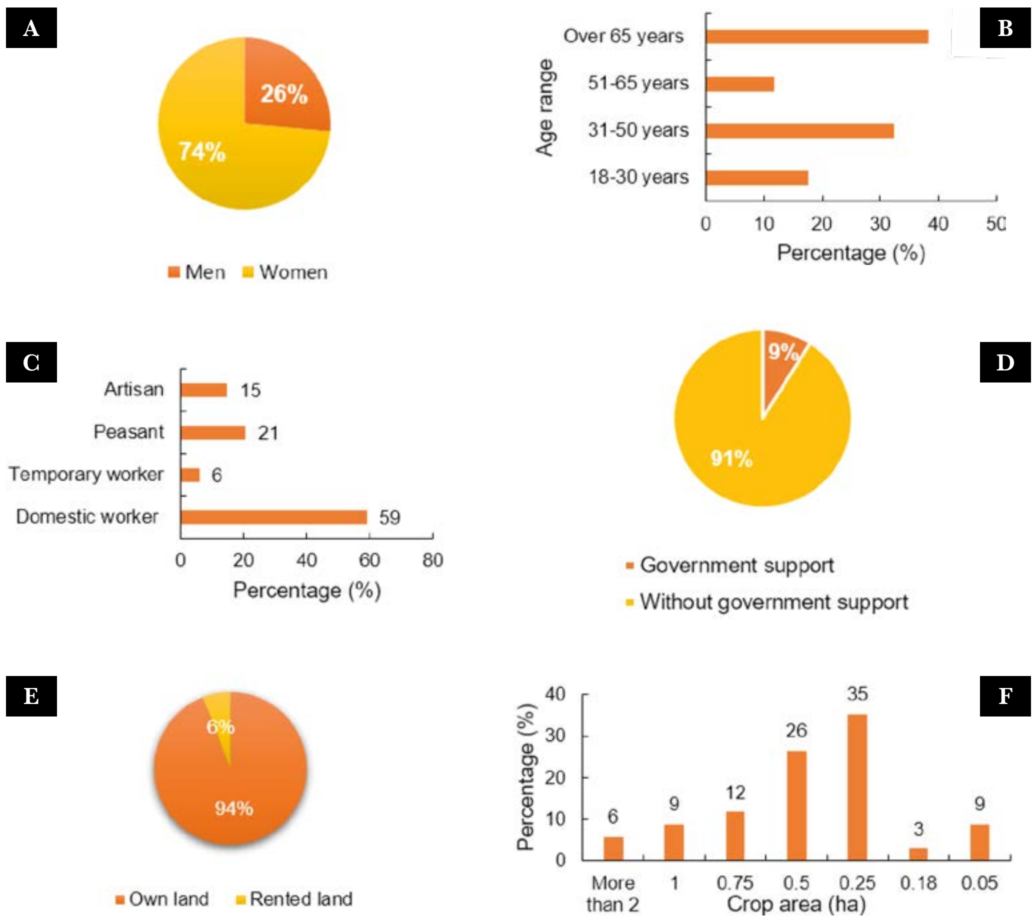

Figure 2. Profile of peasants that cultivated the corn-coffee-allspice agroforestry system in Nahua communities of Cuetzalan, Puebla. Field work 2022. A) Sex; B) Age; C) Additional occupation; D) Reception of government backing; E) Land ownership; F) Farming surface.

Practices associated to the management of the TAFS

As part of the ecological knowledge, the people interviewed described that they identify the Melipona bee (*Scaptotrigona mexicana*) or psilnekmej (in Nahuat), as a beneficial insect associated to the TAFS, because of its role as pollinator insect and honey producer. This confirms that the population interviewed identifies the beneficial action of pollinator insects on the productivity of agroecosystems and turns this knowledge into an agricultural practice associated to the conservation and sustainability of the agroecosystem (García-García *et al*., 2016; Collantes *et al*., 2023).

Stingless beekeeping in Mexico has pre-Hispanic origins as described in the Tro Cortesiano codex from Madrid where it is mentioned that Maya deities would conduct beekeeping practices of the stingless bee, *Melipona beecheii*. For their part, in the Sierra Norte region of Puebla, las Huastecas and Totonacapan, native populations designed pots to breed the *Scaptotrigona mexicana* bee. Nahua people classify insects between predators and reciprocal. In the predation group, parasites are identified which affect human beings and corn, among others. In the other extreme, there are beneficial insects, including bees, the only insect with a mystical relationship with people, demanding good behavior from inhabitants of the household, since they consider that their permanence depends on it (González-Acereto, 2012; Beaucage and Taller de Tradición Oral Totamachilis, 2017).

Considering that traditional understandings lead to agricultural practices associated to TAFS management, the people interviewed mentioned establishing the Melipona bee at the beginning of each production cycle in the plot or near it, as a way to improve production to favor pollination of the plants (Figure 3A and B).

There is evidence of the domestication of the Melipona bee by the Nahua people, who manage hives in two pots, one inverted over the other, with a small orifice (Beaucage and Taller de Tradición Oral Totamachilis, 2017). The communities that manage stingless bees identify their life form and their habitat, the time of day when they go out to feed, the places where they are found, and the plants that they prefer to collect nectar and pollen from: jonote (*Heliocarpus appendiculatus*), mahogany (*Swietenia macrophylla*), carboncillo (*Ocotea puberula*), black cherry (*Conostegia xalapensis*), orange (*Citrus* sp.),

Figure 3. Practices associated to management of the TAFS in Cuetzalan, Puebla. A) Melipona bee (*Scaptotrigona mexicana*), B) Establishment of the Melipona bee within the TAFS to favor pollination.

coffee (*Coffea* sp.), allspice (*Pimenta dioica*), chalahuite (*Inga* sp.), and guava (*Psidium* sp.) (Castillo-Hernández, 2022).

Similarly, it has been recognized that the development of stingless beekeeping helps to take care and propagate biodiversity of specific environments (Contreras-Cortés *et al.*, 2020); in this study, the interview respondents recognize that the species described before are part of the TAFS, which means that the communities have identified the relationship of mutualism between Melipona bees and some agricultural species and have strengthened this relationship through their integration in the composition of the Kuojtakiloyan (Toledo, 2016).

Likewise, the population interviewed described those seeds from perennial plants such as cinnamon, coffee and allspice, which germinate in the plot as part of the natural regeneration of the Kuojtakiloyan, are taken to the family nursery and then used to renovate plants or to occupy available spaces within the TAFS. This practice is constituted as a way to build self-sufficiency of the TAFS by decreasing the dependency on external inputs and where it is recognized that each plant is important, so the conditions for their development are generated (Figure 4 A-B). In addition, the people interviewed indicated that the ash that is produced by cooking with firewood, the manure produced by backyard animals (pigs and birds), the organic residues generated while cooking, in addition to the pulp of cherry coffee when it is processed, are taken to the plot, especially when the perennial plants are renovated (coffee, allspice, cinnamon) in the TAFS, incorporating these organic residues into the stumps. Through anthropological studies, it has been identified that in the states of Tlaxcala and Puebla, inhabitants contemplated the periodic incorporation of organic matter into agricultural systems, such as green fertilizers that are byproducts of the plot and manure obtained from domestic or wild animals (bats) and human feces, since 1000 BC (González-Jacome, 2016) (Figure 4C).

Figure 4. Practices associated to the management of the TAFS in Cuetzalan, Puebla. A) Natural regeneration of the Kuojtakiloyan, B) Family nursery used for the renovation of plants obtained through natural regeneration of the Kuojtakiloyan, C) Addition of organic residues as management practice of the TAFS in Cuetzalan, Puebla.

Farmers explained that they conduct activities for soil conservation through drains in the plot, and earthenware pots or individual terraces for perennial plants (*Coffea arabica*, *Pimienta dioica*, *Cinnamomum zeylanicum*, *Citrus* sp.), which are covered with fallen leaves or branches when the trees are pruned (Figure 5A). Terraces are an ancient system that was used since approximately 1700 BC, until 1200 years BC. In the year 1000 BC, the population of Tlaxcala increased the number of terraces, most of these managed only to farm and they were grouped with drains, dams, and reservoirs to decrease the speed of water and erosion (González-Jacome, 2016). Simultaneously, another practice identified is the conservation of organic matter that is produced in every productive cycle, that is, burning of the harvest residues is avoided, promoting their incorporation into the plot.

On the other hand, the people interviewed described that they promoted the high diversity of associated species (between those cultivated and tolerated) in the TAFS, with the following being the most common: coffee (*Coffea arabica*), allspice (*Pimienta dioica*), corn (*Zea mays*), bean (*Phaseolus vulgaris*), cinnamon (*Cinnamomum zeylanicum*), vanilla (*Vanilla planifolia*), plantain (*Musa balbisiana*), wild banana (*Musa rubra*), roatan banana (*Musa paradisiaca* var. cavendish), mamey sapote (*Pouteria zapota*) chicozapote (*Manilkara zapota*), white cedar (*Cupressus lusitanica*), tomato (*Solanum lycopersicum* L.), chiltepín (*Capsicum annuum* var. *glabriusculum*), chamaki (*Heliconia rostrata*), white pine nut (*Jatropha curcas*), valletilla or huitziquitenqui (*Hamelia patens*), chalahuite (*Inga* sp.), Persian lime (*Citrus latifolia*), orange (*Citrus sinensis*), mandarin (*Citrus reticulata*), lemon (*Citrus limon*), ataulfo mango (*Mangifera indica*) (Figura 5 B).

Finally, they mentioned that they avoid the application of agrochemicals, especially the use of herbicides, since according to their experiences, their use affects the emergence of medicinal plants and wild edible plants; the soil humidity is kept for less time because the plant coverage decreases, in addition to rainfall causing loss of soil when there is no plant cover. The traditional knowledge identified in the Nahua communities of Cuetzalan

Figure 5. Practices of soil conservation in Traditional Agroforestry Systems (TAFS) in Cuetzalan, Puebla. A) Terrace covered with fallen leaves and branches, B) Diversity of associated species.

is related to the ecological understanding and the management of the agroecosystem. According to Vázquez-Pérez *et al.* (2020), the systematization of knowledge and the compilation about traditional management favors the maintenance of biodiversity in the communities and their livelihoods.

Perception and problems of the implementation of agroecological practices

With the intention of identifying the assessment of the population of agroecological practices, there were questions about whether there is interest for the implementation of these practices, if there is prior knowledge about them, and the possible difficulties in the case of their implementation. According to the perceptions recorded, 65% has previous knowledge about the agroecological practices and has used at least one, 12% mentioned that although they know agroecological practices, they have not put them into practice, 24% said they ignore this type of practices or they do not think they are viable and therefore have not performed them (Figure 6A). Of the people interviewed that understand agroecological practices, 42% mentioned that they would be interested in promoting soil conservation practices to avoid soil erosion and keep its fertility, such as fertilizers (biofertilizers, composts, among others), level curves, construction of terraces, etc., 23% mentioned that they would like to know about alternatives to the use of agrochemicals, 19%

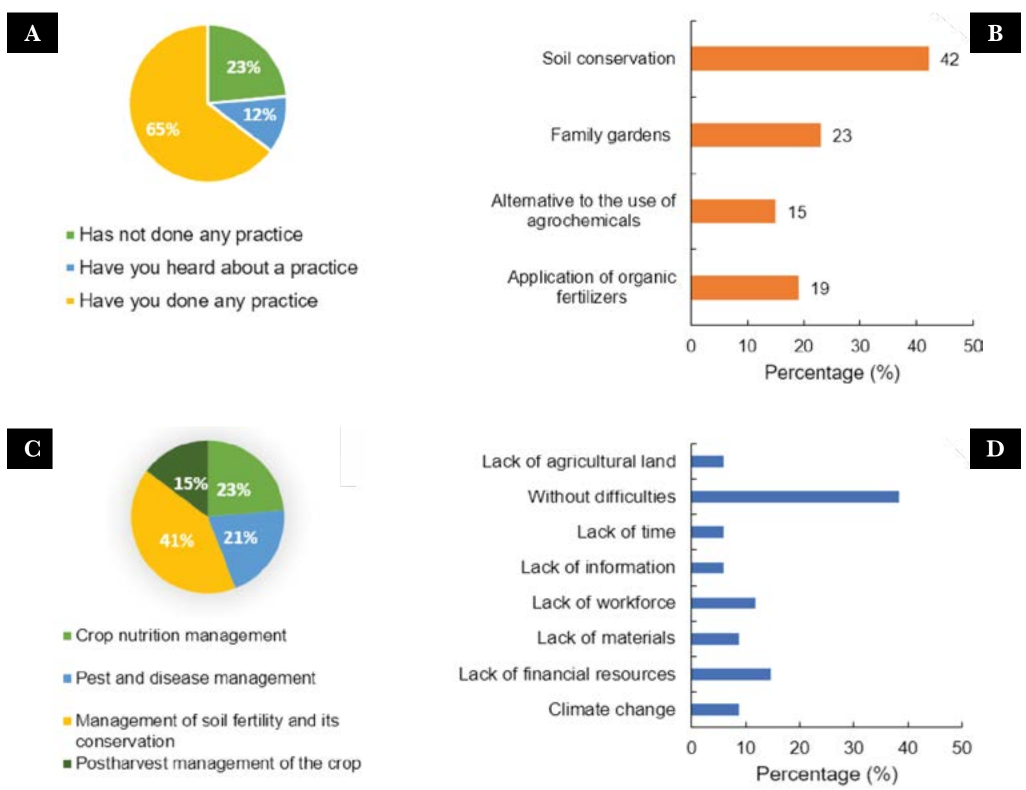

Figure 6. Perception about agroecological practices in Nahua communities of Cuetzalan, Puebla. A) Prior approach to an agroecological practice; B) Agroecological practice that they would like to know; C) Practices that are considered of more importance to maintain the productivity of the TAFS; D) Possible difficulties to face for the application of agroecological practices.

to know practices to improve plant nutrition, 15% want to know practices that strengthen the production of nursery plants since currently the demand for plants that are required in the TAFS are not reached through natural regeneration and they must resort to purchasing plants in local nurseries (Figure 6B).

These appraisals agree with what was reported in other communities of Puebla, where a good assessment about the effects of organic fertilizers on agricultural production is identified and, primarily, on the soil (Huerta-Muñoz *et al.*, 2018). Of the interview respondents, 41% consider that in order to increase and maintain the productivity of the TAFS, it is necessary to devote more time and resources for the management of soil fertility and conservation (Figure 6C). Lastly, some of the difficulties foreseen for the application of agroecological practices are the lack of resources, scarce workforce, and the effects of climate change (droughts, extreme temperatures, hurricanes, torrential rains) (Figure 6D); these aspects have been reported by other researchers as well (Reyes-Reyes *et al.*, 2020).

CONCLUSIONS

There is traditional knowledge associated to the practices that are conducted in the TAFS in Cuetzalan, Puebla, which have been transmitted in a general way, primarily between families and within each community. Some of the traditional practices that stand out are those associated to soil conservation, plant nutrition, and strengthening of symbiotic relationships. The interest in agroecological practices by inhabitants was focused primarily on the management of soil fertility and plant nutrition. There is a vision of Nahua peasants associated to maintaining the agroecosystem and its integral functioning and not only to the increase of productivity, so it could be considered as a vision linked to agricultural sustainability. It is important to continue focusing studies on the perspective of interests and the cultural and environmental pertinence for Nahua communities of Cuetzalan. The practices identified in the TAFS contribute to the construction of sustainable agricultural production, although more studies are required from a holistic view to strengthen their productivity and to maintain the stability of the agroecosystems.

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Estrus syncrhonization in ewes with prostaglandins at different days post CIDR insertion

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ABSTRACT

Objective: To evaluate the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) application on days 0, 3 and 6 of the synchronized luteal phases on reproductive perfomance and its effect on progesterone concentrations of multiparous ewes.
Design/methodology/approach: The experimental design was a completely randomized. Seventy-five ewes synchronized with control internal drug release devices (CIDR) for six days and 250 UI of equine chorionic gonadotropin (eCG) were used at device removal. The ewes were randomly distributed in three treatments in relation to the days of application of $PGF_{2\alpha}$ (125 μg sodium cloprostenol): in the first group of ewes, $PGF_{2\alpha}$ was applied at the time of insertion of the CIDR (D0, n=25); in the second group $PGF_{2\alpha}$ was applied on day three of insertion of the CIDR (D3, n=25) and in the third group it was applied on day six, at the time of the withdrawal of the CIDR (D3, n=25). The presentation of estrus and the gestation rate were analyzed through the χ^2 test. The onset of estrus and prolificacy by a Tukey analysis of variance and comparison of means. The concentration of P_4 was carried out using the mixed design procedure, which included fixed effects of treatment and day, and interaction of both.
Results: Presentation and onset of estrus were different ($P<0.05$) between treatments due to the effect of the interval in the days of $PGF_{2\alpha}$ application. The progesterone (P_4) concentrations in blood serum showed differences during the synchronized luteal phase (D0: 4.8 ng mL^{-1}; D3: 6.0 ng mL^{-1}, D6: 8.8 ng mL^{-1}). However, no differences were found in gestation rate and prolificacy due to the main effects.
Limitations on study/implications: The application of 125 μg of $PGF_{2\alpha}$ on different days of the synchronized luteal phase does not affect gestation rate and prolificacy. Nevertheless, presentation and onset of estrus were different, so it must be considered in laparoscopic artificial insemination programs.
Findings/conclusions: The application of $PGF_{2\alpha}$ during the synchronized luteal phase at short intervals showed better results at the end of treatment. The corpus luteum (CL) and CIDR increase P_4 concentrations; which modifies the estrus response, and it has a positive effect in reproductive performance.

Keywords: Estrus, synchronization, CIDR, Prostaglandin $F_{2\alpha}$.

INTRODUCTION

Hormonal protocols manipulate physiological moments of the estrous cycle and allow for the presence of estrus in a large number of ewes within a short and defined period, thereby increasing flock fertility (Arya *et al.*, 2023); however, factors such as photoperiod, nutrition, breed, facilities, and other elements affecting animal welfare must be considered (Simões *et al.*, 2021).

In ewes, estrus synchronization protocols primarily involve the use of controlled internal drug release devices (CIDR) for 12 to 14 days to simulate the luteal phase (Hameed *et al.*, 2021). However, when combined with gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), equine chorionic gonadotropin (eCG), and prostaglandins ($PGF_{2\alpha}$), the estrus response is enhanced (González *et al.*, 2012). In this context, some researchers have reduced the exposure time to progestogens, proposing short-term synchronization protocols (5 to 7 days) aimed at synchronizing both estrus and ovulation, as well as the follicular wave (González-Bulnes *et al.*, 2020), which allows for higher fertility compared to conventional protocols during the breeding season (Takci and Kivrak, 2022).

Furthermore, the use of progestogens in synchronization protocols combined with $PGF_{2\alpha}$ for a short period is an economical and flexible alternative for field conditions, yielding promising fertility results (Sinimbu *et al.*, 2022). The use of $PGF_{2\alpha}$ is effective for inducing luteal regression in most ewes, with variability observed in the occurrence of estrus and ovulation (Tsai and Wiltbank, 1997). This variability in response is attributed to the timing of $PGF_{2\alpha}$ administration, as the corpus luteum only responds between days 3 and 14 of the estrous cycle (Rubianes *et al.*, 2003). Conversely, it has been reported that the use of $PGF_{2\alpha}$ at short intervals may be an appropriate alternative for synchronizing estrus and performing artificial insemination (AI) in ewes (Contreras-Solís *et al.*, 2009). The present study was conducted with the assumption that the application of $PGF_{2\alpha}$ on different days during a short synchronization protocol (6 days) in pre-synchronized ewes would allow for the identification of changes in reproductive behavior by lysing the corpus luteum (endogenous source of P_4) at the time of CIDR insertion (CL at 3-4 days), three days after CIDR insertion (CL at 6-7 days), or at the time of CIDR withdrawal (CL at 9-10 days). This would explain whether the exogenous source (CIDR) alone is capable of maintaining a sufficient level of P_4 to improve reproductive variables and sustain gestation.

MATERIALS AND METHODS

This study was conducted in October-November 2022 at the Sheep Experimental Unit of the Colegio de Postgraduados, Montecillo Campus, State of Mexico (19° 27' 18" N and 98° 54' 26" W), at an altitude of 2,220 meters above sea level; the climate is temperate subhumid with rainfall in the summer.

Animal management was conducted in accordance with the ethical and biosecurity standards of the Council for International Organizations of Medical Sciences (CIOMS, 1986), in compliance with Mexican law (NOM-062-ZOO-1999) for the use of animals in experimentation (DOF, 2001), and with regulations for the use and care of research animals, approved by the Animal Welfare Committee of the Colegio de Posgrados, Mexico (COBIAN/007/23).

Animals and Treatments

During the breeding season, 75 multiparous ewes of the Dorset × Katahdin cross were used, with an average weight of 50±2.2 kg and an average body condition score of 3 on a scale of 1 to 5. The ewes were previously dewormed and vitaminized, and an ultrasound was performed to confirm that they were not pregnant (CHISON, Eco 6). The ewes were fed *ad libitum* on oat hay (*Avena sativa*) and given 300 g of commercial feed containing 14% crude protein (CP) and 2.4 Mcal kg^{-1} of metabolizable energy (ME), according to the nutritional requirements for sheep (NRC, 2007), in addition to free access to water. All ewes were pre-synchronized with two doses of prostaglandin $F_{2\alpha}$ (125 μg sodium cloprostenol, Celosil®) at an 8-day interval. Six days after the second dose of this hormone, CIDR devices were inserted to control the lifespan of the CL.

For estrus synchronization, the CIDR device (Zoetis®) was used for 6 days, along with the application of 250 IU of eCG (Gonactive®, Virbac) at the time of its removal. Subsequently, the ewes were randomly distributed into three treatments based on the days of $PGF_{2\alpha}$ (125 μg sodium cloprostenol) application: the first group of ewes received it at the time of CIDR insertion (D0, n=25), the second group received it on day three after CIDR insertion (D3, n=25), and the third group received it on day six, at the time of CIDR removal (D6, n=25).

Estrous presence was determined 24 hours after CIDR removal. The rams were randomly assigned by treatment, and controlled mating was carried out, with each ram mating each ewe two times. The first mating occurred upon detection of estrus, followed by one additional mating 12-hour later. Return to estrus was detected between 14 to 17 days after mating, twice a day (morning and afternoon). Pregnancy was confirmed 30 days post-mating via transrectal ultrasonography.

Sample Collection and Laboratory Analysis

Blood samples (5 mL) were collected via jugular vein puncture at 8:00 a.m. To determine serum P_4 concentration, samples were collected one day before CIDR insertion and subsequently every 48 hours for 17 days. All samples were centrifuged at 1500 g at 5 °C for 20 minutes (International Equipment Company, USA); the blood serum was separated and stored in 1.5 mL microtubes (Axigen™) for preservation at −20 °C in a freezer until hormonal analysis was performed. The P_4 analyses were performed using ELISA with the kit (DRG® Progesterone ELISA). The analytical sensitivity was 0.045 ng/mL, and the intra- and inter-assay coefficients of variation were 7% and 9%, respectively.

Statistical Analysis

The experimental design was completely randomized, where each ewe represented an experimental unit. The percentage of estrus occurrence and pregnancy rate were analyzed using the χ^2 test through PROC NPARIWAY. For the onset of estrus and prolificacy index, an analysis of variance was conducted using PROC GLM and a Tukey's mean comparison test ($P<0.05$). For the concentration of P_4, a repeated measures analysis of variance over time was performed using PROC MIXED, which included fixed effects of treatment and day, as well as their interaction. All procedures were performed using the Statistical Analysis System (SAS, 2009).

RESULTS AND DISCUSSION

Estrus Presentation and Onset

In the present study, the presentation of estrus differed among treatments ($P<0.05$) due to the timing of $PGF_{2\alpha}$ administration, with 100% observed in the D3 and D6 groups, compared to 60% in the D0 treatment. The onset of estrus also differed among treatments ($P<0.05$) based on the day of $PGF_{2\alpha}$ application, with early estrus observed in the D0 and D3 groups compared to the D6 group (Table 1).

The D0 group exhibited a lower percentage of estrus (60%, $p<0.05$) compared to the D3 and D6 treatments, which can be attributed to the presence of an immature CL in the females that did not show estrus. In this regard, Wiltbank *et al.* (1995) have mentioned that $PGF_{2\alpha}$ does not affect the development and lifespan of the CL when administered before day 5 post-estrus, attributing this to the absence or low presence of receptors in luteal cells; this is like the D0 treatment and is corroborated by the P_4 concentrations following $PGF_{2\alpha}$ administration in this group of ewes (Figure 1).

The percentages of estrus in the D3 and D6 treatments are similar to those obtained by Contreras-Solís *et al.* (2009) and Balan-May *et al.* (2021), who mention that administering $PGF_{2\alpha}$ to ewes on days 3, 5, and 7 of the estrous cycle can achieve up to 100% estrus. Regarding the onset of estrus, various studies reported that approximately 90% occurs within a 26 to 72 h interval after the removal of the CIDR (Rubianes *et al.*, 2003; Contreras-Solís *et al.*, 2009). However, in the present study, the onset of estrus was influenced by the timing of $PGF_{2\alpha}$ administration during the luteal phase synchronized with the CIDR, as $PGF_{2\alpha}$ causes early regression of the CL and consequently decreases the concentration of P_4 in serum.

Contreras-Solís *et al.* (2009) and Balan-May *et al.* (2021), when synchronizing with $PGF_{2\alpha}$ on days 3, 5, and 7, observed an onset of estrus ranging from 28.0 ± 3.1 h to 38.6 ± 3.3 h, with the earliest onset occurring on day 3, which is similar to the findings of this study. In contrast, Urviola *et al.* (2005) reported an onset of estrus of 34.28 ± 4.26 h and 47.4 ± 7.6 h when administering $PGF_{2\alpha}$ on days 4 and 10 of the estrous cycle. This

Table 1. Response of reproductive variables in ewes synchronized with CIDR for a short period (6 days) with the application of $PGF_{2\alpha}$ on days 0, 3, and 6 during the luteal phase.

Reproductive variable	Treatments		
	D0 (n=25)	**D3 (n=25)**	**D6 (n=25)**
Estrus presentation (%)	60 (15/25)[b]	100 (25/25)[a]	100 (25/25)[a]
Estrus onset (h)[†1]	26.40 ± 1.38^{b}	27.60 ± 1.07^{b}	39.84 ± 1.07^{a}
Gestation (%)[2]	48 (12/25)	72 (18/25)	60 (15/25)
Prolificacy index[†3]	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1

[1] Time referred to the withdrawal of the device. [2] Based on serum P_4 profiles and ultrasound on day 30. [3] Number of lambs born per ewe that lambed. [a, b] Values with different letters between columns are different ($P<0.05$). [†] Means ± standard error.

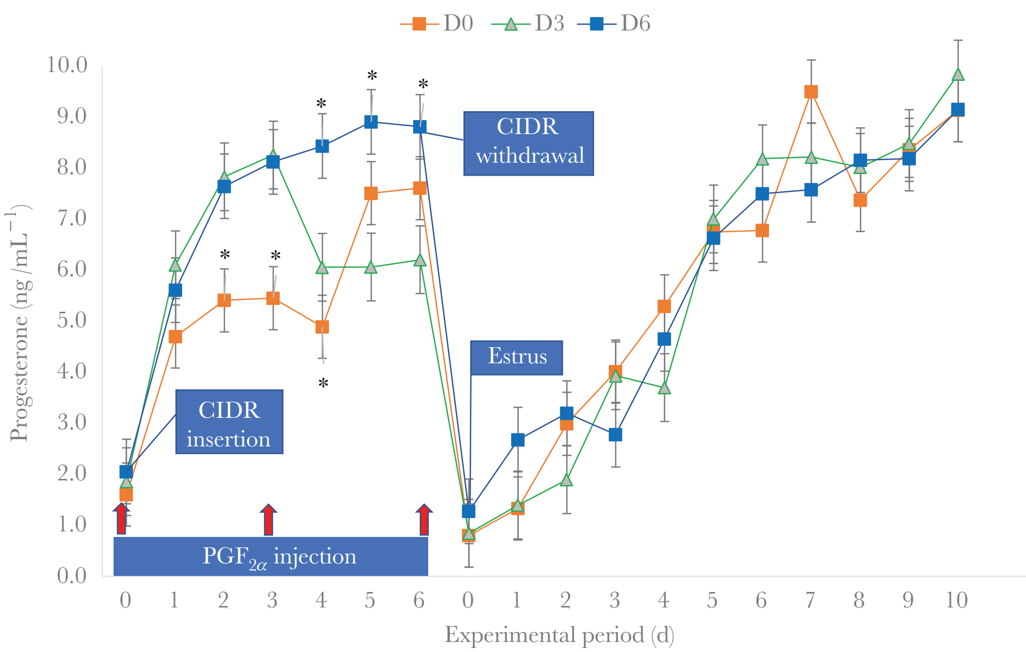

Figure 1. Plasma progesterone concentration (mean ± standard error) during the experimental period in multiparous ewes. * Indicates statistical difference between experimental groups.

variability is attributed to the state of the dominant follicle of the first follicular wave and the developing CL in relation to the timing of synchronization. Champa (2000) reported that estrus and ovulation are less variable during the early luteal phase than during the late luteal phase; that is, when $PGF_{2\alpha}$ induces regression of the CL at earlier stages of its development (Urviola *et al.*, 2005).

Hormonal Profile of Progesterone (P_4)

The concentrations of P_4 in serum were different ($P<0.05$) during the synchronized luteal phase (Figure 1). The results obtained in this study show a higher concentration in the D6 group (8.8 ng mL^{-1}) compared to D3 (6.0 ng mL^{-1}) and D0 (7.5 ng^{-1}). Wheaton *et al.* (1993) reported that serum P_4 concentration rapidly increases after CIDR insertion and decreases after its removal. In the present investigation, P_4 concentrations were above 1 ng/mL prior to device insertion due to prior synchronization and the development of different stages of the CL; the variations in the P_4 curve during the synchronized luteal phase in the D0 and D3 treatments indicated CL lysis following the administration of $PGF_{2\alpha}$ (Figure 1). However, in all three groups, levels fell below 1 ng mL^{-1} 24 h after the CIDR was removed.

Various authors mentioned that the presence of CL and synchronization with CIDR in sheep increases serum P_4 concentrations; Molina-Mendoza *et al.* (2005) reported 8.6 ng mL^{-1} in a group of sheep synchronized for 12 days with the presence of CL; Campero *et al.* (2023) found concentrations of 8.2 ng mL^{-1} in sheep synchronized for 6 days and 10 ng mL^{-1} in sheep synchronized for 12 days, both treatments with the presence of CL. On the other hand, Cordero *et al.* (2023) observed that primiparous ewes have higher concentrations than multiparous ewes.

The increase in P_4 concentrations due to the presence of CL in synchronization protocols must be considered as it modifies the onset of estrus; therefore, $PGF_{2\alpha}$ should preferably be administered before the removal of the CIDR.

Gestation Rate and Prolificacy

The gestation rate showed no differences ($P>0.05$) in the present study; it was 48% for the D0 treatment, while for D3 it was 72%, and for D6 it was 60%. These rates are lower than those reported by Balan-May *et al.* (2021), with 70% and 90% for synchronization protocols in short periods, but similar to those reported by Urviola *et al.* (2005), with gestation rates of 63.6% and 65.0% in ewes synchronized with $PGF_{2\alpha}$ on days 4 and 10 of the estrous cycle; in both studies, the ewes received direct mounting with proven fertile rams. For their part, Contreras-Solís *et al.* (2009) reported rates of 62.5%, 44.0%, and 47.4% in ewes inseminated and synchronized with $PGF_{2\alpha}$ on days 3, 5, and 7, respectively.

Cordero *et al.* (2023) reported a 100% gestation rate in primiparous and multiparous ewes with short synchronization protocols using CIDR (6 days); meanwhile, Ávila *et al.* (2019) mentioned that treatments with $PGF_{2\alpha}$ are effective during the reproductive season, whether in double applications with intervals of 8 days, combined with progestogens, or with GnRH for 6 days. Campero *et al.* (2023) established that regardless of whether a functional CL is present or not, when initiating estrous synchronization in short periods, it is necessary to administer prostaglandin $F_{2\alpha}$ at the end of the treatment to avoid the negative effect of the CL on the estrous response and to achieve steroid feedback.

In the present study, the prolificacy index did not show differences between treatments ($P>0.05$), although it is similar to that reported by Balan-May *et al.* (2021), with prolificacy rates of 1.42, 1.44, and 1.55 for hormonal treatments with $PGF_{2\alpha}$ applied on days 3, 5, and 7. Similarly, Almadaly *et al.* (2023) mentioned that the application of $PGF_{2\alpha}$ on days 7 and 14 results in prolificacy indices of 1.3 and 1.6, respectively.

CONCLUSIONS

The luteolysis triggered by the application of $PGF_{2\alpha}$ at the time of CIDR insertion limits the increase of P_4 during the first 4 days in short synchronization protocols (6 days) in ewes. Meanwhile, the application of $PGF_{2\alpha}$ at 3 days post-CIDR insertion reduces and limits the peak of P_4 concentration, which implies that the presence of the CL exerts a positive effect in synchronization protocols, as lower concentrations of P_4 have been associated with a negative effect on reproductive variables.

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Colegio de Postgraduados

Potential Distribution Models for Predicting Human-Black Bear (*Ursus americanus* var. *eremicus*) Interactions in the Sierra de Zápaliname Natural Reserve, Saltillo, Coahuila

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ABSTRACT

Objective:This research analyzed the interactions between humans and bears in the ejidos of the Sierra de Zápaliname Natural Reserve and the urban area of Saltillo, Coahuila, Mexico.

Design/Methodology/Approach: A database of approximately 481 georeferenced records of Human-Black Bear Interactions (HBI) for the year 2021 was used. Based on these records and their relationship with physiographic variables (slope, aspect, elevation, roughness) and environmental variables (normalized difference vegetation index and a humidity detection index), potential distribution models were developed using the support vector machine algorithm, which is characterized by the assumption of the Greenelian niche theory.

Results: Six potential distribution maps were generated, divided into two rainy and dry periods and three types of interaction: sightings, agricultural-livestock conflicts and captures.

Findings/Conclusions: In all models, a common pattern was found where the sites with the highest probability of IHO are places close to human settlements and areas where agriculture, livestock and landfill activities occur.

Keywords: Human-Bear Interactions, Spatial Analysis, Wildlife Management, Mapping Techniques, activity Patterns.

INTRODUCTION

The Black Bear (*Ursus americanus*) is a species with a broad ecological niche, which has allowed it to distribute across a variety of habitats, such as pine forests and deserts (Garshelis, 2022). This adaptability characterizes the Black Bear as an umbrella species (a

species that, when protected, also protects many others due to its need for large areas to thrive) in the ecosystems of the northern part of the country (Isasi-Catalá, 2011). Despite the importance of the Black Bear in Mexico, its populations are drastically declining, primarily due to land-use changes and habitat loss from fragmentation. This not only affects the Black Bear but also the distribution areas of other carnivores, such as the puma and the jaguar (Balbuena-Serrano *et al.*, 2022). According to NOM-059-SEMARNAT-2010, the Black Bear is classified as "Endangered" within the Mexican Republic and "Subject to special protection" in some populations in northern Coahuila.

Human-Black Bear interactions (HBI) are becoming increasingly frequent in areas of livestock and agricultural production, where the control of the Black Bear has primarily been through hunting (Nuñez-Torres *et al.*, 2020). The main issue with these interactions is that bears alter their behavior by consuming food from anthropogenic sources and become habituated to the presence of human settlements, which increases their interactions (Baruch-Mordo *et al.*, 2014). These HBIs intensify during the summer and fall when bears enter a state of hyperphagia and, therefore, need to maximize food intake to increase their fat reserves before hibernation (McFadden-Hiller *et al.*, 2016).

A useful tool for mapping Human-Black Bear interactions (HBI) and determining potential distribution areas are ecological niche models, which are characterized by analyzing environmental variables that, along with occurrence sites (observations of the bear and HBI), make it possible to calculate the probability of the observation occurring in another location (Escobar-Flores and Sandoval, 2022). Ecological niche models are a vital source of information for planning wildlife management strategies and have currently been applied to identify priority areas for the conservation of critically endangered species, such as the Golden-headed Lion Tamarin (*Callithrix flaviceps*) (Bataillard *et al.*, 2024), or to identify the habitat connectivity of the Black Bear in mountainous and desert valleys in northern Mexico (Lara-Díaz *et al.*, 2021).

The mere presence of the Black Bear has been a catalyst for the creation of new protected natural areas. A clear example of this is northern Coahuila, where the protection of large tracts of land, including the mountains bordering the United States, Big Bend National Park, and Black Gap in Texas, has maintained the natural biological corridor for the Black Bear (McKinney *et al.*, 2006). This research focused on the recent protected natural area of Sierra de Zápaliname, Coahuila, where over 481 records of Human-Black Bear interactions (HBI) have been documented in the last 10 years; it is also considered one of the mountains with the greatest potential habitat for the Black Bear in Mexico (Monroy-Vilchis *et al.*, 2016).

The hypothesis of this study is that potential Human-Black Bear interaction (HBI) areas will depend on the hyperphagia period (May-September), as previously reported for other areas of Black Bear distribution (Baruch-Mordo *et al.*, 2014; Nuñez-Torres *et al.*, 2020). Therefore, areas with the greatest potential habitat for the Black Bear are expected to be located in urban, agricultural, and livestock areas. It is anticipated that during the rainy season, when food is abundant in the pine forest, the potential distribution areas of HBI will shift to the forested and mountainous regions.

MATERIALS AND METHODS

The research was conducted in the protected natural area of the Sierra de Zápaliname State Natural Reserve (Figure 1), established on October 15, 1996, due to its hydrological importance, as it provides more than 30% of the drinking water consumed by the city of Saltillo. The mountain range is located at the following coordinates: latitude 25° 15' 00"-25° 25' 58.35" and longitude 100° 47' 14.5"-101° 05' 3.8", covering an area of 25,768.68 hectares. The Sierra de Zápaliname is part of the Gran Sierra Plegada subprovince, characterized by valleys, plains, and folded elevations. At elevations above 2600 m, forests predominate, while at lower elevations, there are oak-pine forests, oak forests, and chaparral vegetation (Encina-Domínguez *et al.*, 2008).

Human-Black Bear Interaction Records

The records and databases used in this study were obtained from the following sources and correspond to the year 2021: 1) database obtained from the Wildlife Division of the Secretary of Environment (SMA) of the Government of the State of Coahuila, 2) database from the Wildlife Management area of the Mexican Wildlife Protection Association (PROFAUNA), and 3) fieldwork in collaboration with the Secretary of Environment of the State of Coahuila. The data will be divided into three categories of Human-Black Bear interactions (HBI): The first category is called sightings, with 143 records; captures of bears, with 37 records; and agricultural conflicts, with 301 records.

Physiographic Variables

Digital Elevation Models (DEMs) were downloaded from the U.S. Geological Survey (USGS) Global Visualization Viewer (http://glovis.usgs.gov) with a spatial resolution of 30

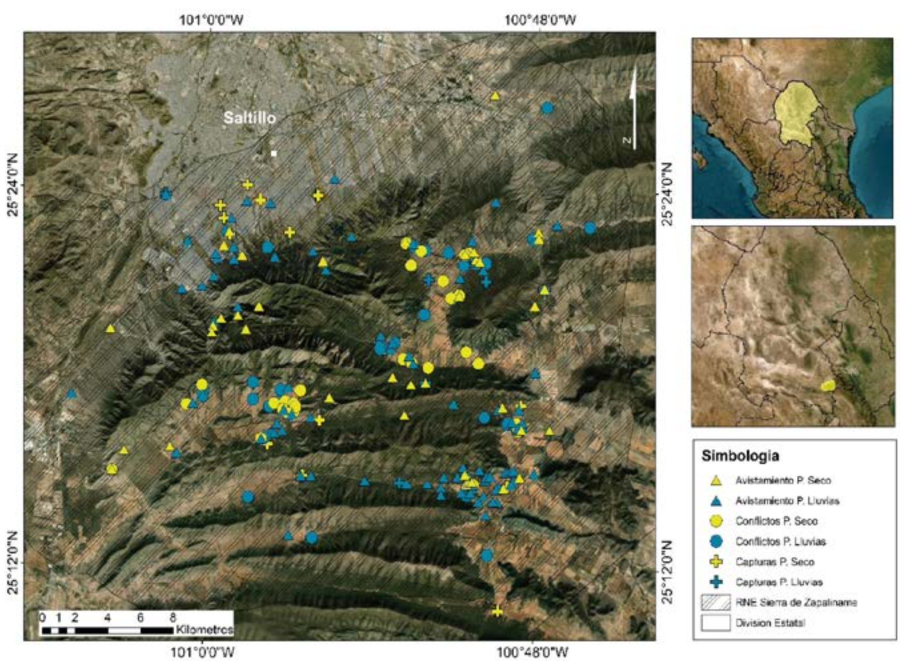

Figure 1. Location of the Sierra de Zápaliname State Natural Reserve and the records of Human-Black Bear interactions (HBI).

m. From the DEMs, four topographic variables were generated using the spatial analysis tools available in ArcGIS 10.8 as follows: (1) elevation above sea level (m); (2) slope (0-90°); (3) vector ruggedness measure (VRM), a measure of terrain roughness, with values ranging from 0 for flat areas to 1 for canyons and ravines (Sappington *et al.* 2007); (4) terrain curvature.

Environmental Variables

The land cover layer for the study area was obtained with a spatial resolution of 10 meters for the year 2021 from the website https://livingatlas.arcgis.com/landcover/. The classification was performed by Karra *et al.* (2021), who used six bands from the Sentinel-2 satellite (red, green, blue, NIR, SWIR1, SWIR2) of surface reflectance-corrected images. These authors propose nine land cover classes, which are presented in the following Table 1.

Two other environmental variables were also obtained: the Normalized Difference Vegetation Index (NDVI) proposed by Rouse *et al.* (1974) and the Normalized Water Index proposed by McFeeters (1996).

To calculate these indices, two Sentinel-2 satellite images with a resolution of 10 meters were first obtained and processed from the United States Geological Survey portal (www.glovis.usgs.gov). The dataset was acquired on November 3, 2021, representing the end of the rainy season, and on May 2, 2021, representing the end of the dry season. These two seasons were chosen to compare the effect of seasonality on the photosynthetic activity of vegetation and moisture.

The NDVI uses the proportions of absorption in the red region (R) and the reflectance of vegetation cover in the near-infrared region (NIR). The NDVI was calculated with the following equation:

$$NDVI = (NIR - R)/(NIR + R)$$

NDVI values ranged from −1.0 to 1.0. On the other hand, the NDWI maximizes the reflectance of water bodies in the green band and minimizes the reflectance of water in the

Table 1. Land cover classes proposed by Karra *et al.* (2021).

ID	Clasification
1	Water
2	Forest
4	aquatic vegetation
5	Crops
7	Urban
8	Bare ground
9	Snow
10	Clouds
11	grasslands

NIR band. The NDWI was calculated using bands three and eight of the Sentinel 2A-MSI sensor. NDWI values >0.10 indicated a probable water body (Escobar-Flores *et al.*, 2019).

Modeling the Potential Distribution of Human-Bear Interactions (HBI)

Based on the georeferenced records of HBI, three groups of presence data were generated for modeling: 1) sightings, 2) captures, and 3) Human-Bear conflicts. The input layers prior to modeling included the four physiographic variables and the three environmental variables, and a common spatial resolution of 30 meters per pixel was defined. The modeling was performed using the open-source program OpenModeller, utilizing the Support Vector Machine (SVM) algorithm. This algorithm is based on Hutchinson's ecological niche theory (Takola and Schielzeth, 2022) and is grounded in statistical learning theory, producing good classification results from complex and noisy data. It separates classes with a decision surface that maximizes the margin between them. This surface can be referred to as the optimal hyperplane, and the data points closest to this hyperplane are called support vectors. The pre-modeling parameters included a gamma type function with a polynomial kernel. The polynomial degree was set to 3, meaning that at least three lines are generated before calculating the potential distribution (Drake *et al*. 2006).

For each model, the area under the receiver operating characteristic curve (AUC) was calculated. AUC values greater than 0.70 were considered to distinguish between observed locations and potentially unsampled ones (Ferson *et al.*, 2000). The model is represented in a map of suitable habitat probability with values ranging from 0 to 1.

RESULTS AND DISCUSSION

The potential distribution models obtained from the different Human-Bear Interactions (IHO) were effective, with AUC values ranging from 0.80 to 0.88 for rainy seasons and from 0.92 to 0.96 for dry seasons. These AUC values indicate that the study area has a high probability of suitable habitat for the Black Bear. These results are consistent with those reported by Monroy-Vilchis *et al.* (2016; Figure 2) in their research on the potential habitat for the Black Bear in Mexico. There was also agreement with Lara-Díaz *et al.* (2020) and Balbuena-Serrano *et al.* (2022; Figure 1), which describe the main ecological corridors for the Black Bear in Mexico, where Sierra Zapaliname is part of the corridor in the Sierra Madre Oriental.

In the model corresponding to the rainy season, it is observed that the urban area in the south of Saltillo has probability values close to 0.80 (Figure 2), indicating a greater likelihood of bear sightings during the hyperphagia period (Nuñez-Torres *et al.*, 2020). This suggests that the high availability of food may be encouraging bears in this region to extend their hyperphagia period possibly until December.

The increase in bear sightings in the urban periphery may raise the incidence of bear-vehicle collisions on roads, as reported by Zarco-González *et al.* (2023), where they identify that roads such as ring roads and intersections where cars travel at higher speeds have a greater probability of collisions. This concern is evident in the study area because there is a ring road that borders the Sierra de Zapaliname and the city of Saltillo.

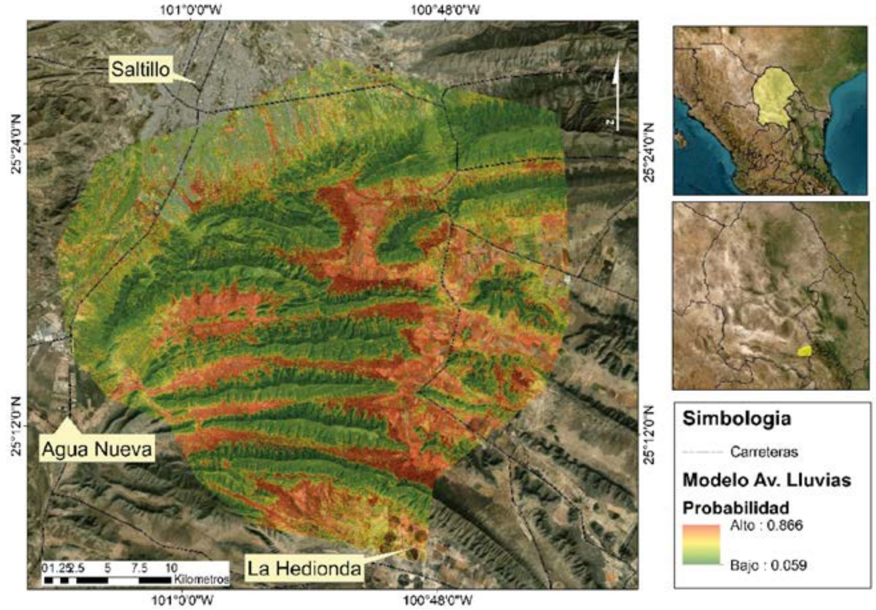

Figure 2. Potential distribution model of Black Bear sightings during the rainy season.

In the dry period (May), the probability of bear sightings in urban areas drastically decreased compared to the rainy season. Habitat suitability values were very low, below 0.1, while values greater than 0.8 were found entirely in the forested region of Sierra de Zapaliname, where bears may be in the hibernation or pre-hyperphagia stage (Johnson *et al*., 2015) (Figure 3).

Regarding the capture model during the rainy season, the model shows a distribution similar to that of sightings, where sites with higher probabilities (>0.8) are common in

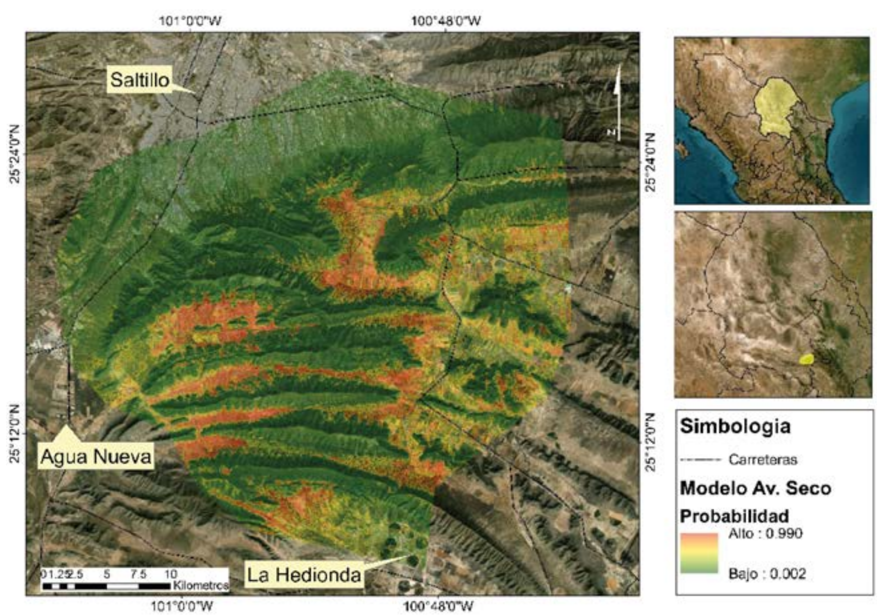

Figure 3. Potential distribution model of Black Bear sightings during the dry season.

urban areas, a result that is similar to that reported by Lackey *et al.* (2018) in various studies from the United States (Figures 4 and 5). In the dry season, the conflict models show a potential distribution focused on the pine-oak forest (Figure 5), which possibly indicates a lower Human-Bear Interaction (HBI) in urban areas (Valdez and Ortega, 2014).

In the capture models, it was found that slopes less than 10 degrees accounted for 100% of captures, regardless of the dry or rainy season. Despite the discrepancy with publications and reports from other areas of bear distribution in Mexico, which indicate that bear

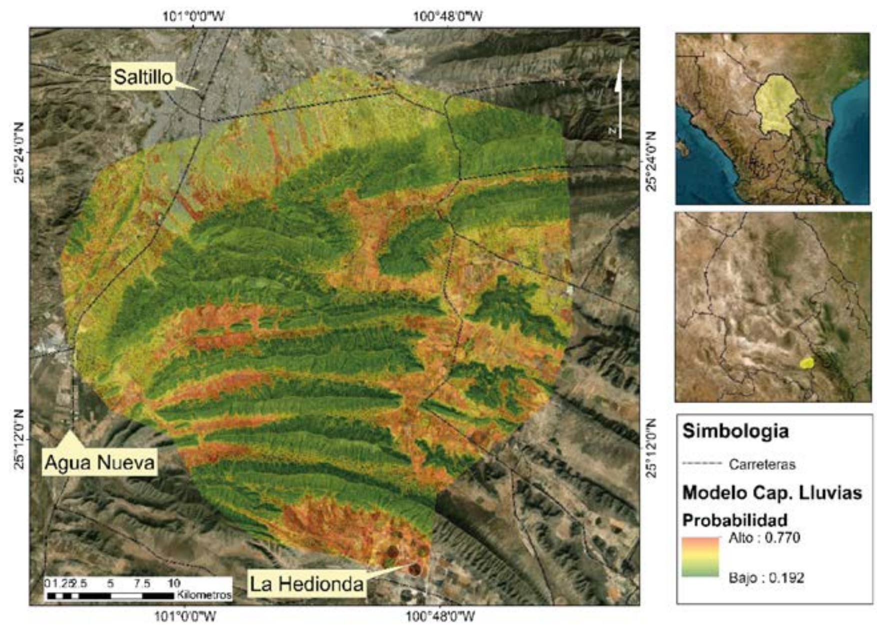

Figure 4. Potential distribution model of Black Bear captures during the rainy season.

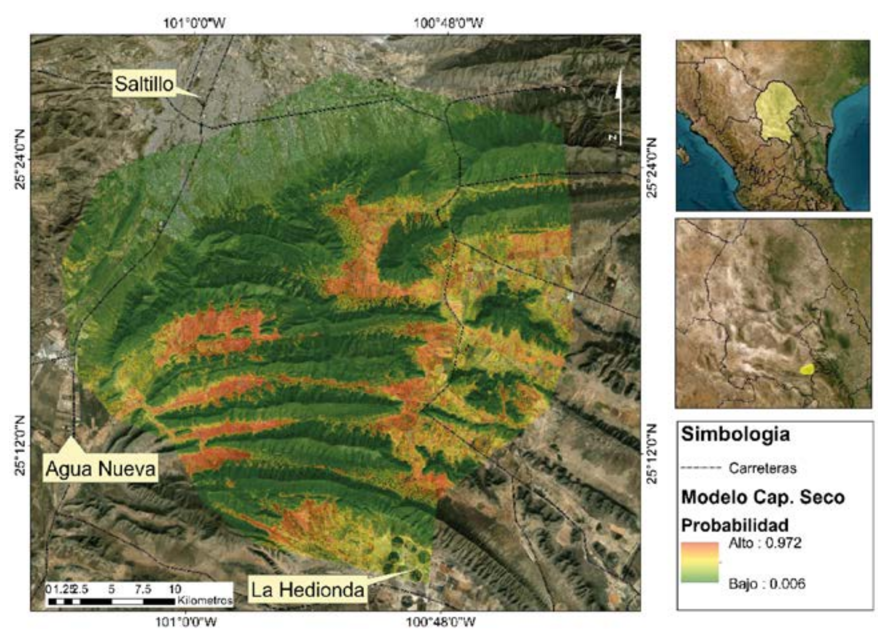

Figure 5. Potential distribution model of Black Bear captures during the dry season.

habitat is associated with steep slopes (Sollmann *et al.*, 2016), the models obtained in this study (Figures 4 and 5) indicate the influence of urban areas and human activities on the increase in bear captures.

The conflict models during the rainy season coincide with the sighting and capture models, where the urban areas of Saltillo show suitable habitat probability values greater than 0.80 (Figures 6 and 7). This result is very similar to that of Núñez-Torres *et al.* (2020; Figure 2), where their Human-Bear Interaction (IHO) model for northern Mexico shows

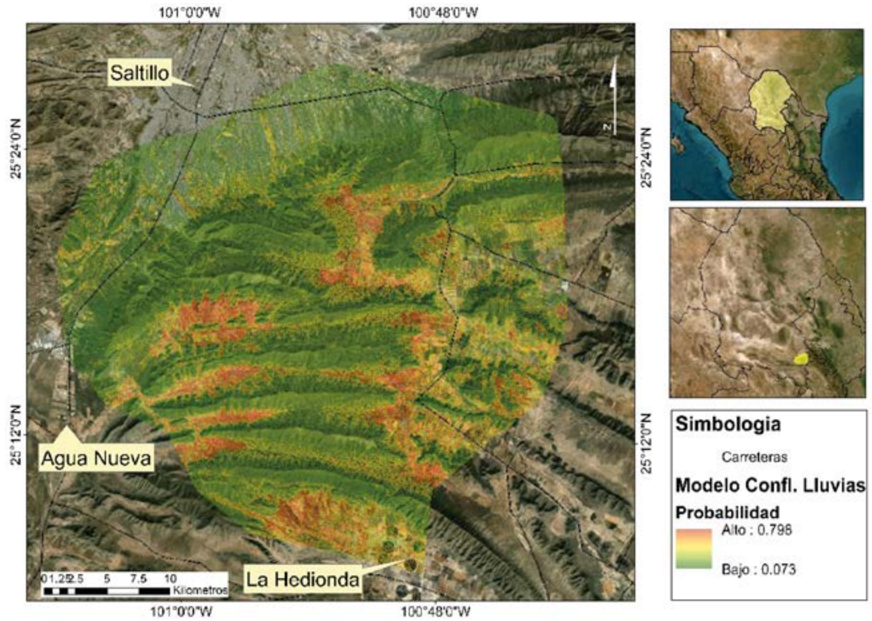

Figure 6. Potential distribution model of conflicts involving the Black Bear during the rainy season.

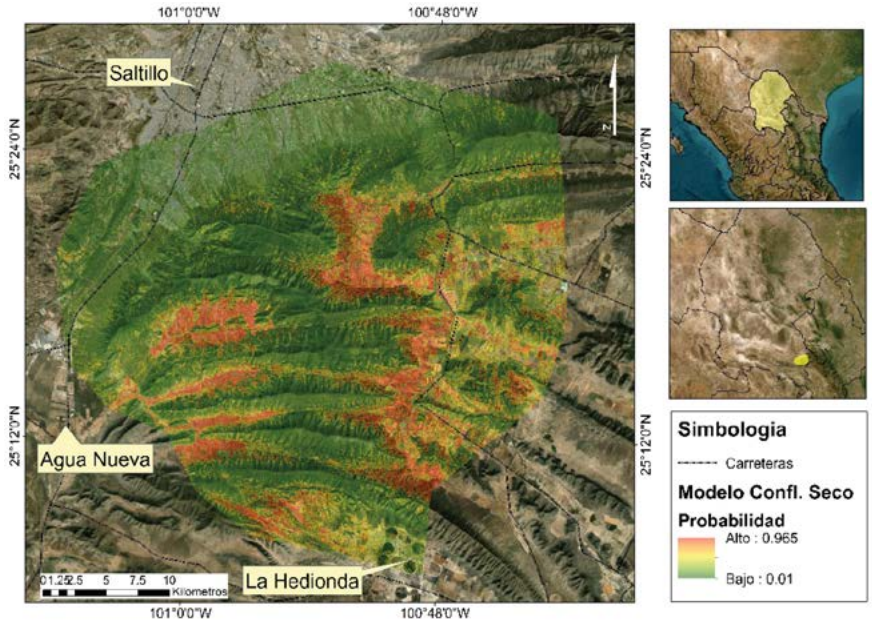

Figure 7. Potential distribution model of conflicts involving the Black Bear during the dry season.

probability values greater than 0.75. The variables with the highest contribution to human-bear interactions are distance to urban areas, distance to forest, and the presence of free-grazing livestock.

CONCLUSIONS

The influence of physiographic variables was decisive in the models of Human-Bear Interactions (IHO). It was observed that records of the bear show a constant relationship with slopes of less than 10 degrees in areas close to urban and rural communities, while in the mountainous region, both proximity to water bodies and sites with high ruggedness (indicating canyons and mountains) are habitat elements that bears may be using as escape routes and access to their food (Carter *et al.*, 2010; Baruch-Mordo *et al.*, 2014).

This research demonstrates that the probability of sightings and captures of black bears varies between the rainy and dry seasons. During the rainy season, an increase in the likelihood of interactions was observed in urban areas, possibly due to greater food availability, while in the dry season, interactions concentrated in forested areas, particularly in pine-oak forests. This indicates that seasonality influences the behavior of the black bear, which should be considered in the management and conservation strategies for the species. Based on the maps generated in this study, landowners and authorities can work together to create Wildlife Management Units (UMAs) that focus on an innovative type of utilization: photographic safaris featuring the charismatic black bear. This activity would be non-extractive according to the General Wildlife Law, which facilitates its implementation in the short term (<2 years). In this way, what is currently a human-bear conflict could transform into an economic opportunity for the landholders. A limitation of using the Support Vector Machine algorithm is that it only models the Grinnellian niche, meaning the relationship between environmental variables and the occurrence records of the bear. It does not consider other factors such as predation, the presence of prey, and population dynamics —factors that are relevant for improving predictions not only for the bear but also for the species that share its habitat.

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Colegio de Postgraduados

Registry of mermithid (Mermithidae) parasites of spittlebug nymphs (Hemiptera: Cercopidae) in sugarcane crops in Veracruz, Mexico

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ABSTRACT

Objective: To make known the presence of parasite mermithids of the spittlebug nymph in different sugarcane localities of the state of Veracruz, Mexico.

Design/methodology/approach: The nematodes were collected in sugarcane soils after observation of the emergence of the pest from the spittlebug nymphs (in situ), while some others were obtained after collecting spittlebug nymphs in the field and the pest's emergence in the laboratory. The death of the nematodes was provoked with hot water (sterile distilled, 60 °C), and they were measured and stored in Eppendorf tubes of 1.5 mL in formaldehyde 4% and kept in refrigeration at 4 °C.

Results: This report is presented as evidence of pest mermithids that emerge from the spittlebug nymph host in sugarcane crops in eight localities of the state of Veracruz. Although their identification at the species level could not be done, the length of each mermithid found was recorded, as well as its relationship with the host.

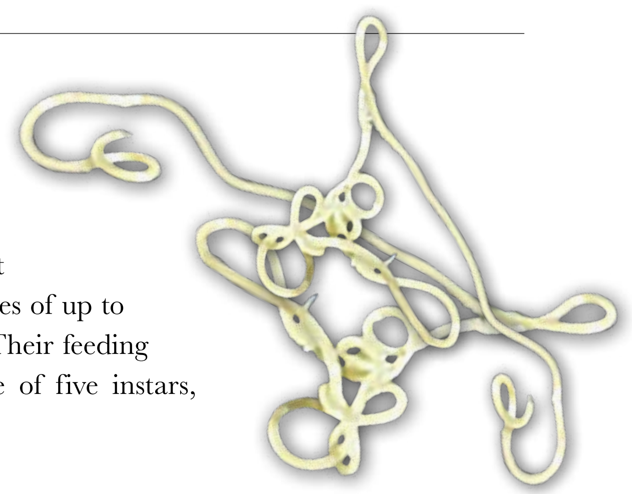
Limitations on study/implications: When the presence of mermithids was verified and their location was identified in diverse localities, the opportunity of continuing with the collection of specimens was justified and their identification could be formally performed, as well as the implementation of biological control studies with the use of these native nematodes; also, the exploration of their roles in the control of nymphs from the sugarcane crop.

Findings/conclusions: The presence of mermithid parasites of spittlebug nymphs in the sugarcane crop in eight localities in the mountainous region of Veracruz was verified *in situ*. Their emergence was classified initially on nymphs of the spittlebug (Hemiptera: Cercopidae) that belong to the genera *Aeneolamia* and *Prosapia*.

Keywords: natural control, *in situ*, nematodes, parasitic, spittlebug.

INTRODUCTION

The spittlebug pest (Hemiptera: Cercopidae) acquired economic importance in the sugarcane crop (*Saccarum officinarum* L.) in Mexico at the start of the 20[th] century, and presently causes losses of up to 9 kg/ha^{-1} (De la Cruz-Llanas *et al.*, 2005). Their feeding begins from egg hatching until the passage of five instars,



where they remain in the root zone and suction the sap from the superficial roots and cause a "physiological disorder" (García *et al.*, 2007). As adults, they feed on the sap of leaves where they introduce their stylet and provoke lesions on the leaves known as "burns" (García *et al.*, 2007).

In Mexico, the presence of this pest involves climate triggers that favor its persistence within the crop, such as temperature (26-45 °C), clay soils, and the presence of natural hosts (grasses) (García-García *et al.*, 2006); as well as high precipitations (June-July) that mark egg hatching and give rise to the nymphs, also called "spittlebugs" (Enríquez *et al.*, 2002).

Control of this pest has required knowledge related to its population dynamics, diapause, chemical products, resistant varieties, and biological agents (Rodríguez *et al.*, 2003). In this strategy, the use of entomopathogenic nematodes has been considered as a viable alternative for their control within the sugarcane crop (Grifaldo-Alcántara *et al.*, 2019).

However, there are other types of nematodes, such as mermithids, which are long, thin and translucent worms that are highly specialized in parasitizing a broad range of invertebrates, and part of their biology involves pre-parasitic infectious juveniles, which enter the tegument until they reach the hemocele where they can finally kill their host (Platzer, 2007).

The findings of mermithids in spittlebug nymphs were reported since 1985 in Venezuela, where the presence of *Hexamermis dactylocercus* parasitizing nymphs of *Aeneolamia varia* (Cercopidae) was discovered, with a level of infection under natural conditions of 50% in nymphs collected in the field (Poinar Jr. and Linares, 1985). In Argentina, the presence of mermithids was reported which emerged from nymphs belonging to the genera, *Deois mourei* and *Notozulia entreriana* (Foieri, 2016). Meanwhile, in Mexico, Flores (1994) reported the first sightings of mermithids of the genus *Hexamermis* sp. as enemies of spittlebug nymphs in the sugarcane crop.

Therefore, the objective of this study is to make known the registry of mermithids that parasitize spittlebug nymphs, as well as the difference in size and their possible relationship with the host. The year and place of discovery in sugarcane crops for the state of Veracruz, Mexico, was also included.

MATERIALS AND METHODS

Records in this study are data accumulated since the year 2009 to the year 2022 in the sugarcane crop within different localities of the state of Veracruz, Mexico, to make known the presence of mermithids that parasitize the nymph (also called "spit") of the spittlebug. The detection and collection of organisms was through monitoring carried out by the field staff from each sugarcane mill within the crop for the control of the spittlebug pest. This was also done from direct collection of nymphs to perform the bioassays or identification of the pest. These nymphs were transported to the laboratory of Colegio de Postgraduados, Campus Córdoba, and fed with pieces of sugarcane plantlets to wait for the development and emergence of adults for their identification.

Both the collections of mermithids in the field and the organisms that emerged from the nymphs that were in the laboratory were placed in test tubes with sterile distilled water and killed at 60 °C for two minutes; then the water was removed and they were placed on a flat surface and measured with a measuring tape to record the total length of the body (cm). To continue with their fixation and conservation, the mermithids were placed in Eppendorf tubes of 1.5 mL in formaldehyde 4% and kept refrigerated at 4 °C.

The mermithids that were recovered in 2019 when the nymphs emerged were kept in a plastic recipient (20×12×7 cm), to which water was added and a superficial layer of previously sterilized river stones, methodology suggested for the massive reproduction of mermithids that are mosquito parasites (Santamarina-Mijares and Berlini, 2000).

RESULTS AND DISCUSSION

In the state of Veracruz, since the year 2009 (Table 1), a registry was started of the presence of mermithids as natural parasites of spittlebug nymphs. The collection of these organisms was carried out with the help of entomological pliers.

Existing data about the mermithids detected are diverse (Table 1), among which there is information by the field staff from Ingenio CIASA (locality Comején), who observed the emergence of mermithids in "spittlebug" nymphs in situ. In the year 2013, in sugarcane areas of Ingenio Central El Potrero, after the collection of nymphs for their identification and then the development and emergence of adults in the laboratory, the exit of mermithids was detected in five nymph carcasses. The species *Aeneolamia albofasciata* was the one of largest population among the adults identified. After collecting spittlebug nymphs in the field to carry out bioassays of two entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) of this pest in the laboratory, Parada-Domínguez (2019) detected natural parasitizing in the nymphs evaluated of up to 10.42% (this is in five out of 48 nymphs).

The presence of mermithids was also recorded in municipalities such as Rancho Tablas, Estrella and Rincón del Otate (Table 1), where, after the evaluation of native entomopathogens (*Heterorhabditis indica* and *Steinernema* sp.) in two sugarcane mills (Grifaldo-Alcántara *et al.*, 2019); the collection of spittlebug nymphs was carried out to identify adults and also to detect the natural emergence of mermithids in the laboratory, data not published in this report.

The last registry was in the year 2022, where after monitoring and placing green traps to capture the spittlebug in the field, the presence of two mermithids that emerged from nymphs was observed, with lengths of 18 to 21 cm, with these being the largest recorded in this study.

The study considers that the dissimilarity in the size of the mermithids found does not show a relation between the variety of sugarcane in the collection sites (Table 1), since the mermithids with lengths of 17 cm and 12 cm were related to the varieties, while the other mermithids, of 12, 14, 18, 21 cm, were present in CP varieties 72-2086; likewise, a relationship with the altitude (masl) is also not considered, or with the proximity between collection sites (Figure 1).

The presence of mermithids in the host *Aeneolamia varia* was recorded in Venezuela since the year 1985: the species *Hexamermis dactylocercus* whose length was 10 cm (Poinar Jr. and Linares, 1985). In contrast, the smaller measurements in this research were of 12 cm in the mermithids found in the municipalities Atoyaquillo, Estrella and Rincón

Table 1. Localities with presence of parasite mermithids of spittlebug nymphs in sugarcane crop and their relationship with spittlebug species (Hemiptera: Cercopidae) in Veracruz.

Localities	Municipality	Sugarcane mill	N	W	Altitude	Variety of sugarcane	Size of mermithids	Number of organisms	Year of presence	Individuals presents in spittlebug nymphs
1) Comejen	Hueyapan de Ocampo, Veracruz	CIASA	95° 11’ 09”	18° 09’ 38”	98 m	CP 72-2086	14 cm	2	Hernández-Rosas, communication personal, 2009	*Aeneolamia albofasciata* (before *A. postica*)
2) California	Amatlan de Reyes, Veracruz	Central El Potrero	18° 54’ 11”	96° 51’ 27”	586 m	ITV 290	17 cm	5	Hernández-Rosas, communication personal, 2013	*A. albofasciata* *Prosapia teapana* *P. simulans*
3) Atoyaquillo	Atoyac, Veracruz	Central El Potrero	18° 55’ 38”	96° 51’ 46”	553 m	Mex 69-290	12 cm	5	Parada- Domínguez *et al.*, 2014	*A. albofasciata*
4) Jareros	Ursulo Galvan, Veracruz	La Gloria	19° 23’ 22”	96° 31’ 04”	148 m	Mex 69-290	17 cm	3	Hernández-Rosas, communication personal, 2018	*A. albofasciata* *P. simulans*
5) Rancho Tablas	Acatlan de Pérez Figueroa, Oaxaca	Constancia	18° 33’ 53”	96° 39’ 00”	156 m	MEX 69290	17 cm	1	Grifaldo-Alcántara *et al.*, 2019	*A. albofasciata* *P. teapana*
6) Estrella	Acatlan de Pérez Figueroa, Oaxaca	Constancia	18° 33’ 34”	96° 38’ 00”	151 m	CP 72-2086 ITV 921424 MEX 69290	12 cm	3	Grifaldo-Alcántara *et al.*, 2019	*A. albofasciata*
7) Rincón del Otate	Amatlan de los Reyes, Veracruz	Central EL Potrero	18° 55’ 25”	96° 53’ 19”	557 m	MEX79-431	12 cm	2	Grifaldo-Alcántara *et al.*, 2019	*A. albofasciata* *A. contigua* *P. teapana* *P. simulans*
8) Paraíso la Reforma	Tezonapa, Veracruz	Central Motzorongo	18° 39’ 28”	96° 44’ 51”	296 m	CP 72-2086	18 cm y 21 cm	2	Hernández-Rosas, communication personal , 2021	*A. albofasciata* *P. simulans*

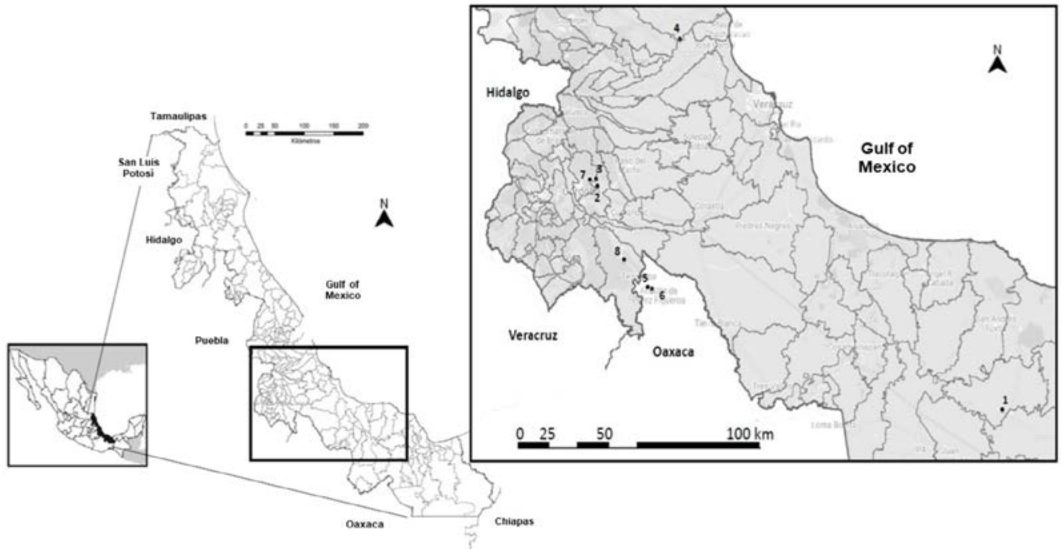


Figure 1. Presence of mermithids in the mountainous region of Veracruz, Mexico. Locality: 1) Comején, 2) California, 3) Atoyaquillo, 4) Jareros, 5) Rancho Tablas, 6) Estrella, 7) Rincón del Otate, and 8) Paraíso la Reforma.

del Otate Hueyapan de Ocampo, where the size of the two specimens was 14 cm; while the larger sizes were found in the municipality Paraíso la Reforma, 18 and 21 cm, followed by California, Jareros and Rancho Tablas with specimens that measured 17 cm. Regarding the interaction of mermithids and spittlebug species, the study assumes that it is possible for the presence of nematodes that range between 17 and 21 cm to be associated with populations of the genus *Prosapia*; meanwhile, the variation of mermithids between 12 and 14 cm would be more recurrent in populations of the genus *Aeneolamia*.

According to what was proposed by Welch (1965), these variations in the size of individuals could be linked to the size of the host that parasitizes it. Thus, it is considered possible for larger mermithids to be recovered from larger hosts/nymphs (*Prosapia*), while the small ones would be related to smaller hosts/nymphs (*Aeneolamia*); it is also regarded that they could be new species, for two reasons. First, because of the distance between *H. dactylocercus* discovered in Venezuela with a length of 10 cm, and the second, because of the variations in size (12 to 21 cm) that were observed in this study regarding the hosts where they were found.

Between the collections conducted in the years 2009 to 2021, all the nematodes discovered parasitizing spittlebug nymphs were killed with hot water and conserved in formaldehyde at 4%. However, the nematodes recovered in 2019 were kept in plastic recipients with sterile river stones and distilled water, trying to seek the survival of these specimens (Figure 2), following the methodology of parasite mermithids of mosquito (Santamarina-Mijares and Berlini, 2000), although the survival was not favorable. Therefore, this study agrees with Welch (1965), who mentioned that it is necessary to carry out studies related with the culture of mermithids, their propagation, and dissemination in the field.

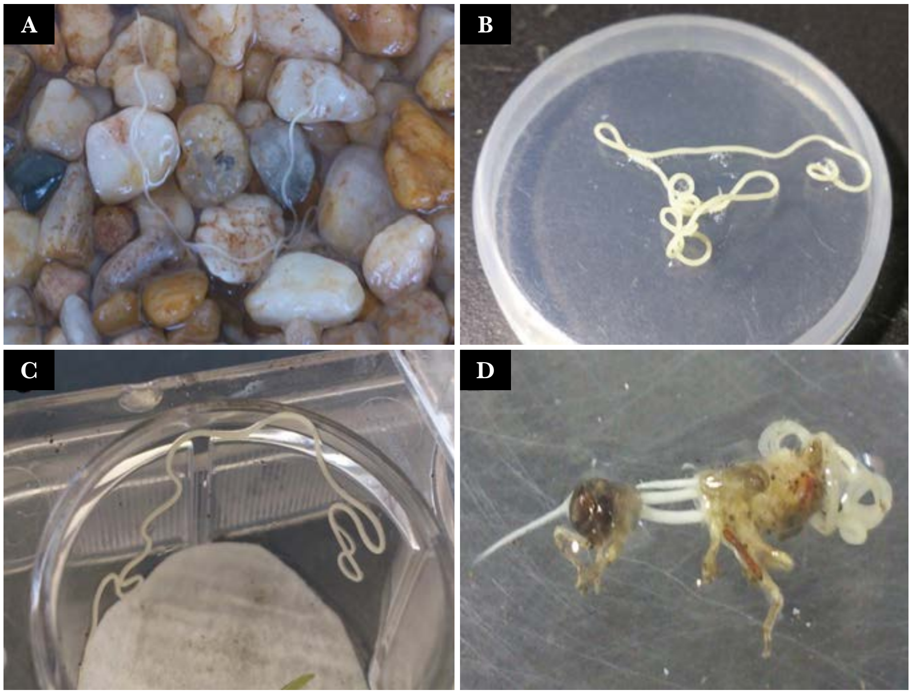


Figure 2. A) Evidence of mermithids, A) in river gravel; B and C) emerging from nymphs collected in the field; D) emergence in a spittlebug nymph (Hemiptera: Cercopidae).

Entomopathogenic nematodes present a favorable potential for control of spittlebugs (Grifaldo-Alcántara *et al*., 2019); however, mermithids are used for biocontrol with high specificity, as is the case of mermithids for the control of mosquito larvae (Menéndez-Díaz *et al*., 2018). Therefore, it is regarded that after the corrective identification of the mermithids present with molecular tools, a technique of multiplication, as well as their incorporation for the reduction of spittlebug nymph populations in sugarcane crops in Veracruz, could become an excellent alternative for the control of this pest.

CONCLUSIONS

The localities with sugarcane crops in the state of Veracruz are presented, where the presence of mermitids has been detected as parasitic nematodes of spittlebug nymphs. The study considers that there is no relationship between the variety of the sugarcane and the altitude (masl). However, hypothetically, it could exist between the length (cm) of the nematodes and the spittlebug genus parasitized. Regarding this, the mermithids of larger size could be related with the genus *Prosapia*, while for other smaller ones, the relationship would be with *Aeneolamia*.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Comparison of the Export Competitiveness of Limes from Mexico, Colombia, Argentina, and Brazil to the European Union

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ABSTRACT

Objective: To determine the competitiveness in the world and in the European Union of the main Latin lime-producing countries through the calculation of the normalized revealed comparative advantage to generate a discussion around the use of the free trade agreement that the countries have with the European Union.
Design/Methodology/Approach: Official sources were used to obtain the information in the period from 2001 to 2021, the normalized revealed comparative advantage was calculated and the behavior in the global market and in the European Union was forecasted. The Prais-Wisten method was used for the correction of autocorrelation.
Results: Competitiveness was estimated through the Normalized Revealed Comparative Advantage Index, both globally and in the European Union, for the main Latin American limes producers.
Study limitations/Implications: The limitation of the study was that it was only compared with Latin American countries, the study could expand its comparison with other regions of the world.
Findings/Conclusions: The main Latin American lime producers were compared using the NRCA. Mexico has maintained a competitive advantage since 2003, while Argentina lost it in 2004. Brazil became competitive in 2021, while Colombia is not. Argentina has the biggest advantage, although declining, and Brazil has surpassed Mexico since 2019. Projections show that Mexico and Brazil will continue to improve their competitiveness, while Argentina will reduce it. The concentration of production in Mexico is an opportunity for other regions with similar conditions to increase production and export to the European market. The proposed hypothesis is accepted.

Keywords: market precariousness, commercial diversification, commercial concentration

INTRODUCTION

The export of citrus fruits is one of the most important economic activities for the agricultural sector in Mexico. The country is a leader in both production and export, with the U.S. being the main destination (Ruiz *et al.*, 2016). According to Nicolás and Favila (2019), limes are one of the products with significant commercial potential because the production system is one of the most competitive in Latin America. In Mexico, limes represent one of the perennial crops with the highest production value (SIAP, 2023).

According to Trademap data (2023), Mexico ranks second worldwide in lime exports (705,596 t), after Spain (634,771 t), Turkey (594,140 t), South Africa (557,482 t), and the Netherlands (201,770 t). In terms of production, according to FAOSTAT (2023), India ranks first (3,548,000 t), followed by Mexico in second place (2,983,802 t), China (2,623,202 t), Argentina (1,826,355 t), Brazil (1,632,109 t), Turkey (1,323,000 t), and the U.S. (938,030 t).

Nationally, the types of lime produced are sour or Mexican lime (*Citrus aurantiifolia*) (43.13%), Persian lime (*Citrus latifolia*) (51.31%), and Italian lime (*Citrus limon*) (5.48%). Veracruz and Michoacán are the states with the highest production, accounting for 23.23% and 27.08%, respectively, followed by Oaxaca and Colima with 10% each (SIAP, 2023). According to SIAP data (2023), in 2022, there was a 4% increase in Persian lime production compared to 2021, while Mexican lime production increased by 3%. The cultivated area for limes overall saw a 2% increase. The highest production of limes occurs between July and December, which influences prices.

Zea *et al.* (2016) conclude that Mexican lime is the preferred variety nationally, although its export volume is limited due to its heterogeneous quality and shorter shelf life compared to other varieties, such as Persian lime. Caamal *et al.* (2017) mention that the Persian variety is easier to manage because the crop has fewer thorns compared to the Mexican lime variety. It is noted that Mexican Persian lime cultivation saw a commercial boom in the 1980s due to a phytosanitary issue with Mexican lime exports. Valencia *et al.* (2016) point out that while Persian and Mexican limes differ in crop management, greater integration of the lime sector is necessary in marketing to find new potential markets. According to Trademap data (2023), in 2022, Mexico exported 689,517 tons to the U.S., meaning that 97.7% of total exports are concentrated in a single destination. According to basic indicators of trade position, the concentration of both destination and origin countries for exports and imports depends on the number of destinations or origins; the more destination countries, the greater the trade diversification (Durán, 2008). The second most important trade agreement after the USMCA is the free trade agreement with the European Union. The modernization of the Global Agreement, which took place in 2020, established the elimination of tariffs on the remaining agricultural products that were not liberalized in the current Agreement (Delegation of the EU in Mexico, 2021).

The European Union is one of the most important markets in the world due to its purchasing power and the size of its population. Known since 1993 as the European Common Market, it had a GDP of 14,522,000 million euros in 2021 and comprised 23 million companies (European Council, 2023). Given the characteristics, the European Common Market is attractive from a commercial perspective, and access to it is of great importance for the diversification of international markets.

A comparison is proposed among Mexico, Colombia, Argentina, and Brazil. The justification for this approach is that they share similar conditions with Mexico in terms of trade balance and relations with the European Union. According to SICE (2023), all the Latin American countries in the study have trade agreements with the European Union. Mexico has an agreement through the Free Trade Agreement between Mexico and the European Union (TLCUEM) since 2001, while Argentina and Brazil are covered by the Free Trade Agreement between Mercosur and the European Union, which has been in effect since 2019. Colombia formalized its relations with the European Union through the Trade Agreement between Colombia, Peru, Ecuador, and the European Union, which has been in effect for Colombia since 2013. Competitiveness is related to the natural conditions of producing regions, market demand, and technological change, which can lead to a geospatial concentration of production (Vargas *et al.*, 2020). This is an undesirable quality; in this case, lime production is highly concentrated in a few countries in the region, and therefore, competitiveness must be promoted.

Contreras and Leos (2021) state that in the agri-food sector, competitive products must meet consumer demand in terms of price, quality, and quantity while also being sustainable for producers. The objective of this study is to determine the competitiveness of the main Latin American lime-producing countries in the world and in the European Union through the calculation of the normalized revealed comparative advantage, to generate a discussion around leveraging the free trade agreement these countries have with the European Union. The hypothesis is that Mexico has competitiveness and that if it continues with the current pace of exports, it will position itself as a significant supplier and competitor in the European market compared to the main Latin American lime exporters.

MATERIALS AND METHODS

Official sources were used to obtain the information: two for international trade and one for the characterization of Mexico. The variables considered for all countries were exports, imports, and production from 2001 to 2021. Global trade data were obtained from the FAO database (2023) (Faostat) and Trademap (2023). For the collection of bilateral trade data, the databases provide homogenized information on the countries, which is pertinent for generating consistent statistical comparisons. The characterization of Mexican production was expanded; for this purpose, the information bank of SIAP (2023) was used.

Data Collection

Lime is a product with various exportable varieties both within and outside of Mexico. For this reason, the tariff classification number 080550 was considered for the documentation of the data, which, according to CAAAREM (2023), refers to lemon (*Citrus limon*, *Citrus limonum*) and limes (*Citrus aurantifolia*, *Citrus latifolia*). The variables used to calculate the global competitiveness of each exporter included the trade flows of each country with the world. The data were obtained through Faostat (2023); this database is pertinent for considering trade without a specific destination market.

Regarding the calculation of the NRCA for Mexico, Colombia, Argentina, and Brazil, exports and imports between each Latin American exporter and the European Union were analyzed, resulting in the competitiveness of each exporter in this specific market. Documentation was done through Trademap (2023), as it allows for the collection of bilateral trade information. The econometric estimates were conducted using STATA software version 17.

Normalized Revealed Comparative Advantage Index

The NRCA is an important tool in formulating trade policies and economic development strategies, as it allows countries to determine areas of specialization and make informed decisions about how to allocate resources and compete in international markets. To estimate competitiveness and identify the best exporter in the European market and globally, the NRCA index created by Yu *et al.* (2008) was used, adapted for a specific market by Contreras and Leos (2021). Furthermore, the index is more precise and consistent than other measures used due to the theoretical properties of normalization.

The calculation was performed over the period from 2001 to 2021; the start is marked by the entry into force of the TLCUEM, and 2021 is the last available year, representing 21 observations, a consistent time series for econometric forecasting (Harrell, 2015). The NRCA was calculated for each of the four countries, both in the European market and globally, and its mathematical formula was as follows:

$$NRCA = \frac{\Delta E_{jm}}{E} - \left[\frac{E_m}{E}\right]\left[\frac{E_j}{E}\right] \quad [1]$$

Where: E_{jm} represents the lime exports of each country in the world and the European Union; E_j is the total supply of lime including exports from the countries plus the internal supply of the destination and imports to the region from the rest of the world. For the global market, global production and exports were used; E_m refers to the exports of the group of fruit from FAOSTAT of the exporters, in both the global and European markets; and E is the total supply of all fruits in the global and specific market.

Trend of the Normalized Revealed Comparative Advantage Over Time

The calculation of the trend through regressions is a statistical technique used to analyze data and predict future values in time series (Moreno, 2008). To calculate the trend of the normalized revealed comparative advantage (NRCA), the following simple regression model was initially used through Ordinary Least Squares (OLS):

$$NRCA_t = \alpha_t + \beta_t + \varepsilon_t$$

Where: $NRCA_t$ is the NRCA index of lime; α_t is the intercept coefficient; β_t is the trend coefficient over time of the lime NRCA; and ε_t is the random error of the regression. The regressions were estimated using OLS, and inconsistencies were found in the t-student test, which demonstrates independence, normal distribution, and homogeneity of variances among the variables (Lugo and Pino, 2022). Therefore, the OLS estimation was not optimal. The Durbin-Watson test was calculated, and it was found that the cause of non-optimality in the regression was autocorrelation, which is a very common problem in regressions with time series data (Luquez *et al.*, 2022). Consequently, an econometric method using first differences was proposed.

Prais-Winsten Correction

Time series regressions are used to predict their behavior. Based on the calculated NRCA, regressions were performed, which had the issue of serial autocorrelation. Due to this, the Prais-Winsten method was employed to correct for autocorrelation.

First-difference methods offer many benefits; among the notable ones for this analysis is that the interpretation of the regressors is made as growth rates and also corrects the regression residuals, transforming them into white noise (Gujarati, 2010). The Prais-Winsten method is an econometric technique used to address autocorrelation in time series by transforming data and estimating adjusted regression models (Vougas, 2021). This method performs a first-difference transformation and generates optimal results. To ensure the correction of the problem, the Durbin-Watson test was applied again.

RESULTS AND DISCUSSION

A positive result indicates that a country has a comparative advantage, while negative results indicate a comparative disadvantage in the production and export of a particular good relative to the world or a specific market. Figure 1 illustrates the results of the calculation of the normalized revealed comparative advantage (NRCA) for Mexico, Colombia, Argentina, and Brazil in relation to global trade, and it also presents a linear projection for the period of 2022-2026.

Mexico has the highest normalized revealed comparative advantage (NRCA) among the countries considered in the study since 2003, and the trend is positive, showing the highest growth rate as well. This can be explained by the specialization in nine states of the republic (Vargas-Canales, 2020). Furthermore, Mexico's position in global exports is consistent with the findings of Canales *et al.* (2019), who state that Mexico accounts for 31% of citrus exports. Argentina was the country with the highest competitiveness from 2001 to 2003; however, starting in 2004, it has lost its competitive edge. From 2004 onward, the trend has generally been negative, which is also reflected in the projected years. Nonetheless, it is still considered competitive due to the positive index. Regarding

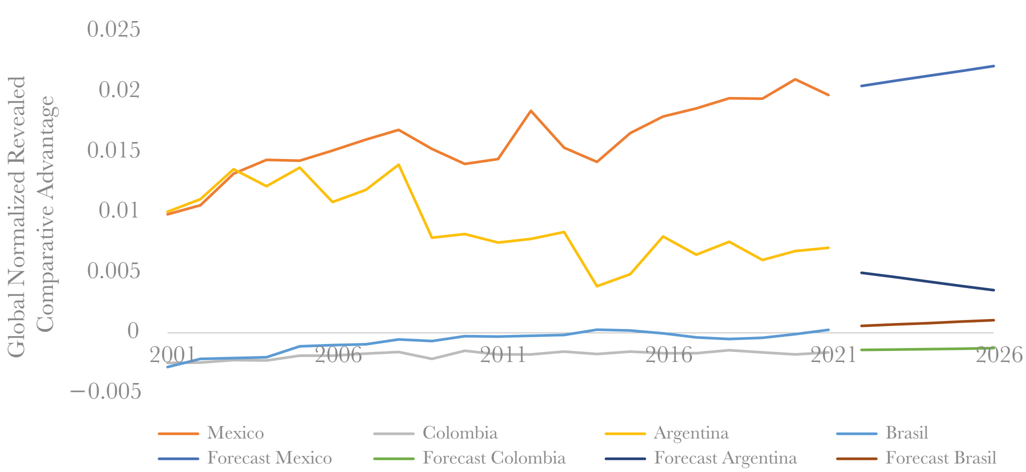

Figure 1. Global VCRN index for Mexico, Colombia, Argentina and Brazil from 2001 to 2021, and its extrapolation from 2022 to 2026.

Table 1. IVCRN in the global market, results of the regressions to first differences.

Independent variable=Time	NRCA Mexico	NRCA Colombia	NRCA Argentina	NRCA Brasil
Coefficient	0.000424	0.000038	−0.000340	0.000141
Constant	−0.838368	−0.078709	0.692626	−0.284378
T-student, time	5.90	4.24	−3.92	4.01
Prob > F	0.0005	0.0000	0.0005	0.0001
R-square	0.47	0.60	0.48	0.55
DW original	1.20	1.51	1.40	0.40
DW converted	1.70	1.98	1.90	1.87

Brazil, it has transitioned from being a non-competitive and therefore deficit country to a competitive one in 2021. In the years 2014 and 2015, it also recorded values indicating competitiveness. The trend is upward, and in the future, it could become a competitor to Mexico. Colombia, on the other hand, does not show a clear trend, but it can be seen from the previous figure that it does not possess a comparative advantage and does not demonstrate the potential to achieve one in the future.

The previous table summarized the regressions. All the t-student values are accepted, and the F probability is lower than the critical value, therefore they are valid, and the Durbin-Watson test values approach their optimal value. The coefficient values found allow us to conclude that Mexico is the country with the highest expected growth in the future, followed by Colombia and Brazil. For Argentina, however, a loss of competitiveness in the global market is expected.

European Market

Figure 2 represents the NRCA of Mexico, Colombia, Argentina, and Brazil in the European Union, which could demonstrate the utilization of the various trade agreements with Europe.

In the previous graph, the results of the NRCA in the European market were presented. Argentina has the greatest advantage, although with a clearly decreasing trend, indicating a loss of competitiveness. Throughout almost the entire time series, Mexico held the second position, but starting in 2019, it was surpassed by Brazil, which experienced accelerated growth. Colombia, on the other hand, does not possess an advantage in this market, despite the fact that the Colombian agricultural sector has been one of the most benefited by the signing of the Free Trade Agreement with the European Union (Tamayo *et al.*, 2017).

Although in the last observed year, Argentina and Brazil were the countries with the highest competitiveness, the trend in the extrapolation of the NRCA can be seen. Extrapolations imply a certain trend; in the estimates, it is evident that the slope of the NRCA is greater for Brazil, followed by Mexico, while Argentina shows a tendency to become less competitive in the near future. Colombia, for its part, does not exhibit a clear trend and therefore is not considered likely to be competitive in the future. In Table 2, the results of the autoregressive regressions of the NRCA are presented.

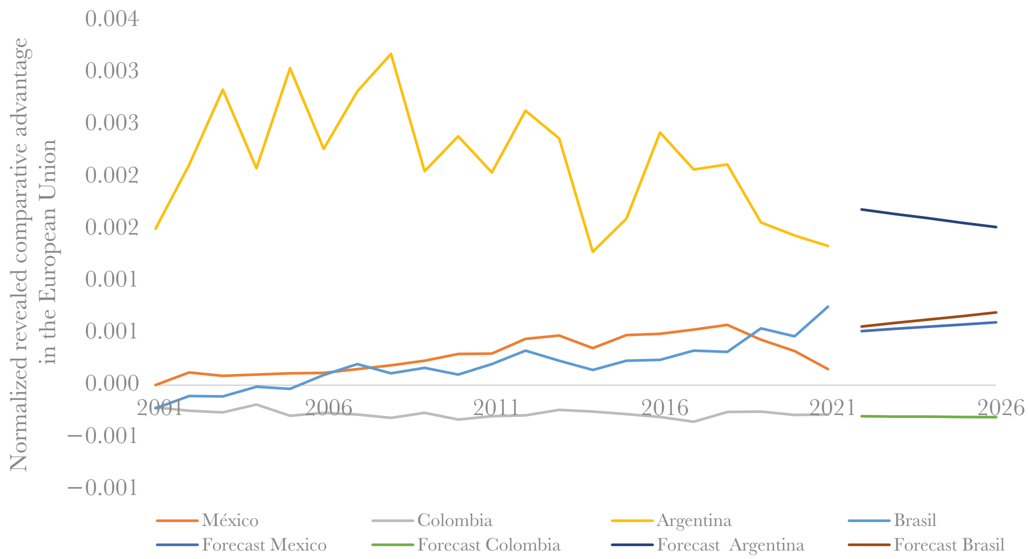

Figure 2. VCRN index for Mexico, Colombia, Argentina and Brazil from 2001 to 2021 in the European market, and its extrapolation from 2022 to 2026.

Table 2. IVCRN in the global market, results of the first difference regressions.

Independent Variable=Time	NRCA Mexico	NRCA Colombia	NRCA Argentina	NRCA Brasil
Coefficient	0.00001150	−0.00000226	−0.00004150	0.00003550
Constant	−0.022864	0.000427	0.085541	−0.071189
T-student, time	1.04	−1.68	−2.14	7.98
Prob > F	1.0000	0.2000	0.0625	0.0000
R-square	-	0.08	0.17	0.75
DW original	0.56	1.83	1.60	1.30
DW converted	1.48	1.93	1.73	1.80

The previous table summarizes the results of four regressions, where each dependent variable is the NRCA in the European Union for each of the American countries considered in this analysis. The independent variable is time, aimed at determining the future evolution of competitiveness. The value of the t-student test indicated that the significant regressions were only for Brazil and Argentina. The regressions for Mexico and Colombia were inconclusive, but the coefficients show that the future trend of competitiveness for Mexico and Brazil is expected to increase, while for Colombia and Argentina, the trend indicates a decline.

CONCLUSIONS

The main Latin American lime producers were compared using the NRCA. Mexico has maintained a sustained competitive advantage since 2003, while Argentina has lost competitiveness since 2004, Brazil became competitive in 2021, and Colombia is not competitive. Argentina maintains the highest advantage, albeit declining, and Brazil surpassed Mexico starting in 2019. Projections indicate that Mexico and Brazil will increase their competitiveness, while Argentina will decrease it. The spatial concentration of lime production in Mexico presents an opportunity for other regions with similar conditions to increase production and, at the same time, enhance exports to the European market. The proposed objective was achieved, and the research hypothesis is accepted.

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Induction of shoot development of *Stevia rebaudiana* Morita II by vitamins and cysteine

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ABSTRACT

Objective: The aim of this study was to evaluate the influence of B vitamins and cysteine on the formation and development of shoots in *Stevia rebaudiana* (*S. rebaudiana*) Morita II, without the addition of plant growth regulators.

Design/methodology/approach: The starting material consisted of nodal segments, subjected to eight different vitamin treatments. The results were analyzed using mean comparisons and Tukey's test to identify statistical differences.

Results: The most effective treatment was treatment number two, which yielded an average of 7.5 shoots per explant, demonstrating its efficiency for multiplication. For explant elongation, treatment five proved to be the most effective.

Limitations of the study/implications: This study aims to establish a multiplication protocol with enhanced efficiency; however, a limitation is the transferability of the results to temporary immersion bioreactors.

Findings/conclusions: Thiamine and cysteine are two organic compounds that induce multiple sprouting, while nicotinic acid and pyridoxine promote the elongation of Stevia explants. This research represents a significant step towards transferring this knowledge to grassroots technological enterprises.

Keywords: *Stevia rebaudiana*, B vitamins, cysteine, Morita II.

INTRODUCTION

Stevia rebaudiana Bertoni is known worldwide for its medicinal properties (González Hernández *et al.*, 2019). This plant belongs to the Compositae family (Ahamad, Blundell, & Mahomoodally, 2020) and is a perennial shrub native to Paraguay (Vázquez Hernández *et al.*, 2019). Its leaves contain steviol glycosides, which make them 100 to 300 times

sweeter than sucrose. This property has led to their consumption as sweeteners and their use as a sugar substitute in the food and pharmaceutical industries (Ahamad, Blundell, & Mahomoodally, 2020), as they are not metabolized by the human body (López Medina, López Zavaleta, & De la Cruz Carrillo, 2017). Stevia has been reported to possess anticancer, antimicrobial, anti-inflammatory, and antitumor properties without toxicity (Ruíz *et al.*, 2015). The genus *Stevia* comprises approximately 150 species (Abdullateef & Mohamad, 2011), among which *S. rebaudiana* variety Morita II and the creole variety are cultivated in the state of Yucatán.

However, the chemical composition of these varieties differs; Morita II has a higher daily biomass production per cm^2 of leaf area (Jarma, Rengifo, & Araméndiz Tatiz, 2006). Additionally, it synthesizes a greater concentration of rebaudioside A in relation to the concentration of steviosides, although its propagation by seed remains unsatisfactory (Ohta *et al.*, 2010). In the case of *S. rebaudiana* Bertoni, it has been demonstrated that in Murashige and Skoog culture medium supplemented with 0.37 mg/L of indole-3-butyric acid (IBA), an average of 14 shoots can be formed per nodal segment (Villamarín Gallegos *et al.*, 2020). This indicates that the type of response exhibited by the nodal segment of *S. rebaudiana* Bertoni will depend on the composition of the culture medium. Moreover, the vitamins used in the culture medium play an important role in the metabolism and development of plants, especially *in vitro*. According to Bhojwani and Razadan (1996), White reported the successful continuous growth of tomato root apices in 1934, initially using a culture medium composed of inorganic salts, yeast extract, and sucrose. However, in 1937, the substitution of yeast extract with pyridoxine, thiamine, and nicotinic acid allowed for the maintenance of tomato cultures for nearly 30 years, thus recognizing the importance of B vitamins. In *S. rebaudiana* Morita II, nodal segments form multiple shoots in the presence of B vitamins (4 mg/L of each), 6-benzylaminopurine (0.5 mg/L), kinetin (0.25 mg/L), and cysteine (4 mg/L) (Domínguez-May A. V., 2018). However, the latter, in the absence of plant growth regulators, increases shoot length in both Vicia faba (EI-Awadi M. E. *et al.*, 2016) and EM26 apple (Sotiropoulos T. E. *et al.*., 2005). This could be advantageous, as it would reduce the *in vitro* production costs of these species and potentially enhance the production of other commercially valuable crops. Therefore, the objective of this work was to evaluate the influence of B vitamins and cysteine on the formation and development of shoots of *S. rebaudiana* Morita II, without the addition of plant growth regulators.

MATERIALS AND METHODS

Biological Material

The initial biological material for obtaining nodal segments originated from *S. rebaudiana* Morita II seedlings produced by the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), located in the city of Mérida, Yucatán, Mexico.

Propagation of Shoots of S. rebaudiana Morita II for the Acquisition of Biological Material

In the preparation of the culture medium, 4.31 g/L of Murashige & Skoog (1962) salts from Sigma was used, supplemented with 100 mg/L of myo-inositol, 4 mg/L of glycine, 4 mg/L of pyridoxine, 4 mg/L of nicotinic acid, 4 mg/L of thiamine, 4 mg/L of cysteine, and 30 g/L of sucrose, along with 7 g/L of agar. The pH was adjusted to 5.8. The Murashige and Skoog culture medium was supplemented with 0.5 mg/L of 6-benzylaminopurine (BAP) and 0.25 mg/L of kinetin (KIN) as growth regulators. Finally, it was sterilized in an autoclave for 20 minutes at 121 °C and 1.032 kg/cm^2 of pressure.

To produce multiple shoots at 41 days of age, 200 nodal segments with a length of 1.5 cm were cultivated in the Murashige and Skoog culture medium in the presence of the previously mentioned growth regulators. The nodal segments were maintained for 41 days in a growth chamber at 25±2 °C under photoperiod conditions according to Domínguez-May *et al.* (2018).

Culture Medium for the Induction of Shoots in Nodal Segments

Nodal segments of 1.5 cm in length were separated from 41-day-old shoots in culture medium. These were cultivated in eight different treatments, with 20 nodal segments inoculated in each treatment. All treatments contained 4.31 g/L of Murashige and Skoog (1962) medium, 100 mg/L of myo-inositol, 30 g/L of sucrose, and 7 g/L of agar, with the pH adjusted to 5.8. The addition of each vitamin (considering that 4 mg/L of thiamine is generally used) was maintained at 4 mg/L, along with cysteine at 4 mg/L. The combinations of these are shown in Table 1. It is important to note that in the evaluation of the effect of vitamins and cysteine, neither BAP nor KIN was added in any of the treatments.

All treatments were sterilized for 20 min at 121 °C with 1.032 kg cm^2 of pressure; afterwards, they were incubated in a growth chamber at 25±2 °C under photoperiod conditions of 16 h of light and 8 h of darkness for a duration of 41 days.

Evaluated parameters

The number and length of shoots of Morita II were evaluated in each of the eight treatments.

Statistical Analysis

The results of the treatments were evaluated using IBM SPSS Statistics version 19, with a one-factor design (treatment) followed by mean analysis and Tukey's test.

Table 1. Treatments in Nodal Segments of *Stevia rebaudiana* Morita II for Shoot Induction.

Treatment	Vitamins, Cysteine o combination (4 mg L^{-1})
T1	Absence of vitamins and cysteine
T2	Thiamine
T3	Cysteine
T4	Nicotinic acid
T5	Pyridoxine
T6	Thiamine+Cysteine
T7	Thiamine+Cysteine+Nicotinic acid
T8	Thiamine+Cysteine+Pyridoxine

RESULTS AND DISCUSSION

Number of Shoots Generated

The application of the treatment in the absence of vitamins and cysteine (T1) to the nodal segments of *S. rebaudiana* Morita II did not allow for adequate shoot formation, exerting a negative effect on shoot development. After 41 days of cultivation, it produced a callus thickening at the base of the nodal segment with notable leaf deformation, resulting in an average of 0.55±0.22 shoots generated.

In the presence of 4 mg/L of thiamine (T2), *S. rebaudiana* Morita II formed an average of 7.55±0.88 shoots per nodal segment after 41 days of cultivation; however, the multiple shoots generated did not develop normal leaves. The addition of 4 mg/L of cysteine solely to the medium (T3) resulted in the formation of 4.30±0.77 shoots per explant, with abnormal stem development and leaf rosettes forming over the callogenic thickening of the explant. The influence of only nicotinic acid in the medium (T4) allowed the development of shoots of *S. rebaudiana* Morita II with normal leaf formation, obtaining 3.55±0.37 shoots per nodal segment. The medium supplemented solely with 4 mg/L of pyridoxine (T5) resulted in the formation of 2.50±0.36 shoots per explant; however, the leaves formed were less expanded than those observed in the presence of nicotinic acid (T4) and exhibited greater internode elongation (Figure 1).

The combined effect of adding thiamine and cysteine to the medium (T6) showed the formation of 3.65±0.39 shoots per nodal segment; however, there was also abnormal development of leaves directly attached to the explant without adequate stem development. The combination of thiamine, cysteine, and nicotinic acid (T7)

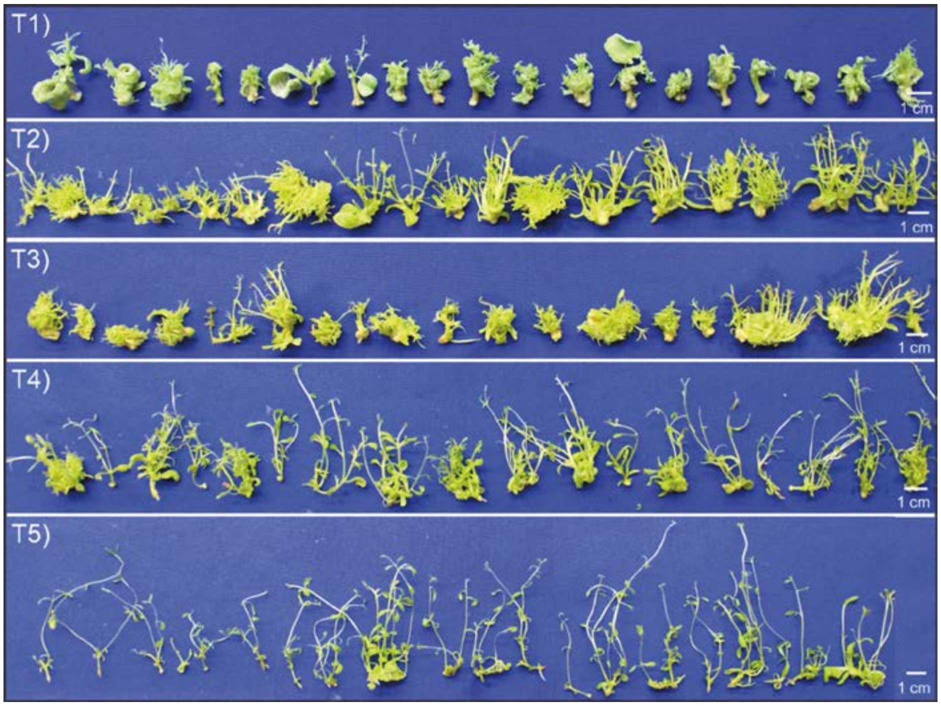

Figure 1. Development of nodal segments into shoots and their elongation. T1 Control, T2 Thiamine, T3 Cysteine, T4 Nicotinic Acid, T5 Pyridoxine.

stimulated the formation of 4.25±0.38 shoots per explant, showing better development of multiple shoots, with normal leaf formation; however, there remained the presence of leaves emerging attached to the explant without stem elongation. Interestingly, the combination of thiamine, cysteine, and pyridoxine in the medium (T8) had a positive effect on the formation and development of shoots of *S. rebaudiana* Morita II, from the nodal segments, resulting in 4.55±0.65 shoots per nodal segment, which exhibited better stem formation and leaf expansion; furthermore, abnormal formations, such as rosette leaves, were reduced (Figure 2).

The analysis of variance was applied to the eight treatments, finding a significant difference among them. Using Tukey's mean comparison test, three different groups were formed, with treatment T2 containing Thiamine showing the highest response in terms of the number of shoots generated, averaging 7.55±0.88 per nodal segment (Table 2).

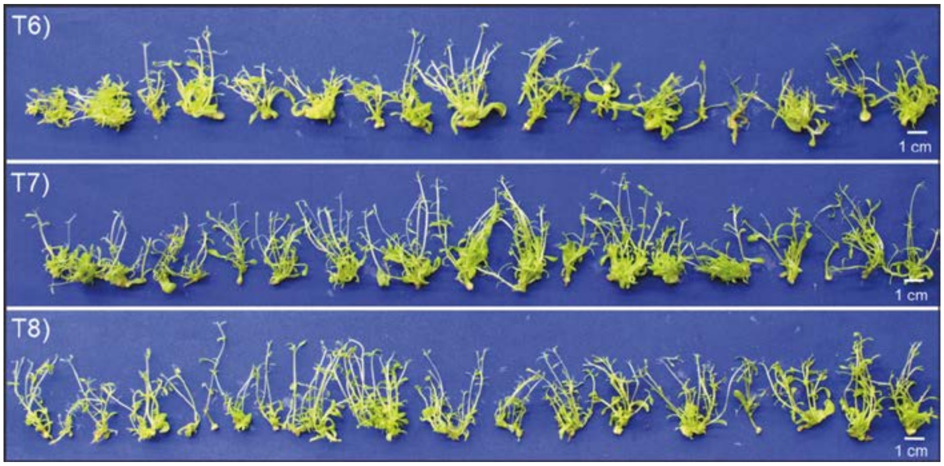

Figure 2. Development of nodal segments into shoots and their elongation. T6, Thiamine+Cysteine; T7, Thiamine+Cysteine+Nicotinic Acid; T8, Thiamine+Cysteine+Pyridoxine.

Table 2. Statistical analysis of number of shoots generated.

Treatment	Average number of shoots*	Tipical error
T2	7.550 c	0.881
T8	4.550 b	0.650
T3	4.300 b	0.771
T7	4.250 b	0.383
T6	3.650 b	0.385
T4	3.550 b	0.366
T5	2.500 ab	0.359
T1	0.550 a	0.223

*Number of shoots generated
Tukey (HSD) α=0.05; Means for groups in homogeneous subsets are shown. N=20 based on the harmonic mean of the sample size. Means for groups in homogeneous subsets are shown.

Although the analysis of variance shows that the highest effect on the number of shoots formation was with the addition of thiamine in the medium (T2), averaging 7.55±0.88 shoots per explant, it exhibited abnormal development of the shoots and rosette leaves attached to the explant. On the other hand, treatment (T8), which consisted of the addition of thiamine, cysteine, and pyridoxine to the medium, demonstrated the development of shoots with better stem thickness and leaf expansion, averaging 4.55±0.65 shoots per explant. This treatment is considered a better alternative for generating shoots of *S. rebaudiana* Morita II from nodal segments.

Shoot elongation

Upon evaluating the effect of the treatments on the shoot length of *S. rebaudiana* Morita II, it was found that the absence of vitamins and cysteine in treatment (T1) resulted in rosette-like shoot formations, without stem development or adequate shoot elongation, reaching an average length of 0.271±0.08 cm. In the treatment with thiamine (T2), the elongation was 1.41±0.05 cm. For the medium supplemented with cysteine (T3), the shoots formed with an elongation of 0.95±0.06 cm, while in the medium supplemented with nicotinic acid (T4), the elongation reached 2.62±0.14 cm. The treatment with pyridoxine (T5) promoted shoot elongation with an average of 4.02±0.26 cm (Figure 2). The treatment with thiamine and cysteine (T6) allowed shoot elongation with an average of 2.61±0.10 cm, showing a response like that of nicotinic acid. The treatment (T7) containing thiamine, cysteine, and nicotinic acid developed shoots with an elongation of 3.34±0.11 cm. Likewise, the treatment (T8), supplemented with thiamine, cysteine, and pyridoxine, resulted in shoot elongation values of 3.24±0.09 cm, with expanded stems and leaves (Figure 2).

For the case of shoot elongation, the analysis of variance shows a significant difference between treatments. Therefore, Tukey's test was applied, determining the presence of five different groups. It was found that the treatment with pyridoxine (T5) had the highest response, with a shot elongation of 4.02±0.26 cm. Even though the numerical value for elongation in treatment T5 was the highest, the treatment (T8) in the presence of thiamine, cysteine, and pyridoxine resulted in shoots with well-developed stems, internodes, and expanded leaves, reaching a length of 3.24±0.09 cm (Table 3).

It is interesting to note that explants from nodal segments of *S. rebaudiana* Morita II have been used in biotechnological tissue culture processes to obtain shoots, but generally with the application of plant growth hormones. According to Macías Hernández (2019), who tested various growth regulators with explants from nodal segments, it was the Murashige and Skoog culture medium with 1 mg/L of BAP that allowed the production of shoots with average values of 4.4 cm in height and 5.8 shoots per explant after 4 weeks of incubation.

In this study, a phytohormone-like effect was observed in the generation and elongation of shoots, exerted by the presence of the B-type vitamins evaluated in the organogenic response of the explant, as shown in Figure 1, treatments T2, T4, and T5 in the induction of shoot formation. According to the results, thiamine increases the number of shoots in *S. rebaudiana* Morita II, but does not promote their elongation. Thiamine in *Calendula officinalis* L. not only increases the number of floral stems but also

Table 3. Statistical Analysis of Shoot Elongation Generated.

Treatment	Average length*	Typical error
T5	4.024 e	0.25939
T7	3.336 d	0.10489
T8	3.239 d	0.07506
T4	2.616 c	0.13998
T6	2.610 c	0.10304
T2	1.413 b	0.05481
T3	0.953 b	0.06241
T1	0.271 a	0.07506

*Length in cm; Tukey (HSD) $\alpha=0.05$; Means for groups in homogeneous subsets are displayed; N=60 based on the harmonic mean of the sample size; Group sizes are unequal. The harmonic mean of the group sizes will be used. Type I error levels are not guaranteed.

enhances their length (Soltani, Saffari, & Maghsoudi Moud, 2014); in *Phoenix dactylifera* L., this vitamin boosts the formation of somatic embryos depending on its concentration (Al Khayri, 2001). In *Triticum aestivum*, thiamine increases seedling length when present at low concentrations; however, at high concentrations, it reduces their length (Misratia & Mansur, 2018).

Cysteine also promotes the increase in the number of shoots of *S. rebaudiana* Morita II, with little stimulation of their elongation. In *Saccharum officinarum* L., cysteine at low concentrations increases the number of shoots, while a contrary effect is observed at higher concentrations (Asad, Arshad, Mansoor, & Zafar, 2009). On the other hand, in *Malus domestica* Borkh., the number of shoots increases; however, it does not stimulate elongation (Sotiropoulos, Dimassi, & Therios, 2005), which aligns with what was observed in Morita II.

Nicotinic acid in the medium (T4) produced a lower number of shoots compared to thiamine and cysteine but allowed for their elongation. In *Solanum tuberosum*, nicotinic acid does not increase the number of shoots in the Mozart variety; however, it promotes the elongation of shoots (Yaseen, Toma, & Carbonera, 2017), as seen in the case of Morita II. Similarly, the elongation effect of nicotinic acid was observed in *Triticum aestivum*, where seedlings increase in length at low concentrations, but their length may decrease if used at high concentrations (Misratia & Mansur, 2018). On the other hand, the medium supplemented with pyridoxine (T5) decreased the formation of shoots per explant of Morita II compared to thiamine; however, it exhibited the highest elongation value. This is contrary to the findings by Yaseen F. K. *et al*. (2017), where *Solanum tuberosum*, variety Mozart, did not see an increase in either the number or length of shoots per nodal segment. It is similar to the response found by Soltani Y. *et al*. (2012), where the foliar application of pyridoxine in *Calendula officinalis* L. plants increased the length of their stems. This suggests that both B vitamins and cysteine can generate a different physiological effect depending on the concentration used and the plant species they act upon.

The combination of B vitamins in *Hypericum perforatum*, when thiamine is at a low concentration (0.1 mg/L) compared to nicotinic acid (50 mg/L) and pyridoxine (50 mg/L), results in a lower number of shoots. However, when the concentration of thiamine is increased (10 mg/L), the number of shoots per explant increases. Conversely, when the thiamine concentration is 0.1 mg/L and the nicotinic acid concentration is 0.5 mg/L, without using pyridoxine, the shoots increase in length; but when thiamine is at 10 mg/L, the length of the shoots decreases. This is contrary to the case where the concentration of nicotinic acid is increased to 50 mg/L while maintaining thiamine at 0.1 mg/L, resulting in a significant increase in shoot length (Khakpour, Motallebi Azar, Bahman, Alizadeh Salte, & Hasani, 2015). In *Solanum tuberosum*, the number of shoots also increases when the thiamine concentration increases from 0.1 to 10 mg/L in the presence of pyridoxine (50 mg/L) and nicotinic acid (50 mg/L). The length of the shoots decreases when the thiamine concentration increases from 0.1 to 10 mg/L with pyridoxine (0.5 mg/L); however, when the concentration of the latter increases from 0.5 to 50 mg/L, the shoots elongate significantly (Kazemiani & Motallebi Azar, 2015). This indicates that thiamine induces the formation of multiple shoots without promoting elongation. In contrast, nicotinic acid and pyridoxine induce elongation in the shoots. A similar combined effect is observed for *S. rebaudiana* Morita II, where thiamine and cysteine induce the formation of multiple shoots, while nicotinic acid and pyridoxine promote shoot elongation.

Even though the numerical value for shoot elongation in treatment T5 is the highest, treatment T8, which included thiamine, cysteine, and pyridoxine, resulted in an average of 4.5 shoots per explant, with well-defined stem development, internodes, and expanded leaves, reaching a length of 3.24 ± 0.09 cm (Table 7). This is like the results obtained by Macías Hernández (2019) using the Murashige and Skoog medium supplemented with 1 mg/L of BAP. Regarding KIN and BAP, it has been documented that these synthetic cytokinins are involved in cell division, callus formation, organ development, and the induction of somatic embryogenesis from plant tissues (Thilaga *et al.*, 2013; Pasternak *et al.*, 2024). Currently, researchers have documented part of the reprogramming mechanism for the induction of somatic embryogenesis in coffee, indicating that a preconditioning period of 14 days in seedlings in the presence of the synthetic cytokinin KIN is necessary to increase the production of endogenous growth regulators such as indole-3-acetic acid (IAA) and conjugated auxins IAA-alanine and IAA-glutamic acid (IAA-Ala and IAA-Glu). Subsequently, during the induction of somatic embryogenesis, when KIN is removed from the culture medium, both free and conjugated endogenous auxins decrease drastically, leading to a reprogramming of transcripts *CcTAA1*, *CcYUC1*, and *CcYUC3* involved in the biosynthesis of IAA, which facilitates the induction of the embryogenic response in the presence of BA (6-benzylaminopurine). Two possible mechanisms of action are proposed: a) de novo synthesis for the formation of IAA, and b) the formation of IAA through the hydrolysis of conjugated auxins. It is suggested that the accumulation of conjugated auxins, which increased during the pretreatment in the presence of KIN, enabled the formation of new IAA during the induction of somatic embryogenesis in the presence of BA (Ayil *et al.*, 2013).

In our study on *S. rebaudiana* Morita II, the nodal segments were pretreated for 41 days with KIN, which was subsequently removed. We could suggest that the endogenous levels of conjugated auxins increased in the presence of KIN, preparing or reprogramming the nodal segments for bud formation through either the de novo synthesis pathway or the hydrolysis of conjugated auxins to form new IAA. Together with the vitamins, these mechanisms would facilitate physiological effects such as elongation and the complete development of the bud in *S. rebaudiana* Morita II.

CONCLUSIONS

The Murashige and Skoog culture medium, in the absence of B vitamins and cysteine, does not promote the formation or elongation of buds from nodal segments of *S. rebaudiana* Morita II. The Murashige and Skoog medium must contain any of these organic compounds for bud development per nodal segment; however, not all generate the same response. Thiamine and/or cysteine induce the formation of multiple buds but do not efficiently promote elongation, while nicotinic acid and/or pyridoxine stimulate elongation. Nevertheless, for the buds from nodal segments to develop adequately in stem and leaf expansion, a combination of thiamine, cysteine, and pyridoxine is required. The absence of plant growth regulators in the culture media allowed for the observation of the inductive effect on bud development, where the combination of B vitamins and cysteine appears to act as inducers in the development and formation of buds.

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Colegio de Postgraduados

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

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ABSTRACT

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

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

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

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

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

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

INTRODUCTION

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

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

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

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

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

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

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

MATERIALS AND METHODS

Study Site

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

Selection and Growth of Seedlings

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

Irrigation System and Nutrient Solution

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

Description of Treatments

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

The experiment was arranged as a randomized complete block design.

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

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

Evaluation of Response Variables

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

Table 1. Description of treatments and applied biostimulants.

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

Table 2. Rates of Biostimulants.

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

For Fresh Biomass

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

Yield Variable

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

Evaluation of Biochemical Components

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

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

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

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

RESULTS AND DISCUSSION

Evaluation of Physiological Variables

Plant Height

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

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

Stem Diameter

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

Number of Branches

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

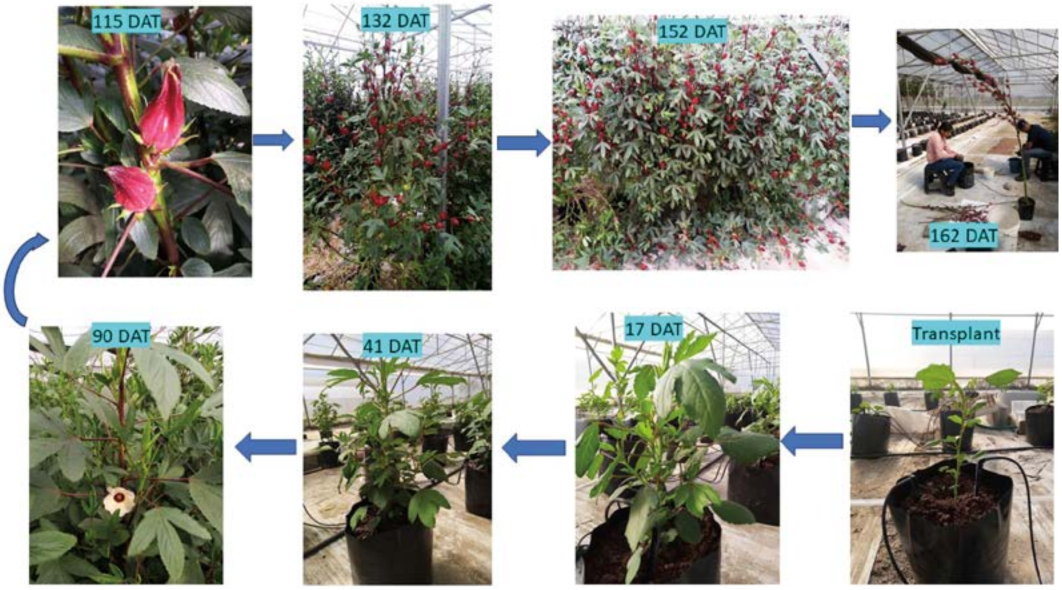

Figure 1. Growth development of roselle Crop.

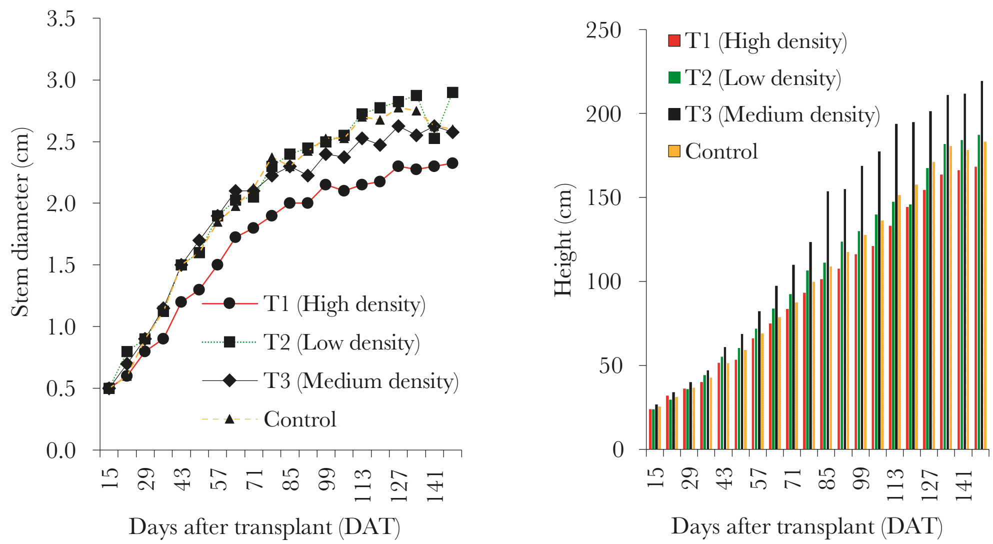

Figure 2. Stem diameter (left) and height (right) of the plant in the treatments.

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

Fresh matter of organs

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

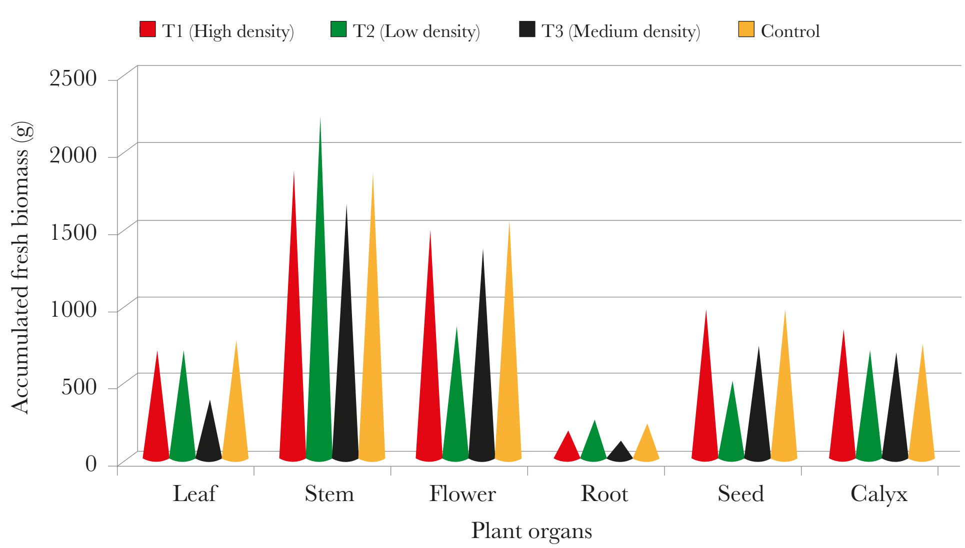

Figure 3. Biomass accumulation in plant organs across treatments.

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

Evaluation of the productive variables

Fresh Calyx Weight

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

Roselle Flower Yield

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

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

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

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

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

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

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

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

Evaluation of quality variables

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

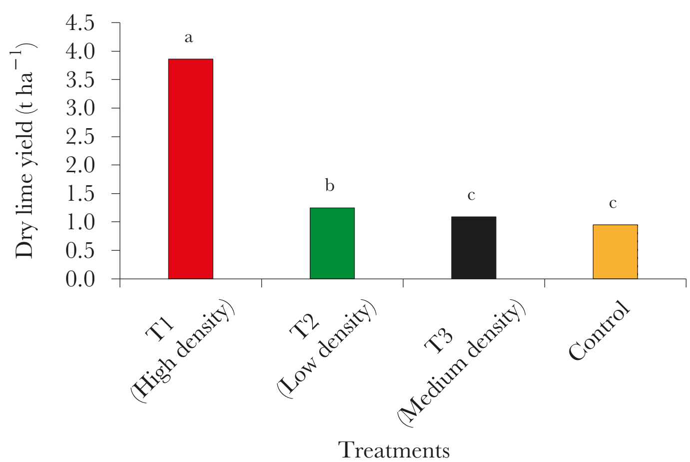

Figure 4. Yields obtained in the treatments.
Bars with different letters are statistically different. Tukey mean separation test (P<0.05).

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

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

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

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

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

CONCLUSIONS

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

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

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

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Colegio de Postgraduados

Hydrolysis of chicken feathers for their use as a protein additive in cattle feed

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ABSTRACT
Objective: To evaluate diverse protein hydrolysis methods of chicken feathers meal on ruminal digestibility for its possible use in the elaboration of protein additives for animal consumption.
Design/Methodology/Approach: Four protein hydrolysis methods of chicken feathers meal were evaluated: thermal hydrolysis (TH), chemical hydrolysis (CH), acid-enzymatic hydrolysis (AH) and allkaline-enzymatic hydrolysis (BH). These methods were compared to chicken feathers meal without any treatment as a control into a completely randomized design.
Results: All hydrolysis methods reduced the protein content in feathers meal when compared to control ($p<0.05$). Crude protein contents were 97.3, 70, 74.1, 75.5 and 87.7 for Control, TH, CH, AH and BH, respectively. However, the highest value in digestibility was observed in CH ($p<0.05$); whereas the other methods showed digestibility values lower to 20% ($p<0.05$).
Implications: Given results show that CH provide higher contents of soluble and digestible protein, as well as higher hydrolysis.
Conclusion: These results demonstrate that chicken feathers meal hydrolyzed by chemical methods shows optimal conditions which makes it suitable for elaboration of protein additives to animal consumption.

Keywords: Keratin, ruminal fermentation, Drought, Chicken feathers meal, Protein additive.

INTRODUCTION

The impacts of climate change are reflected in the agricultural sector in various ways (Kogan, Guo, & Yang, 2019). Prolonged drought periods, coupled with excessive overgrazing, have deteriorated pastures, leading to a reduction in their availability and nutritional value (Allen *et al.*, 2018). In Mexico, 36 million heads of cattle are produced under different systems. However, the droughts experienced in 2023 are atypical and have caused damages not seen in decades (SMN, 2023). Thus, the low nutritional quality of the pastures has forced producers to supplement the diet with protein and energy additives to increase daily weight gains (Itavo *et al.*, 2008; Carvalho *et al.*, 2009). As a result, the demand for protein and energy additives has increased, offering products with a wide range of costs and utilizing a variety of ingredients for their formulation.

On the other hand, the poultry industry in Mexico has experienced significant growth in recent years, reaching a production of 4.6 million tons of poultry meat in 2020 (SIAP, 2022). Consequently, the waste or by-products of the industry have also increased. Such is the case with chicken feathers, which have a production of 192,000 tons per year (Florida-Rofner, 2019; UNA, 2021); these can represent up to 5.2% of the total weight of chicken meat. The main nutrient in feathers is protein, as they contain up to 83% on a dry matter basis; keratin constitutes between 85% and 90% of the total protein found in feathers (Alzamora *et al.*, 2018). However, despite feathers being a potential protein source, their high keratin content limits their use (Parzanese, 2018). Keratin is a fibrous protein that is insoluble and indigestible for monogastric animals, including humans. Additionally, it cannot be broken down by common proteolytic enzymes such as trypsin, pepsin, and papain (Adetunji *et al.*, 2012). However, it can be hydrolyzed by various methods, which may facilitate its utilization in the agricultural industry, particularly in ruminants (Morris *et al.*, 2020). Hydrolysis involves the breaking of the peptide bonds in the protein, leading to the generation of new proteins with lower molecular weight (Sánchez-Villafuerte, 2018). In this sense, hydrolyzed keratin can be used as a source of protein. Based on the above, the objective of this study was to evaluate various methods of hydrolyzing chicken feathers and their potential use as a protein feed for cattle.

MATERIALS AND METHODS

Study Site

The study was conducted in the laboratory of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) at the Juárez University of the State of Durango (N 23° 57' 21.535", W 104° 34' 24.419") and in the Graduate Unit for Research and Technological Development at the Technological Institute of Durango, in Durango, Mexico (N 24° 03' 60.756", W 104° 64' 87.926").

Obtaining and Treatment of Feathers

Chicken feathers were collected from a poultry farm located in the municipality of Durango, Durango. After being washed with running water and commercial detergent, they were dried in a convection oven (FE-294A, Felisa, Mexico) at 55 °C for 48 hours. Subsequently, they were ground using a Willey Mill 4 (Thomas Scientific, USA) and sieved with a 2 mm mesh to obtain feather meal, which was used in the different hydrolysis methods. Additionally, untreated ground feather meal was used as a control.

Thermal Hydrolysis

To carry out thermal hydrolysis (TH), the feather meal was subjected to a sterilization process for 5 hours at 115 °C and 1 atm of pressure. Subsequently, the meal was dried in a convection oven (FE-294A, Felisa, Mexico) for 48 hours at 55 °C for further analysis (Papadopoulos, 1985).

Chemical Hydrolysis

In the chemical hydrolysis (CH) process, 150 g of feather meal were immersed in 600 ml of 1 M sodium hydroxide (NaOH) for 36 hours at 25 °C. Subsequently, the pH was neutralized with a 1 M acetic acid solution (Bauza *et al.*, 2009). The meal was then dried in a convection oven (FE-294A, Felisa, Mexico) for 48 hours at 55 °C for further analysis.

Enzymatic Hydrolysis

Commercial proteolytic enzymes donated by a local company (ENZIQUIM, Mexico) were used. According to the technique described by Viloria *et al.* (2019), the chicken feather meal was immersed in a 0.1 N NaOH solution (pH=8) for alkaline enzymatic hydrolysis (BH) and in a 20% v/v H_2SO_4 solution (pH=3) for acid enzymatic hydrolysis (AH), respectively. For BH, 380 μL of alkaline enzyme (Protease HA 2x, ENZIQUIM, Mexico) was added for every 10 g of feather meal, while for AH, 380 mg of acid enzyme (Acid Protease 25,000, ENZIQUIM, Mexico) was added for every 10 g of feather meal. Both hydrolysis processes were incubated with agitation (200 rpm) at a controlled temperature of 55 °C for 4 hours; the pH was adjusted every 30 minutes during each process. Once the incubation and agitation were completed, the meals were filtered and dried at 55 °C for 48 hours for further analysis.

Protein Determination

The products obtained from the different hydrolysis processes were subjected to crude protein analysis (AOAC, 2001). Additionally, the feather meal was also analyzed for organic protein using the Bradford method, with bovine serum albumin (BSA) used for the calibration curve (Bradford, 1976).

All hydrolyzed meals were also analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) in a Mini-PROTEAN® Tetra cell electrophoresis chamber. The applied potential difference for the electrophoretic run was 25 volts, and the time employed was 2.5 hours. Protein staining was performed using Coomassie Brilliant Blue R-250, and destaining was conducted with acetic acid and methanol diluted in distilled water (Hernández *et al.*, 2012).

***In Vitro* Digestibility**

To evaluate the *in vitro* dry matter digestibility (IVDMD), a Daisy incubator (Ankom Technology, USA) was used. Samples were placed in F57 nylon bags (ANKOM, USA) submerged in a mixture of ruminal fluid and buffer solutions at a 1:2 ratio for 48 hours. The ruminal fluid was donated by two Creole cattle with ruminal fistulas, fed a maintenance diet consisting of alfalfa and concentrate (50:50). The analyses were conducted in triplicate and followed the methodology proposed by the manufacturer (Ankom Technology, USA).

Statistical Analysis

The obtained data were analyzed using a completely randomized design. The Shapiro-Wilk test was used to check for normality, and means were compared using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

According to Table 1, the different hydrolysis methods decreased crude protein content compared to feathers with no hydrolysis treatment (Control) ($p<0.05$). The BH treatment recorded the highest protein content, with a value of 87.7%. Conversely, the treatment that lost the most nitrogen and protein was TH ($p<0.05$). These changes can be attributed to the formation of ammonia during the hydrolysis process, which is released as gas (Duong *et al.*, 2019). Consequently, TH shows nitrogen losses close to 30% ($p<0.05$).

In contrast, the other hydrolysis processes (CH, AH, and BH) recorded a lower loss of nitrogen in the form of ammonia, which suggests a greater degree of hydrolysis to lower molecular weight proteins. This is consistent with the findings of Beaubier *et al.* (2019), who published the characteristics and nitrogen losses in the form of ammonia during the hydrolysis of chicken feathers. In addition, the enzymatic hydrolysates exhibited very low keratin degradation. This may be due to a low degree of specificity of the proteolytic enzymes used; the enzymes employed are exo and endopeptidases, but they are not keratinases, which significantly reduces their effectiveness in this type of assay (Guo *et al.*, 2016).

On the other hand, the Bradford technique is a sensitive method that involves the interaction of Coomassie Brilliant Blue G-250 dye with a protein rather than with nitrogen as an element (Kielkopf, Bauer, & Urbatsch, 2020). Therefore, the organic protein values obtained in this study were higher in CH and BH, with 17.3% and 16.6% organic protein, respectively ($p<0.05$); whereas the control treatment recorded 5.3% organic protein ($p<0.05$). Organic protein values indicate a higher content of highly degradable protein in the rumen, as well as a high solubility of the protein. In this regard, Valencia-Andrade (2018) obtained approximately 14% organic protein in feathers using specific keratinase enzymes. This suggests that, although the enzymes used in this study were not specific keratinases, the degree of hydrolysis is similar to that of other assays.

On the other hand, the degree of hydrolysis affects the ruminal digestibility of dry matter ($p<0.05$). The increase in dry matter digestibility observed can be attributed to an increase in keratin hydrolysis in the chemical treatment; conversely, the lower digestibility observed in enzymatic hydrolysis may be caused by the concentration of keratin in

Table 1. Protein and total nitrogen contents in hydrolyzed chicken feather meals by different methods.

Treatments	Crude Protein (%, DM)	Organic Protein (%, DM)	Total Nitrogen (%, DM)	IVDMD (%)
Control	97.3 ± 0.81^{a}	5.3 ± 0.04^{c}	15.5 ± 0.06^{a}	11.6 ± 0.01^{d}
TH	70.0 ± 1.30^{d}	2.3 ± 0.01^{d}	11.0 ± 0.02^{d}	19.9 ± 0.44^{b}
CH	74.1 ± 0.84^{cd}	17.3 ± 0.19^{a}	11.8 ± 0.13^{c}	86.8 ± 0.22^{a}
AH	75.5 ± 1.96^{c}	1.7 ± 0.04^{d}	11.8 ± 0.09^{c}	10.4 ± 0.07^{d}
BH	87.7 ± 0.91^{b}	16.6 ± 0.12^{a}	14.0 ± 0.14^{b}	16.2 ± 0.41^{c}
SEDM	1.05	0.11	0.08	0.28

^{abcd} Different letters in the same column indicate significant differences ($p<0.05$); DM: Dry Matter; IVDMD: *In vitro* Dry Matter Digestibility; TH: Thermal Hydrolysis; CH: Chemical Hydrolysis; AH: Acid Enzymatic Hydrolysis; BH: Alkaline Enzymatic Hydrolysis; SEDM: Standard error of the difference among means.

the sample (Sypka, Jodłowska, & Białkowska, 2021). Additionally, it is known that the microorganisms contained in the rumen are not capable of degrading keratin. Thus, since keratin is the most abundant component in chicken feathers (approximately 70% DM), its hydrolysis involves the breaking of disulfide bonds contained in keratin and the release of soluble proteins and lower molecular weight proteins, as well as the reduction of amino acid chains capable of being degraded in the rumen (Machuca-Loja *et al.*, 2016). For the above reasons, the chemical hydrolysis (CH) was the treatment that showed the greatest keratin degradation compared to the other treatments ($p<0.05$). However, the other hydrolysis treatments also improved the percentage of digestibility.

Figure 1 shows the results of the SDS-PAGE analysis of the hydrolysates. As can be seen, in lanes 2 and 3 of Figure 1a, a faint band at 75 kDa is observed, which represents the keratin content in the untreated feathers (control); it is worth mentioning that keratin has an approximate molecular weight of 70 kDa (Sypka, Jodłowska, & Białkowska, 2021). In lanes 6 and 7, the degradation bands obtained in the chemical hydrolysis (CH) are shown. In these bands, a greater pattern of degradation or a sweep of proteins with lower molecular weight can be observed. The staining in these lanes indicates a higher

Figure 1. SDS-PAGE of the different hydrolyzed feather treatments. 1a: Lane MP: Standard protein marker; Lane 1and 2: whole feathers; Lane 3 and 4: thermal hydrolysates; Lane 5 and 6: chemical hydrolysates; Lane 7 and 8: alkaline enzymatic hydrolysates. 1b: Lane MP: Standard protein marker; Lane 9 and 10: acidic enzymatic hydrolysates; Lane 11 and 12: liquid alkaline enzymatic hydrolysates; Lane 13 and 14: liquid acidic enzymatic hydrolysates.

concentration of peptides, resulting from a greater degree of hydrolysis. Electrophoresis is a technique that allows for an accurate description of proteins. In this regard, Beaubier *et al*. (2019) stated that an important parameter of protein hydrolysis is the molecular weight distribution of the peptides in the hydrolysate, as revealed in SDS-PAGE gels. However, it is worth noting that the sweeps presented in the migration lanes of SDS-PAGE correspond to soluble protein. That is, even if some lanes do not show degradation or coloration as an effect of hydrolysis, it does not imply the absence of protein, as demonstrated by the presence of total nitrogen and crude protein.

The use of these technologies for obtaining protein from keratin represents a sustainable and viable alternative in bovine feeding. Thus, hydrolyzed chicken feather meals could be incorporated into the diets of confined or even pasture-based cattle as feed supplements that provide the necessary amounts of protein for the proper productive and reproductive development of cattle. In this way, the benefits for producers can be reflected in increased weight gain and reduced costs in protein supplementation. However, it is essential to conduct a feasibility economic analysis to substantiate this.

CONCLUSIONS

The chemical hydrolysis process (CH) proved to be more effective in keratin hydrolysis, showing a clear degradation or degradation pattern; in addition, it maintained the highest amount of total nitrogen or crude protein. Furthermore, CH exhibited a digestibility of over 80%, indicating that it is the technique that provides the highest content of soluble and digestible protein for use in the production of additives for ruminant feed.

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New electrostatic theory for the ascent of sap in tall trees

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ABSTRACT
Objective: to propose a new electrostatic theory for sap ascent in tall trees.
Design/Methodology/Approach: we accomplished a detailed review of the different theories published regarding sap ascent by capillary in trees. The new theory proposed herein is based on some scientific studies of physiologists, biophysicists, and other branches of science, which expose the scientific bases of the water movement in the plant, but who have not proposed an integrated framework.
Results: the proposed theory analyzed prior knowledge, then based on it, a new explanation is proposed for the ascent of sap in trees.
Study Limitations/Implications: no field experiments were carried out to verify the increase in sap ascent in trees with heights greater than 40 m.
Findings/Conclusions: this new theory helps to better explain water loss through transpiration and metabolic processes, since both are closely related to the water content in the mobile solution in conducting vessels.

Keywords: ion hydration shells, ice-like polarized water layer, electrostatic potential, mobile solution column.

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INTRODUCTION

An electrostatic theory is proposed to explain the ascent of the sap in tall trees. It was established by considering the existence of ice-like water structure layers present in soils and plants, reported in scientific literature (Ohe *et al.*, 2007; Smolin *et al.*, 2008; Dhopatkar *et al.*, 2016).

The phenomenon is based on a few characteristics of water molecules and their behavior inside soil and plants, as well as the electrostatic fields which they are in contact. When water molecules are in contact with electrostatic force fields existing in soils, ions, or plant tissues, water molecules form a permanent polarized layer with an ice-like structure. This is free of ions and hydrate dissolved substances (Aceves, 1994). This process occurs in

inorganic crystals of clay and in all the hydrophilic organic substances, which are abundant in plant tissues. This ice-like hydration at ordinary temperatures occurs in proteins (Smolin and Daggett 2008) and in practically all cellular surfaces. Bernal (1965) reported that at distances of 10 to 20 Angstrom from the surface of the tissues, water is bonded to them in the form of ice, free of ions.

The presence of permanent ice-like polarized water molecules in membranes pores and the conductive vessels of xylem is fundamental and indispensable in sap ascent from the roots to the leaves of tall trees (Aceves, 1994). It is important to mention that plant life depends on whether they have in their tissues the adsorbed layer of permanent polarized water molecules, with ice-like structure or not, because this layer retains by hydrogen bonds the mobile solution column in the conductive vessels and restore it inside the xylem by capillarity when it is broken due to water scarcity in the soil.

When a plant or part of it loses its polarized water layer, it dies, because it is not possible to restore the mobile water column inside the xylem. In this paper we propose that the ascent of soil solution in tall trees is related with hydration and dehydration processes, as well as with the form how the water molecules are adsorbed inside the soil and plants tissues. This mechanism of change in hydration also produces changes in the water matric potential in each point of soil and plants.

By definition the pure water potential at atmospheric pressure is equal to zero. Therefore, the water potential in a solution is negative, less than zero. The formula for the plant total water potential, with their components is:

$$\Psi_t = \Psi_m + \Psi_e + \Psi_\Pi$$

where: Ψ_t=total water potential; Ψ_m=matric potential; Ψ_e=electrostatic potential and Ψ_Π=osmotic potential.

When plants transpiration stops during the night, and the electrostatic potential is neutralized by hydrogen bonds from the mobile solution column, the value of electrostatic potential becomes zero: $\Psi_e=0$, and the osmotic potential become part of the matric potential. Then without transpiration in equilibrium conditions: $\Psi_t=\Psi_m$ and the whole mobile column is retained against gravity force by hydrogen bonds and electrostatic charges. This mechanism avoids tension forces and problems of cavitation.

In supporting the proposed theory, regarding the osmotic potential Ψ_Π, it is important to consider the following. Ions in soils and inside the plants does not exist in free state; they always have a hydration shell neutralizing their electric charge. Their free energy is less than that of the water molecules contained in the bulk solution, no linked to the ions. They move in soils and plants as a kinetic unit, always from higher to lower hydration shells, or water content. The water shells of the ions are formed by two layers, (Bernal, 1965; Dang *et al.*, 1991; Gouin, 1998; Kropman and Bakker, 2001). The first one, named distortion layer, is directly in contact with the ion; the second is the coordination layer, united with the first layer by hydrogen bonds, it neutralizes electrostatic ions charges, negative or positive (Lyubartsev and Laaksonen, 1996).

The water molecules of the coordination layer are strongly polarized and fixed with the ion. In the distortion layer the water molecules are less polarized and can be exchangeable with other ions shell or bulk water molecules, as well as another electrostatic force fields inside soils and plants; always producing electro neutrality due to the bipolarity of the water molecules. The thickness of these water layers depends of the ion charge density, solution concentration, temperature and pressure. The hydration shells of the ions avoid the each other attraction; when two ions inside the solution are attracted, they form a salt and precipitate. Inside the solution as far as the ions keep their hydration shells, they don't attract each other. This fact is very important because in many theoretical analyses made in different fields of the science, it is supposed that Coulomb's Law applies to measure the attraction force between ions contained in a solution. For example, the double diffuse layer theory; in this instance, is a wrong consideration (Aceves, 2011).

On the other hand, the classic physiology for measuring the osmotic potential considers a true semi permeable membrane, which allows the pass of water molecules but not ions. This kind of membranes does not exist in plants. In plants, the ions transportation process occurring in all their membranes never independently passes solutes and water molecules; they pass together as a kinetic unit, with their hydration shells. Water molecules exist inside the solution uncoordinated with the solutes, also, they pass through the cell membrane, pulled by the electrostatic forces in a mechanism named water drag. The angle (or coefficient) of plant membranes reflection, also called selectivity, depends on the ions hydration cells thickness and their degree of polarization of water molecules. Ions and solutes with minimum hydration shells and water molecules strongly polarized, generally does not penetrate the plant membranes because are repelled by the strongly polarized water molecules, with ice-like structure existing inside the membranes pores. This ion repulsion by cell membranes is more common in plants growing in saline soils. This phenomenon is known as membrane selectivity (Aceves, 2011).

It is very important to point out that the osmosis phenomena have been studied in membranes of isolate cells, submerged in solutions with different types of tonicity, isotonic, hypotonic and hypertonic to observe their behavior. From this observation, it the osmosis phenomenon and ions movement inside the tissues of plants are inferred. When an increment in soil solution concentration occurs, the degree of ions hydration also changes, as well as the dynamics of ions penetration through the membranes. This also changes the water solutes proportion inside and out the plant, and a mechanism occurs inside the cells, known as cell osmotic adjustment, that avoids plasmolysis (Aceves, 2011).

In regard to ions transportation through plant membranes, the concept of osmosis changes when the ions hydration shells are considered. Example: supposing a solution, external to a vegetal membrane, with ions concentration of 2, given by 24 units of solute and 12 of solvent water: $24/12=2$; the internal cell solution concentration equals 8, given by 24 units of solute and 3 of solvent water (Figure 1).

As the classical chemistry establishes, the concentration inside the cell is four times bigger than outside, and to explain how the ions penetrate the membrane, against a concentration gradient, a lot of theories and complicated mechanism have been established. For example, the ion carriers without considering water properties, as very reactive compounds in all the

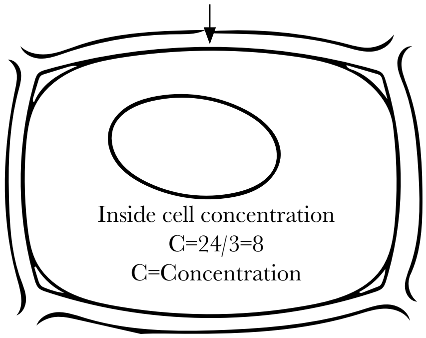

Figure 1. Osmosis as a process of ions hydration shells. Cell model adapted from Azcón-Bieto and Talón (2008).

hydration and dehydration processes including osmosis and solutes transport occurring in soils and inside the plants. The ions keep mobility when they have their hydration shells; as higher is the hydration shell, as bigger the mobility.

Analyzing the example, outside and inside the cell the solute content is the same, 24 units, but there are 4 times more water outside than inside. Considering this situation is reasonable to assume that the ions are four-fold more hydrated outside than inside, and metabolic energy is not needed nor ion carriers for the ions to go inside through the cell membrane channels in a spontaneous process, from higher to lower water content in their hydration shells. Higher concertation inside de cells it does not necessarily mean bigger ions content but less water content. This is another way to see the osmosis phenomenon in the proposed Electrostatic Theory for Sap Ascent in Tall Trees.

Analysis and discussion of the proposed electrostatic theory for sap ascent in tall trees

To explain this theory, it is very important to start by analyzing the mechanism of hydration and dehydration of the ions in the soil and plants solutions (Kropman and Bakker, 2001; Marcus, 2012). Ions in aqueous solution do not exist in a free state; they are surrounded by an envelope or hydration shell with polarized water molecules whose free energy is less than the energy of bulk water molecules (Lyubartsev and Laaksonen, 1996). When an ion with high charge density is in contact with another ion, it can strip it partially or totally from its hydration shell, either in the soil solution or inside the plant (Aceves, 1994; 2011). Water molecules contained in the ion hydration shell can be interchanged with other ions or electric force fields with higher intensity than the ones in contact whit the solution (Aceves, 1994). This is a fundamental principle in dissolved ion mobility and in the explanation of this theory.

The interchange of water molecules between hydration layer ions and dissolved or suspended substances is done constantly. In other words, the ions in the soil solution and inside the plants are subjected to permanent processes of hydration and dehydration (Brownian movements in solutions), and the time of residence of the water molecules in the hydration envelope of an ion, or a dissolved substance is in terms of the charge density or intensity of the electric field; also, concentration, type of ion or substance, temperature, and pressure of the solution.

All ions that lose their minimum hydration shell because of another ion or force field become insoluble and lose their mobility in soils and in plants (Aceves, 1994). Hydration shells of different ions species contained in a solution are different at the same concentration, pressure and temperature. Under the same conditions, some ions get more hydrated than others (the Hofmeister's sequence), in Aceves (1994; 2011). So, in order to explain with greater clarity, the movement and the ascent of ions in solution inside plants, some mechanisms are hypothesized about the way in which water and ions are found inside plant tissues.

Water is found in plant tissues in a similar way as in soils. When water comes into contact with some electric field, a first adsorbed layer of water molecules strongly polarized is formed, with oxygen and hydrogen atoms strongly oriented toward the outside, depending on the electrostatic charge of the tissue surface, and whose structure is similar to that of ice (Aceves, 2011; Dhopatkar *et al.*, 2016). There are not adsorbed ions in this layer, but water molecules are joined, by hydrogen bonds and Van der Waals attraction forces, to the surface of the plant tissues. This cover of polarized water molecules is permanent; its formation starts during seed germination and remains with the approximate same thickness during the entire life period of the plants. This layer of water molecules oriented in an ice-like structure is fundamental for the life of plants and for the passage of organic and inorganic solutes through their plant membranes (Gouin and Kosinski, 1998; Kropman and Bakker, 2001).

The rest of the existing non-strongly polarized ice-like water inside the plant is electrostatic retained and form the mobile water column, associated with mobile solutes, forming their hydration shells or participating in metabolic reactions of hydration and dehydration by water exchange molecules. This mechanism is fundamental for life in all living organisms (Aceves, 1994).

To support the proposed theory, some statements are included:

This entire analysis is based on the classical proposition by Danielli-Davson (1935) as a model of biological membranes, but we modified it to include hydrophilic pores lined by a layer of polarized water molecules and adsorbed in a structure form of ice free of ions (Figure 2).

In order to pass through a biological membrane, an ion must have a suitable hydration shell that allows it to neutralize opposed (of different sign) electrostatic charges which are generated by the adsorbed and polarized water molecules with ice-like structure, within the polar groups of that membrane. If the ion loses water molecules from its hydration shell to another ion or to an electrostatic field, that ion also becomes polarized (by the coordination layer), adopting the same charge (sign) as the polarized water molecules adsorbed in the polar groups of the pore membrane. The ion is then repelled, and does not penetrate (ionic antagonism) since the reflection coefficient equals 1.

The cellular membrane plays the role of regulator of the hydration level of solutes that penetrate the plant. Frequently, the ions do not penetrate through the membrane

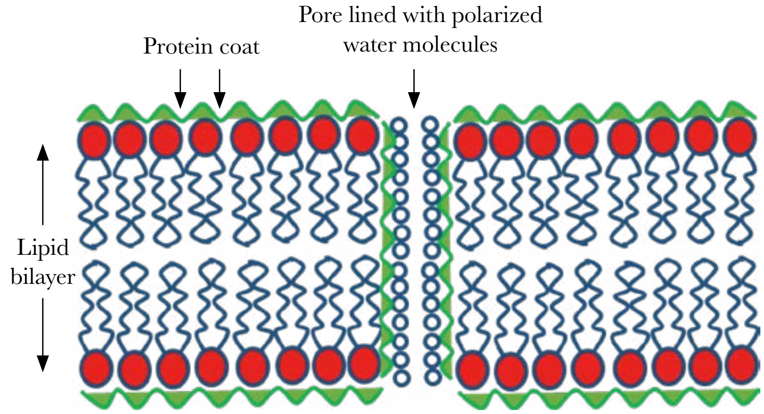

Figure 2. Danielli-Davson (1935) model of the biological membrane, modified to include hydrophilic pores, lined by a layer of polarized water molecules with ice-like structure.

when the soil solution is highly concentrated (saline soils) and the ions just have their minimum hydration shell, which only allows them to remain in solution (Singer and Nicolson, 1972; Aceves, 2011). If ions are found in a diluted solution, as is the case of most soils, the water molecules of their envelopes (distortion layer) can orientate and form hydrogen bonds with the water retained by the membranes in the ice-like structure. This allows the ion to pass through the membrane without being repelled. Hydrated ions always move spontaneously through cell membranes from higher to lower water content on their shells, by diffusion, spontaneous process, without the need of active transport, without ion carriers (Aceves, 2011).

After going through a biological membrane, depending on their charge density, ions can increase or decrease their hydration shells when shared or transferring water molecules; either, interacting with hydration layers of other ions of the internal solution, or with water molecules adsorbed in the tissues. By this means, they obtain or lose mobility inside the plant. Generally, for an ion or organic compound to move through the tissues of any living organism, it must have a minimum required degree of hydration to keep it in solution. That is why membranes become impermeable only when ions do not have the necessary hydration shell to neutralize the electrostatic charges of the polarized water molecules on the inside of membrane pores. In regard to the ion, the degree of selectivity of a membrane depends on its degree of hydration (Green, 1966; Singer and Nicolson, 1972).

New theory proposal

This proposed theory considers that the mechanism of sap ascent in plants, especially in very tall trees, is operating in a permanent and continuous way from the time when seed germinate until the tree reaches considerable heights.

The assumption is that, as the plant grows, one layer of water and one solution column start developing. This layer is formed inside conductive vessels, cell membranes, and tissues by immobile permanent adsorbed water molecules, solutes free, strongly polarized with ice-like structure. This polarized water layer keeps growing together with the plant. This is not a process occurring suddenly, but a continuous and permanent forming layer in all the tissues, during the entire life of the plant (Aceves, 1994; 2011).

The column is formed by mobile solution transporting plant nutrients inside the xylem, it is retained against gravity force by hydrogen bonds formed with the electrostatic charges existing in the permanent layer of polarized water molecules. Due to this, the mobile column is not subject to any acting tension force, produced by plant transpiration; therefore, no cavitation occurs inside the xylem because the only acting forces on the solution column are electrostatic.

This fact is fundamental to understand why no hydrostatic gradients exist that are produced by gravitational water inside the tree, so, no solution gushes out when a trunk is perforated.

The water molecules contained in a mobile solution inside trees, which it is formed by the hydration shells of the solutes, together with the bulk water molecules are in permanent exchange in all directions, in response to the existing electrostatic charges and water content in the tissues. All the solutes move through the cell membranes and in the conductive vessels with their hydration shells. Hydrogen bonds, supporting the whole mobile column by electrostatic forces, can be measured with the pressure chamber designed by Scholander (1958). As thin are the ions hydration shells, the bigger is the retention force due to electrostatic forces. The Scholander pressure chamber measures varies as a function of the water content in the conductive vessels.

The mobile water column is broken when the supplying of the water volume from the soil to the root of a plant occurs at a lesser velocity and amount than that which is lost through transpiration. The plant withers and the continuity of its solution column is broken. When the water level of the soil solution increases again and the bulk water comes into contact with the roots jointly with the hydrated ions, water molecules that do not form part of hydration shells go through with the help of a transport mechanism generated by the tremendous electrostatic potential existing inside roots (acting as water drag force). These hydration envelopes together with hydrated ions that entered the membrane are exchanged from one point to another in an ascending chain process that regenerates the column of the mobile solution, independently of whether the transpiration and metabolic processes are at very low levels. This mechanism upwards, even in conditions of reduced metabolism, is the cause of the appearance of the capillarity phenomena, root pressure, guttation, and exudation of solution in severed stems of trunks. In addition, this mechanism is the cause of the existence of air and water vapor inside the plant ducts, without risking the column of solution would not be reestablished.

When the leaves lose water through transpiration and metabolic process, the water content in the mobile solution decreases inside the upper part of conductive vessels, the electrostatic charges existing in the permanent layer of immobile polarized water molecules show a behavior like small magnets, producing an electrostatic chain reaction by mean of hydrogen bonds, carrying the mobile solution upward, avoiding problems of cavitation and tensile strength; also forming an electrostatic potential gradient inside the conductive vessels and tissues from the leaves to the roots. At the same time a matric water potential is formed from higher to lower water content, from the root to the leaf. The sum of those potentials acting together are what produces the driving force carrying the whole mobile water column upward in small plants and high trees.

It is worth emphasizing that the proposed theory herein stated considers the review of a series of theoretical analyses in regard to molecular form and structure of water and its behavior in all hydration and dehydration reactions, occurring in ions, soil minerals, and physical and biological processes inside the whole plant. It is based on other existing theories and published known facts that have been demonstrated, and accepted as valid, that were cited above in this document. To which we can add Sterling (1966), Garrison and Anderson (1975), Tyree (1997), Canny (1998), Stiller and Sperry (1999), Koch *et al.* (2004), Podgornik (2012), Kumar (2016), and Chaplin (2018).

CONCLUSIONS

The proposed theory states that as the plant grows, a layer of water and a column of particular-behavior solution begin to develop. The water layer is formed within the conductive vessels by permanently immobile, solute-free, strongly polarized adsorbed water molecules with an ice-like structure. The layer of polarized water continues to grow along with the tree. This is why water can ascent in trees taller than one-hundred meters.

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Production Analysis of Corn (*Zea mays* L.) in Agricultural District VII Valle de Bravo, State of Mexico

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ABSTRACT

Objective: Analyze corn production in Rural Development District (RDD) 7 "Valle de Bravo", State of Mexico, focusing on the relationship between yield and sown area, as well as identifying factors that may affect this production.

Design/methodology/approach: Data from the Agri-Food and Fisheries Information System (SIAP) on sown area, production, and yield of corn (2003-2021) were used. Additionally, questionnaires were provided to RRD 7 producers. Statistical analyses were carried out with SPSS software and simple linear regression models were applied to evaluate productivity and associated factors.

Results: Corn yield showed linear growth; however, the sown area decreased as farmers have dedicated themselves to producing more profitable crops. Guaranteed prices per ton were deemed inadequate and high input costs hindered agricultural production.

Limitations: Farmers faced challenges such as high production costs and limited access to financial resources. Government programs offer some support for corn production; however, this is insufficient to address the structural problems of the sector.

Conclusions: A comprehensive strategy is needed to address the systemic challenges facing the corn sector. This includes measures to improve productivity, reduce production costs, ensure fair prices, promote crop diversification and enhance access to resources and technologies that guarantee the long-term sustainability of the agri-food sector.

Keywords: Corn, production, yield, government subsidies.

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INTRODUCTION

Corn, an agricultural crop of global importance, stands out for its multiple uses in human and animal food, as well as in industry. Its versatility and nutritional value have made it a highly influential product in international markets (Bada-Carbajal *et al.*, 2021). The United States and China lead global corn production, while Mexico ranks as the

eighth-largest producer worldwide. The historical importance of corn in Mexico is undeniable; as the center of origin of this cereal, its *per capita* consumption is significant, feeding more than 100 million Mexicans. In addition to its cultural relevance, corn plays a crucial role in the industry, serving as a raw material for a wide range of products and by-products (Basilio-Peña *et al.*, 2019).

Despite Mexico's significant corn production, the country faces a challenge in meeting domestic demand. Approximately 41% of national consumption is met through imports from the United States and Brazil (Tadeo Robledo *et al.*, 2015; SIAP, 2019). This dependence on imports has been increasing, highlighting the influence of the expected rural average price on the domestic market (Moreno Sáenz *et al.*, 2016). In 2021, national corn production reached 27,503,477 tons (t), cultivated over an area of 7,309,546 hectares (ha). The states of Sinaloa and Jalisco stood out as the main producers in Mexico, while the State of Mexico ranked third nationally. However, productivity in the State of Mexico remains below other states, with an average yield of 3.96 t ha^{-1} (SIAP, 2022). This productivity disparity results in a significant deficit in corn consumption in the State of Mexico, which is the second-largest corn consumer in the country after Jalisco. This gap between production and consumption is reflected in an unmet demand of 389,310 tons of white corn and 741,740 tons of yellow corn, accounting for 34% and 66% respectively (García Salazar *et al.*, 2016).

The State of Mexico, with its 125 municipalities and 11 regional delegations, plays a crucial role in the primary sector, contributing 5.7% of the country's employment. It is divided into 8 Rural Development Districts (RDD), with RDD 7 "Valle de Bravo" standing out in the state for its corn production, ranking third in 2021 with a total of 153,935.94 tons and an average yield of 3.71 t ha^{-1}. This yield is slightly above the national average but significantly lower than the yields in Sinaloa and Jalisco (SIAP, 2022). The historically low productivity in Mexico, especially in regions such as the central and southeastern parts of the country, is attributed to spatial, temporal, and cultural issues. Additionally, 80% of corn producers in these areas farm under rainfed conditions on small plots, using low-cost and minimally mechanized farming systems (Santillán Fernández *et al.*, 2022).

Therefore, addressing this challenge requires a comprehensive approach that includes improving corn production and yield through the use of improved seeds, fertilizers, and agrochemicals. Technical assistance also plays a crucial role in this process, promoted through legislation, federal subsidy programs, and the creation of institutions such as the Agricultural, Aquaculture, and Forestry Research and Training Institute of the State of Mexico (ICAMEX) (SADER, 2018; Ramírez Jaspeado *et al.*, 2020). In this context, the present article focuses on analyzing corn production in RDD 7 "Valle de Bravo," concentrating on the relationship between yields and the planted area, as well as identifying the factors that may affect such production.

MATERIALS AND METHODS

Study Site

The study was conducted in RDD 7 "Valle de Bravo," composed of 12 municipalities in the State of Mexico (Table 1). The corn grown in this RDD is adapted to high valley areas, with elevations ranging from 2200 to 2600 meters above sea level, a temperate subhumid

Table 1. Geographic Coordinates of the Municipalities of RDD 7 "Valle de Bravo," State of Mexico.

Municipality	Latitude	Longitude
Amanalco	19.1936 N	100.0753 W
Donato Guerra	19.3506 N	100.2072 W
Ixtapan del Oro	19.1819 N	100.2100 W
Otzoloapan	19.1112 N	100.3054 W
Santo Tomás	19.2834 N	100.2093 W
Valle de Bravo	19.1924 N	100.1330 W
Villa de Allende	19.3573 N	100.1259 W
Vila Victoria	19.4383 N	100.0189 W
Zacazonapan	19.0833 N	100.2667 W
Temascaltepec	19.0378 N	100.0283 W
San Simón de Guerrero	18.9917 N	100.1783 W
Luvianos	18.9765 N	100.3083 W

Note: The coordinates are approximate and correspond to the location of the centers of each municipality according to the data source INEGI, (2022).

climate with an average annual temperature of 15 °C, and annual precipitation ranging from 900 to 1600 mm (INEGI, 2022).

Data Collection

The productivity analysis was conducted using data from SIAP on the variables of planted area (ha), harvested area (t), production volume (t), and yield (t ha^{-1}) from the eight Rural Development Districts that make up the State of Mexico during the period 2003-2021. In addition, a directed sampling was carried out during the first semester of 2021 using questionnaires for corn producers. These questionnaires addressed various aspects, such as the area allocated for corn planting, the destination of the production (self-consumption or sale), the seed varieties used (local or hybrid), field yield (t ha^{-1}), production value (\$ t^{-1}), and production costs (\$ ha^{-1}).

Statistical Analysis

The differences in productivity among the districts were determined through an analysis of variance and a Tukey mean comparison test with a 95% confidence level. To delineate the evolution of corn production in RDD 7 "Valle de Bravo," simple linear regression models were fitted to the variables of planted area, production volume, and yield. The statistical analyses were performed using IBM SPSS software.

RESULTS AND DISCUSSION

Corn grain yield in RDD 7 "Valle de Bravo" showed a linear growth trend during the period 2003-2021, with a minimum yield recorded in 2006 (2.73 t ha^{-1}) and a maximum in 2016 (4.52 t ha^{-1}). This upward trend in yields was associated with the National Program for the Sustainable Modernization of Traditional Agriculture (MasAgro), which was formalized in the State of Mexico in 2012, promoting the use of improved seeds and fertilizers (CIMMYT-MASAGRO, 2012; Ramírez Jaspeado *et al.*, 2020). However, starting in 2017, the average yield in the State of Mexico decreased to 3.66 t ha^{-1}, while in RDD 7 "Valle de Bravo" the difference was smaller (3.89 t ha^{-1} in the 2013-2017 period and 3.76 t ha^{-1} in 2017-2021). Despite the increase in productivity, yields in the State of Mexico and in RDD 7 "Valle de Bravo" remained lower compared to the main producing states in the country, such as Jalisco (6.76 t ha^{-1}) and Sinaloa (11.32 t ha^{-1}). In contrast to the historical increase in yield, production values showed a slight downward trend over the same period, decreasing from a production of 208,707 t in 2003 to 153,936 t in 2021. However, there were years in the last decade with high production levels, such as in 2016 and 2017 (220,434 t and 202,364 t, respectively). The gradual decrease in production, even considering the increase in yields, may be mainly due to the decline in the linear trend of the planted corn area (R=0.813), which decreased from 61,702 ha in 2003 to 41,507 ha in 2023 (Figure 1).

The decrease in the area allocated to corn, among other factors, occurred due to the crop change implemented by farmers in search of higher economic returns.

In the last decade, crops such as forage oats increased from a planted area of 12,344 ha in 2010 to 19,971 ha in 2020. Other examples include vegetables, whose cultivation is of interest to farmers because, although production costs are higher, production values also significantly increased. Another example is the potato crop, which reached 836 ha planted in 2010, while by 2020 this area increased to 1,578 ha. The production value showed an upward trend due to the increase in the price per ton of corn, which rose from $1,424.83 MXN in 2003 to $6,051.30 MXN in 2021 (CEDRSSA, 2019) (Figure 2). However, this increase does not necessarily translate into higher profits for producers, as inflation and the rise in input costs must also be considered.

The productivity of RDD 7 "Valle de Bravo" compared to other districts in the State of Mexico shows statistically similar yields, with the exception of Tejupilco. The RDDs of Atlacomulco and Toluca lead in planted area, harvested area, and production volume, although this is due to a larger cultivated area and concentrated agricultural resources. Meanwhile, the RDDs of Coatepec Harinas and Texcoco have smaller cultivated areas but higher yields, comparable to other RDDs, suggesting greater productivity that can be attributed to favorable edaphoclimatic conditions (Table 2).

The questionnaire results showed that farmers in RDD 7 "Valle de Bravo" plant an average of 2.5 ha per cropping cycle, with 77% using conventional machinery, although a significant percentage still uses manual tillage (17%). Currently, it is possible to request financial subsidies for the acquisition of tractors, motorized machinery, and precision agriculture implements through the Ministry of Agriculture and Rural Development (SADER) and the Ministry of the Field (SECAMPO). However, 86% of the interviewed producers have not received this type of support, due to a lack of financial capital to cover the non-subsidized portion by government institutions. Regarding seed supply, only 26% of farmers obtain seeds through the self-production of native varieties, while 56% of producers do so through two companies: Asgrow Monsanto, with varieties "H50," "Niebla," and "Faisán"; and the Mexican company Aspros with cultivars "722" and

Figure 1. Variation in production values (a), planted area (b), and yield (c) during the period (2003-2021) in RDD 7 "Valle de Bravo," State of Mexico. The graphs show the values for hectares planted (a), total tons harvested (b), and the average tons harvested per hectare (c), respectively.

"Cherokee" (Figure 3). This represents an oligopoly issue, as according to the ProNacE (National Strategic Programs) "Soberanía alimentaria" a small group of companies sell the inputs for the agricultural cycle, which disadvantages self-consumption production and product commercialization (CONAHCYT, 2022). The reduction of self-production leads to increased production costs, as each cycle requires purchasing seed due to the fact that hybrid cultivars are male-sterile.

The government support program PROCAMPO, now called "Producción para el Bienestar" (PpB), has mainly focused on the acquisition of fertilizers and seeds, and has been

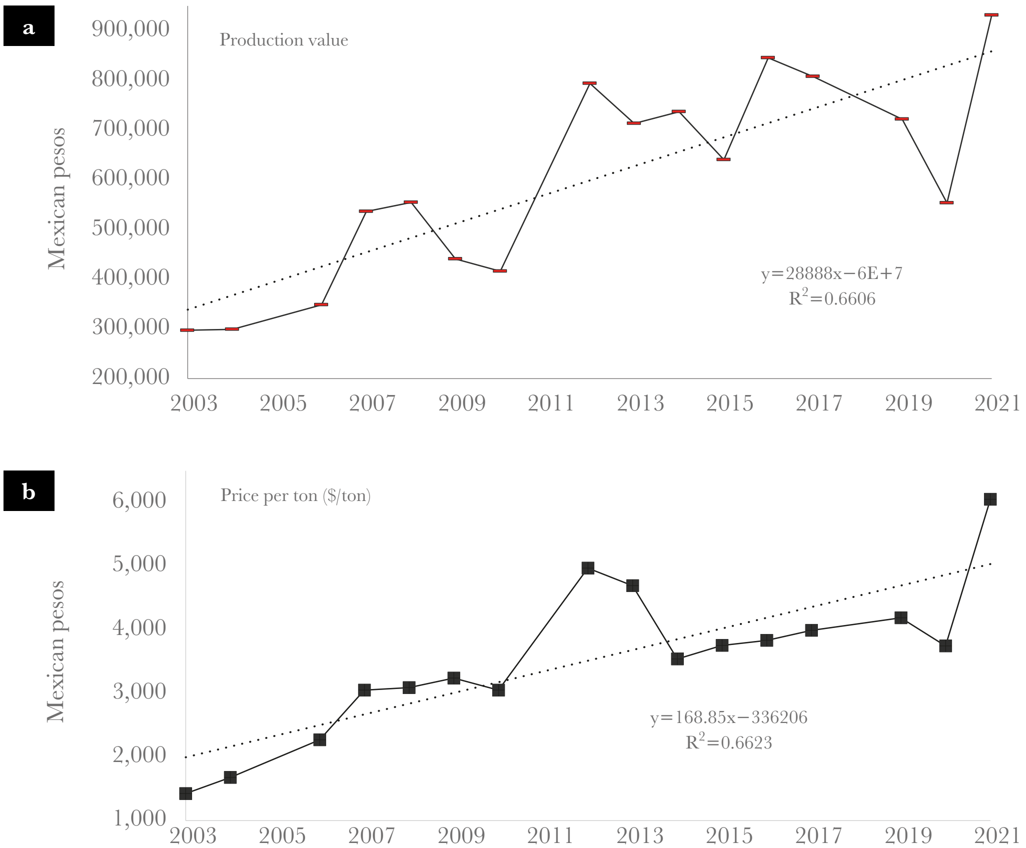

Figure 2. Variation in production value (a) and price per ton of corn (b) during the years 2003-2021 in the Valle de Bravo district, State of Mexico. Both graphs express their values in Mexican pesos.

received by 84% of the surveyed farmers. However, 96% of the beneficiaries considered that the amounts provided did not cover all the costs associated with agricultural production. The subsidies granted amounted to $1,600.00 pesos per hectare, representing 12.29% of the total maize production value. The government support program PROCAMPO,

Table 2. Historical comparison by RDD of the State of Mexico in corn production (2003-2021).

	District	Sown area (ha)	Harvested area (ha)	Production value (Thousands of $ mxn)	Production (ton)	Yield (ton ha^{-1})	Price per ton ($ ton^{-1})
					***	***	ns
1	Toluca	122598.79	115359.25	1467274.57	455330.40b	3.95a	3246.4a
2	Zumpango	44987.24	41812.99	361885.66	109413.94c	2.60bc	3367.32a
3	Texcoco	34784.52	34404.58	379774.37	112420.80c	3.28ab	3333.56a
4	Tejupilco	49716.79	49574.38	469165.08	124815.98c	2.50c	3880.12a
5	Atlacomulco	154715.52	148832.17	1797786.11	542908.81a	3.58a	3225.15a
6	Coatepec	33315.17	303094.65	392374.83	110777.31c	3.35a	3478.87a
7	Valle de Bravo	49461.24	47917.72	555683.12	158885.71c	3.33ab	3493.20a
8	Jilotepec	49181.17	45899.85	494451.72	151623.98c	3.22abc	3282.52a

Means with different letters in a column are statistically different ($p \leq 0.05$). *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ns: not significant.

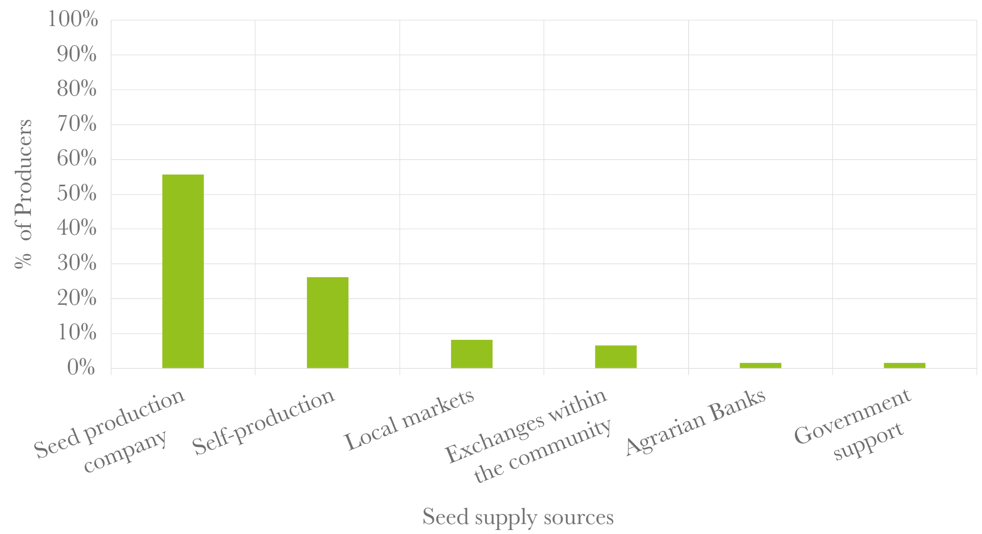

Figure 3. Sources of seed supply for farmers in the DDR 7 "Valle de Bravo."

now called "Producción para el Bienestar" (PpB), has mainly focused on the acquisition of fertilizers and seeds, and has been received by 84% of the surveyed farmers. However, 96% of the beneficiaries considered that the amounts provided did not cover all the costs associated with agricultural production. The subsidies granted amounted to $1,600.00 pesos per hectare, representing 12.29% of the total maize production value. The divided opinions on the usefulness of technical advice reflect the need for greater dissemination and quality in its delivery, with 19% of farmers perceiving it as exclusively focused on product sales. In this regard, according to CEDRSSA (2020b), farmers face multiple productivity challenges that require a comprehensive strategy, suggesting coordination between the Production for Wellbeing Program (PpB) and other SADER programs to address these difficulties and move towards food self-sufficiency. CONEVAL (2020), on the other hand, proposes allocating more resources to the PpB to improve access to training, technical assistance, and formal credit, as programs focused solely on economic incentives, such as PROAGRO, have not shown a significant increase in agricultural yield.

Financing for corn production, according to producers, comes primarily from their own resources (54%), combined with public subsidies (40%), and through research institutions (6%). However, despite the efforts made by the producers, 98% of them believed that the prices are inadequate as they leave little profit margin. Additionally, 24% thought that a fair price would be $10,000.00 pesos per ton of grain, followed by $7,000.00 and $8,000.00 pesos (12% each), while 10% believed a fair price would be $25,000.00 pesos per ton. The guaranteed price for a ton of corn in 2022 was $6,278.00 pesos, according to the Diario Oficial de la Federación (SADER, 2022). It is important to note that 3% of the respondents believe that the liberalization of corn prices is ideal; however, this may not be the best strategy, as leaving the price to the discretion of sellers would automatically raise the price of the crop and, consequently, the price of products made from the grain. At the same time, the cost of food products derived directly and indirectly from corn, such as meat, would increase, creating a chain reaction of rising prices across various products. Ultimately, according to the interviewed producers, the factors that they consider most difficult for the development of agricultural activities are: the high costs of seeds and fertilizers (76%), followed by the high risks and low profitability of the crop (68%), as well as the lack of financial resources for inputs (58%) (Table 3).

Table 3. Factors hindering the development of agricultural activities.

Factors	High	Low	Medium	Irrelevant	Total
lack of technological information.	34%	30%	28%	8%	100%
High risk and low profitability of the crop.	68%	6%	26%	0%	100%
Inadequate infrastructure.	50%	4%	26%	20%	100%
Presence of intermediaries.	52%	16%	14%	18%	100%
Lack of resources for inputs.	58%	4%	34%	4%	100%
High seed and fertilizer costs.	76%	8%	12%	4%	100%
Lack of access to inputs and machinery.	28%	22%	34%	16%	100%
Weather claims.	62%	14%	24%	0%	100%
Difficulty in accessing financial support.	16%	32%	22%	30%	100%
Other Factors.	0%	4%	0%	96%	100%

CONCLUSIONS

The disparity between the supply and demand of maize in the State of Mexico remains a significant issue, leading to a heavy reliance on imports, especially from the United States and Brazil. This highlights the need to strengthen domestic production to ensure food security. The DDR 7 "Valle de Bravo" has experienced a gradual increase in maize yields, partly due to programs like the Sustainable Modernization of Traditional Agriculture. However, yields remain lower than in other leading production states, such as Jalisco and Sinaloa.

The decrease in the area dedicated to maize in the DDR 7 reflects a shift in farmers' preferences towards more profitable crops, such as oats and vegetables, due to high input costs and low maize sale prices. Farmers face challenges that require a comprehensive strategy to increase productivity, reduce costs, and promote crop diversification to ensure the sustainability of the sector.

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Colegio de Postgraduados

Genetic Improvement of Miahuateco Chili (*Capsicum annuum* L.) (Solanaceae) through Gamma Radiation of ^{60}Co

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ABSTRACT

Objective: To improve genetic attributes in Miahuateco chili (*Capsicum annuum* L.) through gamma irradiation with ^{60}Co.

Design/Methodology/Approach: Seeds were irradiated at doses of 0, 100, 200, and 300 Gy at the National Institute of Nuclear Research. The evaluated variables were LD_{50}, agronomic yield, plant height, polar and equatorial diameters, percentage of aborted flowers, number of branches, and plant color, which were assessed under a completely randomized design (4×4)=16 experimental units.

Results: The results indicate that the application of 200 Gy induces higher agronomic yield. The LD_{50} is reached at 145 Gy. High doses of gamma irradiation caused a high percentage of aborted flowers, as well as chlorosis in the plant.

Study Limitations/Implications: The seeds used in this project were landraces, which exhibited significant heterogeneity that could have affected flowering and fruiting. However, this was not a substantial factor for this project.

Findings/Conclusions: It is concluded that 200 Gy of gamma radiation from ^{60}Co induces genetic variability in the species, improving certain agronomic attributes of interest.

Keywords: LD_{50}, agronomic yield, flower abortion, color.

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INTRODUCTION

Mexico, according to Vavilov, is the center of origin for many cultivated plants, among which chili (*Capsicum annuum* L.) can be cited (Toledo *et al.*, 2011). This plant is a vegetable whose fruit is consumed, and after the red tomato, it is the most cultivated in Mexico (Acosta and Chávez, 2003). Moreover, it has been known since pre-Columbian times and was cultivated by ancient pre-Hispanic cultures in Mesoamerica (Castellón *et al.*, 2012). There are multiple varieties of this fruit, varying in shape and color (yellow, green, red, and brown), as well as in their pungency and flavors (Aguirre and Muñoz, 2015). Some are endemic to certain areas in the central region of the country because they are adapted to the ecological characteristics of the places where they are cultivated, such as habanero chili, which is adapted to the soil conditions, precipitation,

and temperature specific to the Yucatán Peninsula (Ruíz *et al.*, 2011). Miahuateco chili is an example of the above, as it is endemic to the Santiago Miahuatlán area in Puebla. The species is highly valued by the local inhabitants, as it is used in the preparation of traditional mole Poblano, typical of the region (Pérez *et al.*, 2017). Currently, this species has been losing its typical fruit characteristics, such as shape, color, and flavor, due to genetic erosion, which threatens the loss of agricultural plant species due to cross-hybridization with other chiles, such as Poblano (Secretariat of the Convention on Biological Diversity, 2008). A morphological characteristic of the fruit that has been observed in recent years in Miahuateco chili is the presence of a "pedicel cavity," commonly referred to as "Cajete" by the local producers, which is typical of Poblano chili. This demonstrates the hybridization between the two species. This characteristic causes water accumulation at the base of the fruit, leading to rot, and as the pathogen translocates, it also damages the root due to the interaction between water and the pathogens *Fusarium* and *Pythium*, which reduce agronomic yield and quality (Pérez *et al.*, 2017; Velásquez *et al.*, 2001). Gamma rays from certain radioactive elements, such as ^{60}Co, have proven to be an important tool in genetic improvement for both vegetable species and staple and ornamental crops like Polianthes. Additionally, it is free from the restrictions and regulations imposed on genetically modified organisms (Álvarez *et al.*, 2017; Estrada *et al.*, 2011), as it induces somatic mutations through irradiation of the DNA molecule, resulting in molecular mutations that can be observed in the short term directly in the phenotype (Caro *et al.*, 2012), saving time compared to other techniques such as crossing. This genetic improvement technique has been termed radiation-induced mutagenesis. Therefore, the main objective of the present research was to improve certain agronomic attributes in the fruit of Miahuateco chili through four levels of gamma irradiation with ^{60}Co. The proposed hypothesis was that the application of 300 Gy of gamma irradiation from ^{60}Co would induce morphological changes in the V1 generation of Miahuateco chili fruits.

MATERIALS AND METHODS

Location of the Study Area

The research was conducted under greenhouse conditions at the Technological University of Tehuacán, located at 18° 24' 51" north latitude, 97° 20' 00" west longitude, and at an altitude of 1,409 meters.

Germplasm, Seed Irradiation, and Planting

The germplasm consisted of open-pollinated Miahuateco chili seeds, derived from a stratified mass selection, which were irradiated at the National Institute of Nuclear Research (ININ, its acronym in Spanish) using the Transelektro LGI-01. The irradiated seeds were sown in polystyrene trays with 200 cavities, using peat moss as the substrate. When the plants reached a height of 12 cm, they were transplanted into a Mini greenhouse under a topological arrangement of (0.80×0.30 m), resulting in a population density of 4.16 plants per m^2.

Fertilization and Crop Management

The fertilization used was 150-100-200 of (NPK), using urea (46% N), single superphosphate (21% P_2O_5), and potassium chloride (60% K_2O) as sources of these nutrients, which were applied 14 days after transplanting. Weed management was done manually. Copper oxychloride was applied at a rate of 1 g L^{-1} as protection against fungal diseases. To control *Bemisia tabaci* (HEMIPTERA), imidacloprid was applied at 0.3 L ha^{-1}.

Treatments and Experimental Design

The treatments consisted of gamma irradiation doses of ^{60}Co: 0, 100, 200, and 300 Gy, with four replications (4×4)=16 experimental units, which were evaluated under a completely randomized design using the mathematical model

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where: Y_{ij} is the response variable of the *i*-th gamma irradiation level of ^{60}Co in the *j*-th repetition; μ is the true general mean; T_i is the effect of the *i*-th gamma irradiation dose of ^{60}Co, and ε_{ij} is the experimental error of the *i*-th dose of irradiation in the *j*-th repetition (Cochran and Cox, 2005).

Response Variables

Median lethal dose (LD_{50}) was determined based on survival as a function of irradiation, using a quadratic regression to interpolate the dependent variable at 50% survival. The result was the LD_{50}. Agronomic yield was determined by harvesting the total yield, weighing the total fresh fruits per plant using an analytical balance model USS-DBS-3 (Barrios *et al.*, 2014). Plant height was measured from the base of the epicotyl to the apical bud of the last branch using a measuring tape model 30088. Polar and equatorial diameters were determined using a digital caliper model 500-195-30. For the polar diameter, measurements were taken from the base of the fruit to the apex, and for the equatorial diameter, the measurement was taken at the center of each fruit. Pedicel length was measured from the base of the fruit to the point where the pedicel connects to the plant branch. The number of branches was calculated by counting the total number of primary and secondary branches per plant. Aborted flowers were counted as the total number of pollinated flowers that were aborted per plant. SPAD units were measured by taking five readings on the leaf petiole using a SPAD-502 chlorophyll meter. The fresh fruit color was measured with the Munsell color chart for plant tissues, comparing the fruit color to the different shades on the chart. When the response variables were found to be significant, a Tukey multiple comparison test was performed at a significance level of 5% ($P \leq 0.05$).

RESULTS AND DISCUSSION

LD_{50}

The median lethal dose (LD_{50}) was fitted to a descending quadratic model, with a coefficient of determination of 0.89, which is significant. This indicates that 89% of the survival is influenced by the increase in irradiation. Upon interpolation in the quadratic model, it indicated that the LD_{50} is reached at 145 Gy. Regarding maximum survival, it was achieved in the control group with 79.45%, being this value the intercept. In relation to high doses, they had a negative effect on survival as it drastically decreased to a value of 4.45%. This study differs from that reported by Ramírez *et al.* (2006), who reported a survival rate of 60% with the application of 300 Gy of X-rays and concluded that there is a negative correlation between survival and the increase in irradiation dose. These differences are primarily attributed to the different species used in both studies, as well as the different sources of irradiation utilized in each study (Figure 1).

Agronomic Yield

The yield per cut and total yield is presented in Figures 2A and 2B, where the maximum yield per cut was 680.9 g plant^{-1} for the second cut, achieved with 200 Gy. The maximum total yield resulted from the application of 200 Gy of ^{60}Co, with 1470.25 g plant^{-1}. This indicates that this parameter is stimulated by the application of 200 Gy; thus, applications above this level of irradiation cause a decrease in agronomic yield, reaching only 235.99 g plant^{-1}. These results are consistent with those reported by Gómez *et al.* (2017), who worked with irradiated seeds of wheat (*Triticum aestivum* L.) var. T-89 and reported that the yield components, including the number of grains and spike length, are stimulated by the application of 200 Gy. They mention that the application of gamma irradiation in seeds of any species produces new genetic variability with advantages such as higher yield, pest resistance, and improved nutritional quality.

Effect of gamma irradiation on morphological parameters

The morphological parameters of plant height, polar diameter, and equatorial diameter were affected by irradiation. Thus, the greatest plant height was at 200 Gy with 1.24 m, followed by T_0 and T_{200} with 1.04 and 0.95 m, respectively, which were statistically similar. This behavior indicates that plant height is affected by radiation; as it increases, height

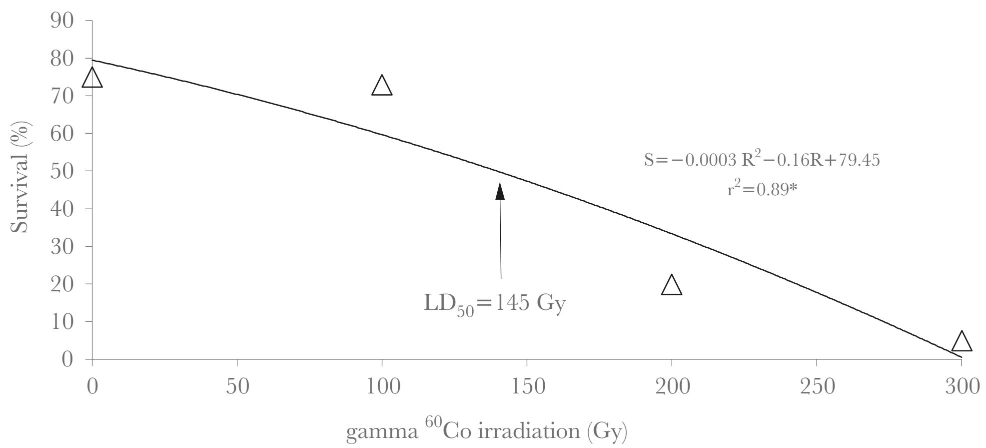

Figure 1. The median lethal dose (LD_{50}) in Miahuateco chili seedlings (*Capsicum annuum* L.) under four levels of gamma irradiation from ^{60}Co. Technological University of Tehuacán. Spring-Summer cycle, 2020.

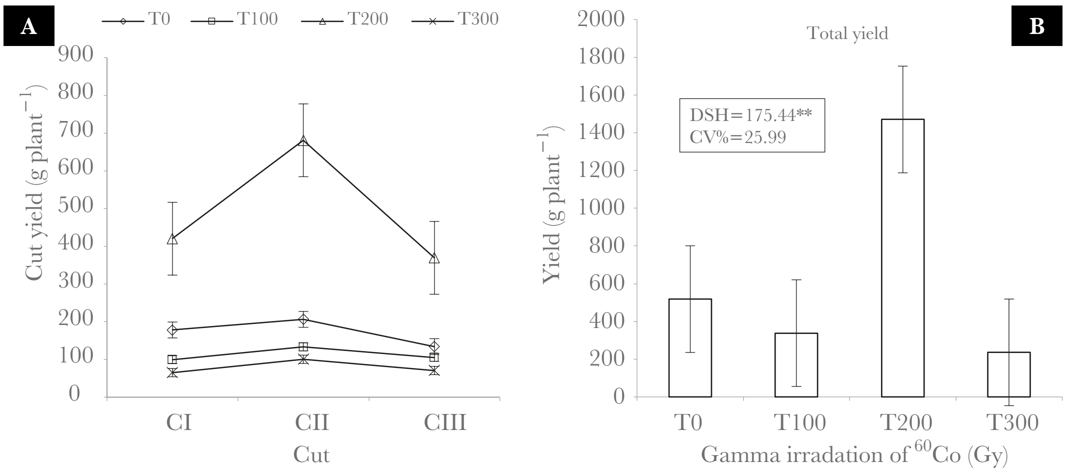

Figure 2. A: Dynamics of yield per cut and B: total yield from three cuts in Miahuateco Chile (*Capsicum annuum* L.) (SOLANACEAE), in relation to four levels of gamma irradiation of ^{60}Co. Spring-Summer cycle, 2020. CI, CII, CIII: cuts; DSH: Honest Significant Difference; CV: coefficient of variation; **; *; n.s: significant at 0.01; 0.05, and not significant.

tends to decrease, as noted by Álvarez *et al.* (2017), who, when working with Navajita, Banderita, Buffel, and Llorón grasses, observed a decrease in seedling height when subjected to gamma irradiation of ^{60}Co. Regarding the polar and equatorial diameter of the fruit, the maximum values were recorded at 200 Gy, measuring 8.83 and 4.31 cm, respectively (see Table 1).

Aborted Flowers, Number of Branches, SPAD Units, and Color

The highest number of aborted flowers was a consequence of the high irradiation dose; thus, the application of 300 Gy induced a 29% abortion rate in the flowers, while in the control treatment, as well as at 100 and 200 Gy, the percentage of aborted flowers was 12.75%, 13.00%, and 19.00%, respectively. The highest number of branches, 6.25 and 9.75, was observed for the control treatments and T_{100}, which were statistically similar

Table 1. Analysis of variance and multiple comparison test for four response variables in Miahuateco chile (*Capsicum annuum* L.), under four levels of gamma irradiation with ^{60}Co. Technological University of Tehuacán. Spring-Summer cycle, 2020.

Treatment Gy	**PH**	**PD**	**ED**	**PL**
	cm			
T_0	1.04 b	7.29 bc	3.62 b	0.436 a¶
T_{100}	1.24 a	7.56 b	2.84 c	0.356 a
T_{200}	0.95 b	8.83 a	4.31 a	0.445 a
T_{300}	0.60 c	8.12 b	3.56 b	0.398 a
HSD	0.12**	0.60**	0.50**	0.090$^{n.s}$
CV%	20.12	15.33	16.37	22.14

¶ Means within columns with the same letter are statistically equal according to Tukey at P≤0.05; AP, plant height; DP, polar diameter; DE, equatorial diameter; LP, pedicel length; T_0, T_{100}, T_{200}, and T_{300}, treatments; DSH, honest significant difference; CV, coefficient of variation; *; ** n.s, significant at 0.01; 0.05 and not significant.

despite numerical differences (Table 2). Regarding the SPAD units, the minimum values were recorded in T_{200} and T_{300}, with 59.6 and 48.9, respectively, which was reflected in a decrease in color tone, primarily in T300, showing a reduction in tone as indicated by the fresh color 2.5 GY 6/8. This response was reported by Hernández *et al.* (2017), who stated that the color of photosynthetic protocorms of *Loelia autumnalis* tends to decrease with the increase in irradiation under *in vitro* conditions. On the other hand, Martínea *et al.* (2018) mention that seedlings from seeds irradiated with gamma rays of ^{60}Co showed chlorosis at doses of 60 Gy and attribute this response to the fact that high doses of gamma irradiation cause an imbalance in growth regulators, which manifests as chlorosis.

Table 2. Analysis of variance and multiple comparison test for four parameters of Miahuateco Chile (*Capsicum annuum* L.) under four levels of gamma irradiation from ^{60}Co. Spring-Summer Cycle, 2020.

Treatment Gy	AF	NB	SPAD	Color in fresh
	%			
T_0	12.75 bc	6.25 a	65.4 a[¶]	5.0 GY 3/4
T_{100}	13.00 b	9.75 a	62.5 a	5.0 GY 3/4
T_{200}	19.50 b	4.74 ab	59.6 b	5.0 GY 3/4
T_{300}	29.00 a	3.00 b	48.9 c	2.5 GY 6/8
HSD	7.14**	3.55**	5.3**	---------------
CV%	15.99	28.33	14.33	---------------

[¶] Means within columns with the same letter are statistically equal according to Tukey at $P \leq 0.05$; FA, aborted flowers; NR, number of branches; SPAD, SPAD units; T_0, T_{100}, T_{200}, and T_{300}, treatments; DSH, honest significant difference; CV, coefficient of variation; *; ** n.s, significant at 0.01; 0.05 and not significant.

CONCLUSIONS

The median lethal dose for Miahuateco chili is reached with the application of 145 Gy of ^{60}Co. The maximum yield, as well as the number of fruit cuts, is stimulated by 200 Gy. Applications of 300 Gy lead to an increase in flower abortion, as well as a decrease in plant color, which manifests as chlorosis. Gamma irradiation of ^{60}Co is an important source that induces genetic variability in Miahuateco chili, thus it can be used in future improvement programs involving the species.

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Colegio de Postgraduados

Changes in physicochemical and antioxidant properties over one year of *Apis mellifera* honey

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ABSTRACT

Objective: Quantify the physicochemical and antioxidant properties of honey harvested in the four seasons of the year, to determine the variation in its quality with respect to official standards.

Design/methodology/approach: Honey samples were collected in the municipality of Tantoyuca, during the four seasons of the year, in the presence or absence of rain. For each sample, color, electrical conductivity, moisture, pH, free acidity, diastase activity, total reducing sugars, Brix degrees, caloric content, phenolic and total flavonoid content and antioxidant capacity were determined by the FRAP and ABTS assays.

Results: The Brix degrees and moisture were found within the limits accepted by NOM-004-SAG/GAN-2018 and CXS 12-1981 throughout the year, however, in the rainy period these variables were higher (82.1 °Brix and 19.6 g 100 g^{-1}). The FRAP and ABTS values showed variation depending on the absence or presence of rain. The highest antioxidant content occurred in the winter season (63.91 and 68.82 μmol TE 100 g^{-1}). The results obtained are attributed to the geographical origin and the floral species present during the bees' foraging.

Limitations on study/implications: Climate change in the region has decreased rainfall, reducing the floristic resource.

Findings/conclusions: The effect of the season of the year affects the characteristics of the honey evaluated, however, it complies with the parameters established in the Mexican standard and the codex alimentarius, which can encourage and support its commercialization in the international market.

Keywords: honeybee, *A. mellifera*, antioxidant compounds, quality, seasonal variation.

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INTRODUCTION

Honey is defined as a sweet and natural food generated from the nectar of plants, collected and transformed by bees. This product consists of various types of carbohydrates, water, minerals, amino acids, proteins, and organic acids. The composition of honey depends on the floral species from which the bees sucks (monofloral, multifloral, and honeydew) (Martínez *et al.*, 2017), as well as the characteristics of the soil, species of the bee, colony physiology, among others (Jean-Prost, 2007; Bogdanov *et al.*, 2008). The nutritional value and therapeutic and stimulating qualities of honey position it as a highly demanded product in the international market (Escobar and Manresa, 2005). The identity of honey can be determined by analyzing its physicochemical properties, such as moisture content, electrical conductivity, free acidity, pH, and color (Pineda *et al.*, 2019), as well as the total sugar content and other related substances, such as diastase activity. On the other hand, polyphenolic compounds, recognized as responsible for the health benefits provided by honey, are produced in plants and have been studied due to their antioxidant, antimicrobial, and anti-inflammatory activity (Viuda-Martos *et al.*, 2008), as a consequence of their ability to inhibit and/or reduce the production of free radicals that cause oxidative damage to molecules such as carbohydrates, lipids, proteins, and genetic material (Nascimento *et al.*, 2018). Honey production faces various challenges, such as climate change, deforestation, and toxic agrochemicals that threaten the lives of bees. These factors affect production yields and raise doubts about honey quality. La Secretaría de Agricultura y Desarrollo Rural " (SADER) of México, aiming to regulate honey market behavior, issued the Official Mexican Standard NOM-004-SAG/GAN-2018, which outlines the conditions for honey production and marketing (García-Pérez and Fong-Reynoso, 2023). This standard strengthens beekeeping activities in the region and encourages honey commercialization in the international market. Therefore, the objective of this research was to determine the physicochemical properties and evaluate the nutraceutical potential of *A. mellifera* honey produced in a region of the Huasteca Veracruzana to determine the existing variation during the four seasons of the year.

MATERIALS AND METHODS

Study Location

This research was conducted in the Huasteca Veracruzana, located in the northern part of the state. It borders Tamaulipas to the north, Hidalgo, the Gulf of Mexico, and the Totonac region to the south. The Huasteca region lies between the parallels 97° 59' and 98° 24' west longitude and 21° 06' and 21° 40' north latitude. It has a warm sub-humid climate with summer rains and an average annual precipitation of 1100 mm. One of the main productive activities is cattle ranching focused on milk and meat production. In agriculture, the production of maize, citrus fruits, and sugar cane is prominent. The coexisting ecosystems include subtropical evergreen forest types, with species such as guarumbo, jonotes, guanacaxtle, and sangre de grado, along with important crops like sesame, peanuts, zucchini, sweet potatoes, beans, watermelon, sorghum, wheat, tobacco, tomatoes, coconuts, mangoes, and papayas (Alan and Martínez, 2010; INEGI, 2021).

Sample Collection

A total of 32 honey samples were collected from the localities of San Jerónimo, San Sebastián, Pensador Mexicano, Zapote Largo, Ixcanelco, Las Martas, and Mincuiní, all belonging to the municipality of Tantoyuca, Veracruz. Samples were obtained during the winter of 2022 and the spring, summer, and autumn of 2023, with eight samples taken from each apiary. The supers were selected randomly, and a manual extractor was used to avoid honey contamination. The collected samples (approximately 500 mL) were stored at room temperature and protected from light until analysis.

Reagents and Instrumentation

The Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium bisulfite, quercetin (95% purity), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ferric chloride (III) hexahydrate, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Absorbances for the quantification of phenols, flavonoids, and antioxidant capacity were measured using a Synergy 2 Microplate Reader, with Gen5 software (BioTek Instruments Inc., Winooski, VT, USA). Physicochemical parameter color was measured using a Hunter colorimeter (MiniScan XE Plus 45/0-L, HunterLab, Reston, Virginia, USA) on the CieLab scale (L*, a*, b*). The chromatic coordinates a* and b* are expressed on a scale of −100 to 100. For a*, the negative end indicates green, while the positive end indicates red. In the chromatic coordinate b*, the negative end indicates blue, and the positive end indicates yellow. The values for hue (Hue; h*) and chroma (Chroma; C*) were calculated using equations 1 and 2, while the value L* was taken as luminosity (Karabagias *et al.*, 2017).

$$h^* = \mathrm{Tan}^{-1}\left(b^*/a^*\right) \tag{1}$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{2}$$

Electrical conductivity, moisture content, pH, free acidity, and diastase activity were measured according to the procedures described by Bogdanov *et al.* (2002). All determinations were performed in triplicate.

The concentration of reducing sugars was determined following the method of Miller (1959) with some modifications: 0.5 mL of the aqueous honey solution (0.8 mg mL^{-1}) was mixed with 0.5 mL of 3,5-dinitrosalicylic acid (10 mg mL^{-1}). The mixture was heated to 90 °C for 10 minutes and then cooled in ice water for 10 minutes. The absorbance of the reaction mixture was read at 540 nm on a microplate reader. A glucose calibration curve was prepared in the concentration range of 0.2 to 1.0 mg mL^{-1}. The results were reported as grams of glucose equivalents per 100 grams of honey (g GluE 100 g^{-1}).

The percentage of soluble solids (°Brix) was determined using a pocket digital refractometer PAL-3 from Atago, with a range of 0.0 to 93.0%, calibrated with distilled water. An approximate volume of 1 g of sample was introduced, each in triplicate.

The caloric content of the samples was determined in a Parr 6400 calorimeter, and the results were reported in kilocalories per 100 grams of honey (kcal 100 g^{-1}).

Antioxidant properties

The antioxidant capacity was evaluated in aqueous solutions of honey (1:5 w/v). Total phenolic content was determined using the Folin-Ciocalteu method, adapted to microplates

(Hernández-Rodríguez *et al.*, 2016). Absorbance was measured at a wavelength of 760 nm. The results were expressed in milligrams of gallic acid equivalents per 100 grams of honey (mg GAE 100 g^{-1}). The calibration curve was prepared from a stock solution of 0.5 mg mL^{-1} of gallic acid in the linear concentration range of 0.02 to 0.21 μg mL^{-1}. The samples were analyzed in quadruplicate, taking four absorbance readings.

Total flavonoid content was quantified according to Chang *et al.* (2002). Absorbance was measured at a wavelength of 415 nm. The results were expressed in milligrams of quercetin equivalents per 100 grams of honey (mg QE 100 g^{-1}). The calibration curve for quercetin was prepared in a concentration range of 0.5 to 10 μg mL^{-1}. The samples were analyzed in quadruplicate, taking four absorbance readings.

Antioxidant capacity was determined using the FRAP (Benzie & Strain, 1996) and ABTS (Re *et al.*, 1999) assays, adapted to microplates. The results were reported in micromoles of Trolox equivalents per 100 grams of honey (μmol TE 100 g^{-1}). The calibration curve for Trolox was prepared in concentration ranges of 4 to 46 μM for the FRAP assay and 5 to 60 μM for the ABTS assay. The samples were analyzed in quadruplicate, taking four absorbance readings.

Experimental design and statistical analysis

A completely randomized experimental design was used, considering the season and the presence or absence of rainfall as sources of variation. A one-way ANOVA was performed for statistical analysis. Homogeneity was evaluated using Bartlett's test, and normality was assessed using the Shapiro-Wilk test. Mean comparisons were conducted using Tukey's test ($p<0.05$). The statistical analysis was carried out using Statistica v.10 software.

The proposed general linear model for this research is as follows:

$$y_{ij} = \mu + \alpha_i + \varepsilon_i$$

where: y_{ij}=response variable; μ=overall population mean; α_i=effect of the *i*-th season and presence or absence of rainfall during the year; εj=Associated experimental error.

RESULTS AND DISCUSSION

Physicochemical properties

Color is an important characteristic in the quality of honey. It can vary from light yellow, light amber, amber, reddish amber to almost black (Tuberoso *et al.*, 2013). The color parameters L*, a*, and b* of the samples evaluated in the four seasons and two periods of the year are presented in Figure 1. It was observed that autumn honey showed the highest luminosity value (L*=13.8), indicating that honey collected in this season was lighter. Honey from spring and winter was slightly darker (L*=9.9 and 9.3), and the lowest luminosity value was recorded in summer honey (L*=7.5), resulting in the darkest samples compared to the other seasons. The chromatic coordinates (a* and b*) of the honey in winter, spring, and autumn were similar. The honey samples evaluated over the periods showed that honeys with elevated L*, a*, and b* values were present during the rainy season, indicating that the samples collected during this period were lighter than those collected during the dry season.

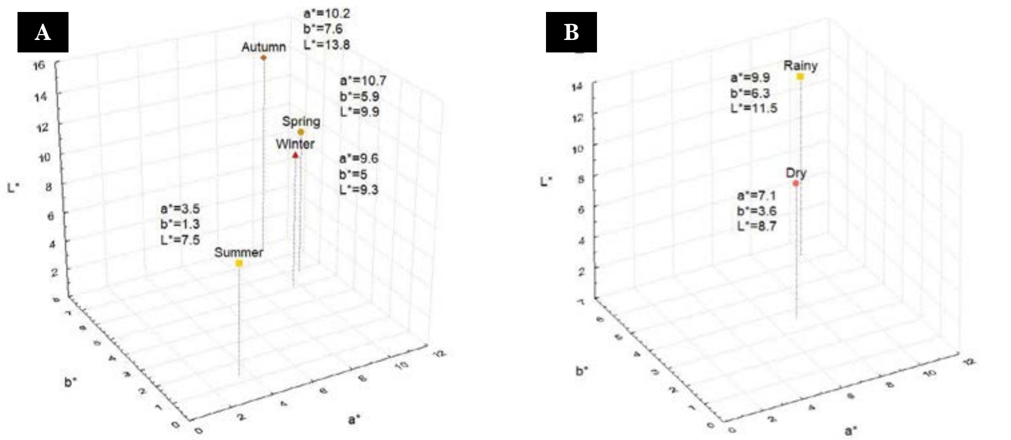

Figure 1. Chromatic coordinates and luminosity (a, b, and L*) of *A. mellifera* honeys in the CIE Lab space. A. Honey color by season. B. Honey color by period of the year.

The color of honey is a physical property influenced by the floral composition of the nectar, the extraction process, and the storage temperature. It is described by three chromatic attributes: luminosity (the color closest to white or black), hue (the perceived color: yellow, red, blue, green), and chroma (saturation and purity of the color). The luminosity of the honey samples evaluated was higher in autumn than in the other seasons ($p<0.05$) (see Figure 1). In this regard, Starowicz *et al.* (2021) studied the relationship between browning index and phenolic content, color, and antioxidant capacity in honey from Poland. Using the CIE L* a* b* method, they reported different luminosity values (8.7, 9.17, and 47.52) in monofloral honeys (*Acacia*). The similarity of the values found in the Huasteca region with those of these authors is noteworthy, given that they evaluated monofloral honeys, unlike this research. The edaphoclimatic conditions in Poland determine the local floral resources that could be responsible for the coloration of the honey.

The hue attribute had a higher value in autumn honey (0.761) compared to other seasons ($p<0.05$). In contrast, the hue of honey collected in summer showed the lowest

Table 1. Color attributes h* and C* in honey samples from the Huasteca Veracruzana.

Season / Period	Color	
	h*	C*
Spring	0.540^{b}	12.35^{a}
Summer	0.343^{c}	3.75^{b}
Autumn	0.761^{a}	12.99^{a}
Winter	0.453^{bc}	10.99^{a}
Standard error	0.04	1.23
Rain	0.442^{b}	8.05^{a}
Dry	0.607^{a}	11.99^{a}
Standard error	0.03	0.97

Note: Means within a column with different letters indicate statistical differences (Tukey $p<0.05$).

value (0.343), while the honeys harvested in spring and winter seasons (0.540, 0.453) did not differ from each other ($p>0.05$). The hue values were higher during the rainy season (0.607) than in the dry season (0.442). The results obtained are lower than those reported by Piotraszewska-Pająk and Gliszczyńska-Świgło (2015) in multifloral honeys from Poland. On the other hand, the chroma values for autumn, spring, and winter honey were 12.99, 12.35, and 10.99 ($p>0.05$), respectively. The saturation value in summer honey was lower (3.75) compared to the other seasons, indicating a statistical difference. The chroma values during the dry and rainy periods showed no statistical differences, with values of 8.05 and 11.99, respectively ($p>0.05$). These results are slightly lower (18.64, 9.71, 19.45, 23.11, 25.33, and 27.44) than those reported for multifloral honeys in temperate climates (Piotraszewska-Pająk and Gliszczyńska-Świgło, 2015).

The fluctuations found in this study regarding the color attributes could be attributed to the beekeeper's management practices, the conditions under which the honey is extracted, the storage duration, and the temperature at which the honey is kept. Various authors state that the color intensity in honey is related to the content of phenols, flavonoids, and antioxidants, which are provided by the plants (Becerril-Sánchez *et al.*, 2021).

The electrical conductivity of honey samples collected in the spring and summer was the highest (0.63 and 0.58 mS cm^{-1}) and did not show significant differences ($p<0.05$). The samples with the lowest conductivity were obtained in winter (0.18 mS cm^{-1}). The data indicate that there were no differences between the spring and summer seasons ($p<0.05$). However, this group exhibited higher conductivity compared to autumn and winter ($p<0.05$). The electrical conductivity of honey is related to its mineral content. Estimating this property is important for classifying honey and determining its origin. The variation in the obtained results may be a consequence of the presence of mineral substances from the botanical source, the degree of maturity at the time of extraction, and the storage conditions (Campo and Hincapié, 2023). On the other hand, the electrical conductivity of honey was higher ($p<0.05$) during the dry period (0.61 mS cm^{-1}) than during the rainy season (0.302 mS cm^{-1}). According to the Mexican Official Standard (NOM-004-SAG/GAN-2018) and the Codex Alimentarius (CXS 12-1981), the electrical conductivity of honey should not exceed 0.80 mS cm^{-1}. Thus, the honeys studied were within the established range.

Ezin *et al.* (2018) conducted a physicochemical characterization of honey produced in Benin during two seasons (dry and rainy) and found that during the dry season, the electrical conductivity was measured at 0.63 mS cm^{-1}, which is similar to the findings of this study. The similarity in results may be attributed to the climatic conditions and the sampling periods in both research studies.

The moisture content of the summer and winter samples was higher than that of the spring and autumn samples ($p>0.05$). Humidity is an important parameter that determines the maturation of honey and its physicochemical properties (García-Chaviano *et al.*, 2022). Humidity can vary according to the relative humidity at the collection site and the storage conditions. Percentages above 20%, as established in NOM-004-SAG/GAN-2018, facilitate the fermentation of honey, leading to changes in its physicochemical characteristics (Moyano *et al.*, 2023). The humidity values during the dry and rainy periods show a statistical difference. These results are similar with those reported by Albú *et al.* (2022), who found humidity values ranging from 15.41% to 19.49% in multifloral honey from honeybees. The humidity range obtained is within the parameters established by NOM-004-SAG/GAN-2018. Environmental conditions and the collection of samples from capped frames could explain the similarity between these studies.

As shown in Table 2, the pH values of the samples collected in spring and summer did not show significant differences ($p>0.05$). No differences were observed between the autumn and winter samples either. The results obtained in autumn and winter honey are agree with those reported by Al-Ghamdi *et al.* (2019), who conducted a comparison of the chemical composition of honey samples from *A. mellifera* and *A. florea* subjected to different thermal processes. In their research, they found pH values of 3.62 in honey from *A. mellifera*. The appropriate pH range is between 3.2-4.5. This range is capable of inhibiting the growth of microorganisms (Da Silva *et al.*, 2016). The pH values of the samples evaluated during the dry and rainy periods showed no statistical difference and were similar to those reported by Ezin *et al.* (2018), who assessed the variation of the physicochemical properties of honey during two seasons (dry and rainy) and reported pH values ranging from 3.7-4.1. On the other hand, Albú *et al.* (2022) found pH values ranging from 3.25 to 5.03 in monofloral honeys from the east and southeast of Romania. The values reported in the studied samples could be associated with the floral resources available to the bees during foraging. The variation in pH may be due to the salivary secretion of the bees, which is responsible for the enzymatic and fermentative processes during the nectar processing (Ezin *et al.*, 2018).

Table 2. Electrical conductivity, moisture, pH, free acidity, diastatic index, reducing sugars, °Brix, and calorific value of *A. mellifera* honey samples from the Huasteca Veracruzana and the standard quality value.

Season/ Period /Quality standards	Electrical conductivity (mS cm^{-1})	Moisture (g 100 g^{-1})	pH	Free acidity (mEq kg^{-1})	Diastatic index (Schade units)	Reducing sugars (g GluE 100 g^{-1})	°Brix	Calorific value (kcal 100 g^{-1})
Spring	0.63 ± 0.17^{a}	19.1 ± 1.2^{ab}	4 ± 0.2^{a}	50.3 ± 12.4^{b}	19.5 ± 13.3^{a}	42.3 ± 15.6^{c}	81.8 ± 1.1^{b}	311.6 ± 3.2^{a}
Summer	0.58 ± 0.22^{a}	18.5 ± 1^{b}	4 ± 0.2^{a}	49.5 ± 7.6^{b}	13.8 ± 6.8^{a}	61 ± 13.7^{a}	81.1 ± 0.9^{b}	312.1 ± 3.4^{a}
Autumn	0.42 ± 0.18^{b}	19.1 ± 1.3^{ab}	3.6 ± 0.1^{b}	60.9 ± 7.9^{a}	16.2 ± 6.4^{a}	52.9 ± 9.7^{b}	81.6 ± 1.7^{b}	312.7 ± 5.3^{a}
Winter	0.18 ± 0.07^{c}	20.1 ± 2^{a}	3.6 ± 0.06^{b}	62.5 ± 8.4^{a}	16.2 ± 4.9^{a}	64.9 ± 8.1^{a}	82.6 ± 1.5^{a}	315.1 ± 3.1^{a}
Rainy	0.61 ± 0.20^{a}	18.8 ± 1.2^{b}	4.02 ± 0.2^{a}	49.9 ± 10.2^{b}	16.7 ± 10.9^{a}	49.9 ± 10.2^{b}	81.4 ± 1.0^{b}	311.8 ± 3.2^{b}
Dry	0.30 ± 0.18^{b}	19.6 ± 1.7^{a}	3.6 ± 0.1^{b}	61.75 ± 8.13^{a}	16.2 ± 5.7^{a}	58.9 ± 10.7^{a}	82.1 ± 1.7^{a}	313.9 ± 4.4^{a}
NOM-004-SAG/GAN-2018	Max. 0.8	Max. 20	SVR	Max. 50	Min. 8	Mín. 60	NRV	NRV
CXS 12-1981	Not more than 0.8	Not more than 20	SVR	Not more than 50	Not less than 8	Not less than 60	NRV	NRV

Note: Means in the same column with different letters indicate statistical differences (Tukey $p<0.05$). NRV=no reference value.

The free acidity values in autumn and winter honey were higher and statistically different from those in spring and summer ($p<0.05$). Free acidity is a parameter that indicates the freshness and deterioration of honey. The results reported in the four seasons are similar to those found by Al-Ghamdi *et al.* (2019) in multifloral honey from *A. mellifera* with values ranging from 51.80 to 84.6 mEq kg^{-1}. The honeys evaluated during the dry period showed lower acidity compared to those collected in the rainy season (see Table 2). Da Silva *et al.* (2016) mention that this parameter can be affected by the location and time of harvest.

The diastase index values showed no significant differences; however, they were higher than the maximum allowable range according to the Official Mexican Standard for Honey Production and Specifications and CXS 12-1981. The diastase index is an indicator of honey freshness. This indicator is determined by the floral resources accessible to the bees. Its content can vary based on the age of the hive, the nectar collection period, and the high concentration of sugars. A diastase level below 8 Schade units (DN) could indicate the premature collection of honey (Da Silva *et al.*, 2016).

The diastase index of honey during the dry and rainy periods showed no statistical difference (16.70 and 16.08 DN, respectively). These results are similar to those reported by Velásquez and Goetschel (2019), who determined the physicochemical quality of honey sold in markets south of Quito, Ecuador. The diastase index reported in their research ranged from 11.04 to 16.44 DN. Both studies prioritize understanding the physicochemical qualities of the evaluated honeys.

The reducing sugars contained in the samples collected in winter and spring (61 and 64 g glucose 100 g^{-1}) showed no significant difference between them ($p>0.05$). The determination of sugars in honey is used to assess its quality and possible adulteration. According to quality standards, the content must be at least 60 g glucose+fructose 100 g^{-1}. The concentration of sugars in the dry and rainy periods (51.66 and 58.91 g glucose 100 g^{-1}) was statistically different ($p<0.05$). Castillo *et al.* (2022) compared the sugar composition and °Brix in multifloral honeys from *A. mellifera* and *Melipona beecheii* from different states in Mexico. In that comparison, they found values of 28.9 g glucose 100 g^{-1} for *A. mellifera* and 28.2 g glucose 100 g^{-1} for *Melipona beecheii*. Their results were lower than those reported in this research. The variation between results can be attributed to the different floral sources present in each state. The soluble solids content (°Brix) in winter was higher (82.69) than in the other seasons. The values estimated in spring, summer, and autumn samples did not show statistical differences ($p>0.05$). Soluble solids represent the percentage of sugars present in honey. The results from the dry and rainy periods showed a statistical difference, with the rainy period (82.15) being higher than the dry period (81.49). Castillo *et al.* (2022) reported values between 76.7 and 81.5 °Brix in multifloral honeys from *A. mellifera*, coinciding with the values found in this study. This could be attributed to the diverse floral origin of honey in both investigations.

The caloric content does not show statistical differences ($p>0.05$). The monosaccharides glucose and fructose are rapidly absorbed and allow honey to provide energy. The energy content of honey fluctuates between 294-320 kcal 100 g^{-1} (García-Chaviano *et al.*, 2022). The heat of combustion showed a statistical difference between the dry and rainy periods; however, it is similar to the values reported for honeys from La Patagonia Verde, Chile

(Lobos *et al.*, 2021). The similarity of the obtained results can be attributed to environmental conditions, floral resources, and the timing of collection in both investigations.

Phenolic content and Antioxidant Capacity

The content of phenols and flavonoids, as well as the antioxidant capacity in the honey samples, is shown in Table 3. According to Adaškeviciute *et al.* (2019), phenolic compounds in honey are responsible for its antioxidant, antimicrobial, and anti-inflammatory properties. The phenolic content of summer honey (28.87 mg GAE 100 g^{-1}) was higher than that of the other seasons ($p<0.05$).

The flavonoid content of the evaluated samples indicated that the highest value was obtained in summer honey (0.757 mg QE 100 g^{-1}). Flavonoids are part of phenolic compounds. Their origin varies according to the floral source, which could explain the variation of its concentration between seasons.

The FRAP method is used to measure the antioxidant capacity of foods, beverages, and dietary supplements containing polyphenols. The results obtained for this parameter indicate that the calculated value in summer honey (93.39 μmol TE 100 g^{-1}) was higher than in the other seasons ($p<0.05$). In the samples of autumn and winter, the values obtained showed no statistical differences (57.75 and 63.91 μmol TE 100 g^{-1}, respectively).

The ABTS method measures the capacity of antioxidants to eliminate the $ABTS\bullet^{+}$ cation radical (oxidizing agent) (Mercado-Mercado *et al.*, 2013). The autumn and spring samples showed higher values of antioxidant capacity (100.12 and 98.46 μmol TE 100 g^{-1}) ($p>0.05$).

The results for phenolic content, flavonoids, and antioxidant capacity during the dry and rainy periods are presented in Table 3. In the dry period, a value of 27.31 mg GAE 100 g^{-1} was found, which was higher than that found in the rainy period (25.32 mg GAE 100 g^{-1}) ($p<0.05$). The content of polyphenolic compounds in honey can vary according to geographical origin, floral source, and climatic conditions (Becerril-Sánchez *et al.*, 2021). The results obtained during the studied periods were higher than those presented by Perna *et al.* (2013). They evaluated the antioxidant properties, polyphenol content, and colorimetric characteristics in mono- and multifloral honeys from different regions of southern Italy. In their research, they found values of 11.79 mg GAE 100 g^{-1} for multifloral honeys and 12.15 mg GAE 100 g^{-1} in citrus honeys. Becerril-Sánchez *et al.*

Table 3. Content of Phenolic Compounds and Antioxidant Capacity of Honey Samples Produced in a Region of Huasteca Veracruzana During the Four Seasons of the Year.

Season	Phenols mg GAE 100 g^{-1}	Flavonoids mg QE 100 g^{-1}	FRAP μmol TE 100 g^{-1}	ABTS μmol TE 100 g^{-1}
Spring	25.74 ± 3.94^{b}	0.562 ± 0.18^{b}	72.18 ± 25.35^{b}	98.46 ± 32.70^{a}
Summer	28.87 ± 4.83^{a}	0.757 ± 0.17^{a}	93.39 ± 23.90^{a}	79.85 ± 23.83^{b}
Autumn	25.92 ± 7.95^{b}	0.518 ± 0.22^{b}	57.57 ± 22.24^{c}	99.84 ± 59.96^{a}
Winter	24.86 ± 4.65^{b}	0.503 ± 0.18^{b}	63.91 ± 23.77^{c}	68.82 ± 20.59^{b}
Rainy	27.31 ± 4.67^{a}	0.660 ± 0.20^{a}	82.78 ± 26.78^{a}	89.15 ± 30.04^{a}
Dry	25.39 ± 6.51^{b}	0.510 ± 0.20^{b}	60.76 ± 23.20^{b}	84.21 ± 47.23^{a}

Note: Means in each column with different letters indicate statistical differences (Tukey $p<0.05$).

(2021) concluded that the content of phenols and flavonoids is related to the botanical origin. They assume that variations in phenolic content are related to conditions at the sampling site (country and/or region). In their study, they found that monofloral honeys can have higher phenolic content, ranging from 203 to 217.0 mg GAE 100 g^{-1}. However, they mention that multifloral honeys have been reported with values of 20.32 and 28.26 mg GAE 100 g^{-1}, respectively. These results align with those found in the honey samples evaluated in this study during the dry and rainy periods (27.31 mg GAE 100 g^{-1} and 25.32 mg GAE 100 g^{-1}). The similarity in results is attributed to the floral resources available to the bees during foraging.

The flavonoid content was higher during the dry period (0.660 mg QE 100 g^{-1}) than during the rainy period (0.507 mg QE 100 g^{-1}) ($p<0.05$). The results obtained were lower than those reported by Perna *et al.* (2013), who found values of 8.94 mg QE 100 g^{-1} in multifloral honeys and 5.49 mg QE 100 g^{-1} in monofloral (citrus) honeys. On the other hand, Cabrera *et al.* (2017) evaluated phenolic compounds, flavonoids, and antioxidant capacity in relation to color. In their study, they reported values ranging from 6.94 to 37.47 mg QE 100 g^{-1}. The variation in results can be attributed to the different floral sources and geographical origin in each study.

The calculated values for FRAP and ABTS in the evaluated periods indicate that the dry period (82.78 and 89.15 μmol TE 100 g^{-1}) was higher than the rainy period (60.84 and 84.41 μmol TE 100 g^{-1}) ($p<0.05$). These results are similar to those of Rodríguez *et al.* (2011), who evaluated the antioxidant and antimicrobial properties in multifloral and monofloral honeys from Mexico. The FRAP values for multifloral and monofloral honeys were 182.6 and 749.4 μmol TE 100 g^{-1}, respectively. Likewise, the ABTS results were 76.8 and 910.2 μmol TE 100 g^{-1}. The floral resources available to the bees are likely the reason for these differences.

Correlation Between Color and Antioxidants

The chromatic coordinates L*, a*, and b* of the color indicate a negative correlation with the flavonoid content (Table 4).

It was observed that as the values of L*, a*, and b* increased, the flavonoid content decreased. The presence of phytochemicals is greater in dark honey samples. This finding is consistent with the studies by Anklam *et al.* (1988), Frankel *et al.* (1998), and Vanhanen *et al.* (2011), who have studied and found that dark honeys have a higher concentration of minerals and pigments (phenols, flavonoids, carotenoids) that provide antioxidant properties to the honey.

Table 4. Correlation Between Color Attributes and Flavonoid Content.

Color attributes	Phenols compounds y antioxidant capacity	Goodness-of-fit-attributes		
		r	r^2	Valor p
L*	Flavonoids QE mg 100 g^{-1}	−0.753	0.568	0.001
a*	Flavonoids QE mg 100 g^{-1}	−0.867	0.752	0.001
b*	Flavonoids QE mg 100 g^{-1}	−0.797	0.636	0.001

Note: r=Correlation; r^2=Determination coefficient.

CONCLUSIONS

The physicochemical properties studied in *A. mellifera* honey showed statistical differences due to the season and time of year. However, the reported values were within the parameters established by NOM-004-SAG/GAN-2018 and CXS 12-1981. The presence of phenols and flavonoids was evidenced. The FRAP and ABTS assays confirm that throughout the year, the honey produced in the region has antioxidant properties; however, that produced in summer stands out in this regard. It is important to investigate the quality of honey in other regions of the state in order to promote its properties, generate a designation of origin, and encourage its commercialization in international markets.

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Colegio de Postgraduados

Neltuma laevigata and its influence on soil fertility in the Valle del Mezquital, Hidalgo, Mexico

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ABSTRACT

Objective: The physicochemical characteristics of soil fertility, based on the influence of mesquite (*Neltuma laevigata*) were evaluated in an agricultural community of Ixmiquilpan, Hidalgo, Mexico.

Design/Methodology/Approach: Based on NOM-021-SEMARNAT-2000, physical and chemical parameters were evaluated. Nine samples were taken from each of the four sites with and without mesquite ($n=36$). The relationship between soil fertility and the presence or absence of mesquite mesquite was determined with a paired t- test ($p<0.05$).

Results: A significant difference in organic matter content was recorded. Humic substances and acids are higher in soils with natural vegetation with mesquite than in soils with natural vegetation without mesquite.

Study Limitations/Implications: The study does not consider changes in microbiological biodiversity or the influence of other plant species in organic matter content and humic substances.

Findings/Conclusions: Mesquite promotes soil fertility in the area; consequently, proposing management strategies for this species is fundamental in the Valle del Mezquital.

Keywords: mesquite, deforestation, nitrogen fixation, organic matter.

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INTRODUCTION

Deforestation has rapidly impacted more than half of the Mexican territory (Godínez-Montoya *et al.*, 2022). Overgrazing, land use change, post-harvest tillage, and poor management of agricultural soils have speeded the process (Hernández-González *et al.*, 2018). The introduction of agricultural irrigation systems to the Valle del Mezquital that use sewage water from urban areas has caused an almost complete change in the semiarid landscape, significantly reducing the original plant cover. This process has diminished the regional extension of mesquite forests, potentially impacting soil structure and fertility (Domínguez-Narváez *et al.*, 2023).

Arid and semiarid ecosystems -which cover approximately 60% of the Mexican territory- are fundamental, as a result of their plant diversity (6,000 species) and their endemism (50%) (Montaño *et al.*, 2016). These ecosystems have processes that maintain soil function and microbial activity, favoring biogeochemical cycles (Jurado-Guerra *et al.*, 2021). Some

of the major species in these ecosystems belong to the genus *Neltuma* sp., including mesquite (*Neltuma laevigata*), formerly known as *Prosospis laevigata* (Hughes *et al*., 2022). Depending on soil depth, this evergreen thorny tree can grow up to 10 m. This legume belongs to family Fabaceae and it is a valuable resource for the inhabitants of semiarid areas, such as El Alberto community (Del Valle-Martínez *et al*., 2023). *Neltuma laevigata* is a key species of the semiarid ecosystem, because it helps secondary vegetation to thrive (nurse effect), favoring the ecological succession of these systems (Estrada-Castillón *et al*., 2024).

The properties of mesquite are not limited to their environmental benefits. Some parts of this multipurpose species, such as roots, trunk, branches, leaves, pods, flowers, and gums, have medical uses. Batista-Roche and Huerta-Campo (2021) pointed out that the extracts of mesquite leaves have analgesic, anthelmintic, antiemetic, antioxidant, and healing effects, as a result of its flavonoids, tannins, alkaloids, and phenolic compounds. Meanwhile, Armienta *et al*. (2008) studied the genus *Neltuma* sp. and highlighted its soil bioremediation and restoration potential; these capacities are ideal to mitigate the volatile component dispersion in open-pit mining areas. Therefore, some physicochemical characteristics of soil fertility, resulting from the influence of mesquite (*Neltuma laevigata*), were evaluated in an agricultural community of Ixmiquilpan, Hidalgo, Mexico.

MATERIALS AND METHODS

Study area

The study was carried out in El Alberto community, located at 20° 29' N and 99° 13' W, at 1,700-2,250 m.a.s.l., 12 km away from Ixmiquilpan, Hidalgo, Mexico (Figure 1). The community has an extension of approximately 22 km^2. It borders with Cantinela (north), Panales (west), the municipality of Alfajayucan (southwest), Dadhó, Boxaxni, and Loma (south), and La Estancia (southeast) (Del Valle-Martínez *et al*., 2023). The climate is semiarid, with a mean annual precipitation of 389 mm. The area has a predominant xerophytic vegetation.

Figure 1. Map of El Alberto, Hidalgo, Mexico.

Sampling and physicochemical characterization of the soil

The analysis of the samples included a selection of agricultural soils with mesquite (ASWM) and without mesquite (ASWOM), as well as soils with natural vegetation with mesquite (NWM) and without mesquite (NWOM). A 20-cm deep sampling was carried out with a selection by convenience process, obtaining nine samples per site (n=36) (Cabrera-Rodríguez *et al.*, 2024). Soil samples were air dried under shade conditions; afterwards, they were sieved with a 2 mm mesh and stored in polypropylene bags, awaiting their chemical and physical analyses. Gravimetry was used to determine their moisture content (AOAC, 2005) and a pressure plate was used to establish moisture retention capacity (Richards, 1956). The pH, electric conductivity, and total nitrogen content of each sample was determined (Rowell, 1994). The silver thiourea method was used to establish the interchangeable bases (SEMARNAT, 2000). In addition, the Walkley-Black method was used to determine organic matter and organic carbon content. The Bouyoucos method was used to determine the texture of the samples (SEMARNAT, 2000). Humic and fulvic acid content was established following the protocol proposed by Sánchez-Monedero *et al.* (2002). The statistical analysis included a verification of the assumption of normality and homoscedasticity of the variables. In addition, a student's t-test ($p<0.05$) was performed with the SPSS software to determine any differences between the physical and the chemical variables of the agricultural soils and the soils with natural vegetation, with and without mesquite.

RESULTS AND DISCUSSION

The Valle del Mezquital is made up of a vast network of agricultural plots, both under irrigation and rainfed systems. Hernández-González *et al.* (2018) carried out a comparative study about soil fertility changes caused by anthropic activities. In their study, these authors pointed out that anthropic activities influence physicochemical parameters of soils, regarding organic matter, pH, and aggregate stability. In the case of El Alberto, soils with natural vegetation with mesquite (NWM) and without mesquite (NWOM) have a slightly acid pH (pH=6.7). Agricultural soils with mesquite (ASWM) and without mesquite (ASWOM) have a strongly alkaline content (pH=8.23). An alkaline pH can indicate that there are less nutrients available for the crops (Riaz *et al.*, 2020). In both cases, soils have a sandy and sandy loam texture (Table 1).

Table 1. Soil texture in El Alberto, Ixmiquilpan, Hidalgo.

Variable	With natural vegetation with (NCM) and without mesquite (NSM) (g kg^{-1})	Agricultural with (ACM) and without mesquite (ASM) (g kg^{-1})
Texture	Sandy and sandy loam	
Particle size distribution	Clay 25.3, silt 105 and sand 869.6	Clay 57.2, silt 150.8 and sand 792
pH	6.7	8.23

NWM (NCM): soils with natural vegetation with mesquite; NWOM (NSM): soils with natural vegetation without mesquite; ASWM (ACM): agricultural soils with mesquite; ASWOM (ASM): agricultural soils without mesquite. Source: table developed by the authors.

Variable With natural vegetation with (NCM) and without mesquite (NSM) Agricultural with (ACM) and without mesquite (ASM)

Table 2 shows the physical and chemical characteristics of the soil. The organic matter and organic carbon percentages, as well as the nitrogen and phosphorous concentrations, were higher in the sites with mesquite, regardless of soil use (α=0.05). Mesquite favors organic matter and organic carbon concentrations in the soil, as a consequence of litterfall decomposition (Cuss and Guéguen, 2013). The mineralization of these organic compounds increases nutrient concentration (phosphorous and nitrogen); in addition, Carrillo-Flores *et al.* (2007) pointed out that mineralization allows a greater fixation of atmospheric nitrogen. Table 2 shows another positive element of *Neltuma laevigata* in agricultural areas: the improvement of water retention capacity and moisture in the soil (α=0.05). Soils with higher water retention capacity and higher moisture percentages guarantee a constant supply of water to the roots, even during draughts (Rodríguez-Sauceda *et al.*, 2019).

Soils with mesquite recorded an enhanced water retention capacity and moisture content. Organic matter (particularly humus) is a natural sponge that can absorb and retain large amounts of water, improving water availability in the soil (Lehmann and Kleber, 2015). This phenomenon could explain the higher moisture and water retention capacity of soils with mesquite, both in areas with natural vegetation or in agricultural soils.

The average concentrations of calcium, magnesium, and sodium recorded higher concentrations (3.8, 2.1, and 7.8, respectively) in agricultural soils (with and without mesquite) than in soils with natural vegetation (with and without mesquite). These values

Table 2. Physicochemical parameters evaluated to determine soil fertility in El Alberto, Ixmiquilpan, Hidalgo.

Physical and chemical tests Sites	Sites			
	Soils with natural vegetation		Agricultural soils	
	with mesquite	without mesquite	with mesquite	without mesquite
rganic carbon (%)	3.15±1.30*	1.67±0.26	1.72±0.33*	0.72±0.03
Organic matter (%)	5.43±2.24*	2.88±0.45	2.97±0.98*	1.24±0.09
Total nitrogen (%)	0.32±0.12*	0.18±0.04	0.18±0.04*	0.11±0.04
Electrical conductivity (dS/m)	3.56±3.88 *	0.42±0.17	2.77±2.56*	3.12±3.11
Water holding capacity (mm/m)	81.44±7.66*	91.64±1.27	108.17±12.6*	82.16±3.15
Moisture percentage (%)	7.91±2.59*	13.79±2.67	13.55±4.40*	14.12±7.91
Phosphorus (mg kg^{-1})	54.85±15.0*	29.75±3.35	48.85±11.6*	25.48±5.43
Calcium (Cmol (+) kg^{-1})	14.43±4.96˜	14.27±6.11	29.69±12.6˜	24.56±9.78
Magnesium (Cmol (+) kg^{-1})	4.91±1.63˜	3.86±1.39	11.69±4.79*	7.05±1.82
Sodium (Cmol (+) kg^{-1})	0.24± 0.22˜	0.14±0.93	0.98±0.88	2.03±0.95*
g CAH / kg humic acids	6.11±2.41*	3.26±1.75	3.12±2.14˜	1.51±0.25
g CAF/ kg fulvic acids	4.18±1.78*	2.68±1.64	4.29±3.39˜	3.94±3.97
g C humins /kg	23.59±11.7*	20.67±7.55	22.49±14.1˜	11.42±11.3

Asterisks (*) show significant differences between soils with and without mesquite, while the (˜) shows no significant differences.

are related to the use of fertilizers in agricultural areas, although their long-term use can cause salinity in soils. Armienta *et al.* (2008) proved that this species can grow in areas with a high salinity content and regulate the number of these ions in soils. Consequently, *Neltuma* sp. is a viable option for the restoration of soils degraded by salts.

The results of humic substances showed a significant difference of humic acid content between NWM and NWOM. However, there was no significant difference (α=0.05) between ASWM and ASWOM sites. The same results were obtained in the comparison of fulvic acid and humic substance contents. Moraes-Tavares and Nahas (2014) pointed out that the land use influences humic substance content and the humification process that takes place in the first 20 cm of the carbon reservoir.

CONCLUSIONS

The sampling areas with mesquite (*Neltuma laevigata*) are more fertile as a result of their higher organic matter and humic acid content. In addition, the total nitrogen and phosphorous levels available are higher in this soil than in areas without mesquite, both in soils with natural vegetation and mesquite and in agricultural soils. In conclusion, mesquite conservation in the semiarid ecosystems of the Valle del Mezquital can help to maintain soil fertility and improve water retention.

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Non-toxic *Jatropha curcas* L. residual paste as a protein supplement in poultry diets

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ABSTRACT

Objective: To evaluate the effect of the non-toxic residual meal from Mexican jatropha (*Jatropha curcas* L.) as part of a protein supplement in broiler chicken feed.

Design/methodology/approach: An experimental diet was designed by incorporating meal obtained from the non-toxic residual jatropha paste, which was administered to a group of 20 one-day-old Cobb 550 chicks. The chicks were weighed every three days, and their feed was adjusted weekly according to their development over a 28-day period. Another group of chicks, with the same number of individuals, characteristics, and follow-up, was fed a commercial diet for comparison purposes.

Results: The inclusion of non-toxic residual jatropha paste in the broiler chickens' diet produced similar performance to that obtained with the commercial diet.

Study limitations/implications: The scale of the experiment could affect the generalization of the results to a larger poultry population.

Findings/conclusions: The non-toxic residual meal from Mexican jatropha could be a viable and sustainable alternative for enriching poultry diets.

Keywords: Poultry feed; Production alternatives; Broiler chickens; Meal.

INTRODUCTION

Poultry farming in Mexico is in constant growth, driven by the demand for chicken meat as the main source of animal protein. In 2019, chicken meat production reached 909 thousand tons, representing 55% of the animal protein supply in the country (SIAP,

2019). This expansion calls for the search for sustainable alternatives in animal feed, where Mexican jatropha emerges as a viable option. This native Mexican plant, with high protein and lipid content in its seeds (Makkar, Becker, Sporer, & Wink, 1997), offers promising potential as a feed supplement for the poultry sector. The existence of non-toxic varieties of Mexican jatropha in Mexico (Martínez-Herrera, Siddhuraju, Francis, Dávila-Ortíz, & Becker, 2006) allows for the use of its residual meal as a protein supplement in poultry diets. This study analyzes the effect of non-toxic residual meal from Mexican jatropha on the growth of broiler chickens, contributing to the search for sustainable alternatives in poultry feed.

MATERIALS AND METHODS

Obtaining and processing inputs for the preparation of the experimental diet. The Mexican jatropha seeds, sourced from Papantla, Veracruz, were pressed to extract the oil. The residual meal was processed in an autoclave at 121 °C and 15 psi for 15 minutes to inactivate thermolabile antinutrients (Martínez-Herrera, Siddhuraju, Francis, Dávila-Ortíz, & Becker, 2006). The diet inputs (residual meal, corn, soy, wheat, sorghum, fish, and beef bone) were ground into a fine meal and then pelletized.

Toxicity. The residual meal was subjected to HPLC analysis to determine the content of phorbol esters, following the procedure described by Makkar (Makkar Harinder, Aderibigbe, & Becker, 1998).

Characterization of the diet inputs. The moisture, protein, and crude fiber content were determined according to Mexican official standards (PROYECTO de Norma Oficial Mexicana NOM-116-SSA1-1994, 1994) (Norma Oficial Mexicana NOM-F-68-S-1980, 1980) (NORMA Oficial Mexicana NOM-F-90-S-1978, 1979).

Experimental diet. The study compared the growth of chickens fed a commercial diet (Alpesur) and an experimental diet. The experimental diet was divided into three phases: pre-initial (week 1); providing 22% protein, initial (weeks 2-3); providing 20% protein, and finishing/final phase (week 4 onwards); providing 18% protein (Table 1).

The Pearson square method (Núñez González, Barcenas Mompeller, Mejías Caba, & Marrero García, 2020) was used to determine the quantity of each ingredient. Trouw Nutrition™ Axtra® PHY TPT 10,000 G phytase was added at 0.005% (200 g) to improve mineral absorption and eliminate phytic acid.

Experimental development. A total of 40 one-day-old Cobb 550 chicks were used. They were vaccinated against Newcastle disease on days 1 and 15. A shelter was established

Table 1. Elaboration of the non toxic formulation (%).

Inputs	Week 1 Pre-starter	Week 2 Starter	Week 3 Starter	Week 4 Fattening
J. curcas non- toxic	41.14	35.05	35.05	28.95
Beef bone flour	10.21	7.16	7.16	4.11
Wheat	42.21	45.26	45.26	48.30
Corn	3.21	6.26	6.26	9.31
Sorghum	3.21	6.26	6.26	9.31

with four blocks (two per group) of 1 m^2, with 10 chicks in each block, and the temperature was controlled using a heater. 100 W bulbs were used to maintain constant illumination.

Feeding program. The amount of feed was calculated for 20 chicks per group. The feeding program was developed over 28 days, increasing the amount of feed each week: week 1: 0.900 kg per day; week 2: 1.90 kg per day; week 3: 3.90 kg per day; and week 4: 5 kg per day.

Ethical management. The guidelines of NOM-051-ZOO-1995 (PROYECTO de Norma Oficial Mexicana NOM-051-ZOO-1995, 1996) were followed for the humane treatment of animals throughout the experiment.

RESULTS AND DISCUSSION

Toxicity of the residual meal. The HPLC analysis of the residual meal showed a phorbol ester content of 0.02 mg/g, confirming its non-toxic nature, which is consistent with previous studies (Makkar, Becker, Sporer, & Wink, 1997).

Nutritional content of the inputs. The residual meal from Mexican jatropha showed a protein content of 24.11% and a lipid content of 38.96%, standing out as a viable protein and energy source for chickens. In comparison, corn and sorghum, traditional ingredients, had lower percentages of protein (8.3% and 8.7%, respectively) and lipids (4.6% and 3.1%). Wheat provided 15.3% protein, and beef bone meal contributed 49.7%. These results indicate that Mexican jatropha can partially replace conventional inputs, offering an adequate nutritional balance and contributing to sustainability in poultry feeding.

Total and average daily feed consumption. The average feed consumption was similar in both groups during the 28 days of the study. Although the control group consumed more feed in the first week (0.303 kg compared to 0.266 kg in the experimental group), the differences narrowed by day 28, with totals of 2.769 kg and 2.316 kg, respectively. Neither group reached the theoretical consumption of 3 to 4 kg per chick (DANE (Departamento Administrativo Nacional de Estadística), 2015), and the differences were not statistically significant ($F=5.88$; $P=0.79$). The slight preference for the commercial diet may be attributed to palatability factors such as color and texture, as noted in previous studies (Nesseim *et al.*, 2019). Overall, the inclusion of Mexican jatropha did not negatively affect the amount of feed consumed by the chicks.

Increase in body mass. Figure 1 shows the increase in body mass of the chicks fed the experimental diet compared to those fed the commercial diet. The data indicate that both groups experienced similar growth throughout the study, with no statistically significant differences in average weight ($F=2.61$; $P=0.9$; $DF=2$).

During the 28 days of the study, both groups showed a consistent weight increase, with more pronounced gains starting from day 7, and by the end of the period, the average weights were similar. These results contrast with previous studies (Aguirre Jaramillo, 2011; Granda Paz, 2012), which reported lower weights due to the use of lower concentrations of Mexican jatropha (10%). In this study, the concentrations were higher (45% to 28%), which may have limited efficient metabolism and, consequently, body mass gain. Future research could evaluate intermediate concentrations to optimize growth and metabolic efficiency.

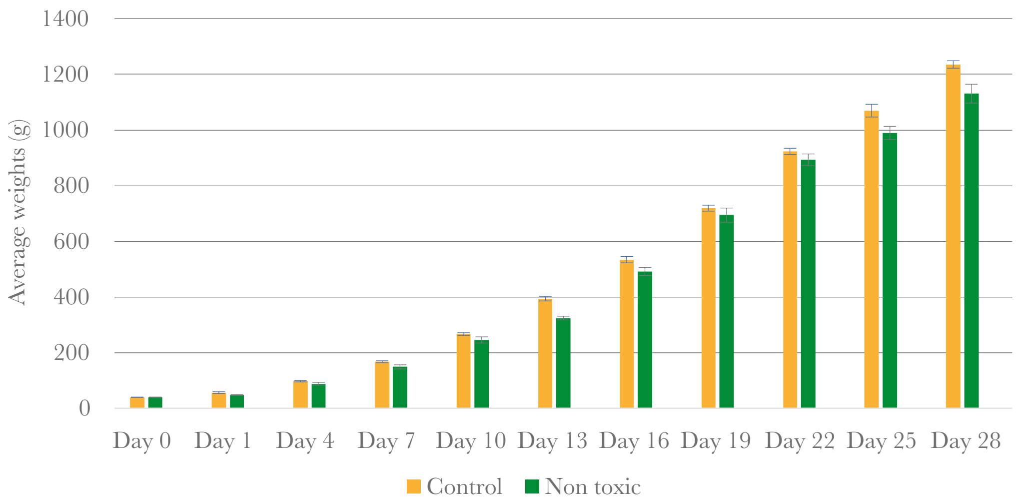

Figure 1. Average body mass gain.

Feed conversion ratio and productive efficiency factor. The results on the feed conversion ratio (FCR) for the groups fed the commercial diet and the experimental diet are presented in Table 2.

The data indicate that there was no significant difference in the feed conversion ratio (FCR) between the two groups ($F=6.39$; $P<0.05$; $GL=2$). This result suggests that the experimental diet is as efficient as the commercial diet in terms of feed conversion. Although optimal conversion ratios are considered to be below 1.8, the results of this study were higher: 2.24 for the group fed the commercial diet and 2.05 for the group with the experimental diet. The conversion ratios obtained in this study differ from those reported by Granda (Granda Paz, 2012) with a value of 1.50 and Aguirre (Aguirre Jaramillo, 2011), who achieved an efficiency of 1.48. The main difference lies in the concentrations of Mexican pinion or *J. curcas* seeds used: this study implemented higher levels (45% to 28%), while the aforementioned studies used a concentration of 10%. Various factors can influence conversion ratios, such as the genetics of the birds, the composition of

Table 2. Comparison of commercial and experimental diet.

Day	Commercial Diet	Experimental Diet
1	5.39±1.19	5.53±1.24
4	3.78±1.19	3.76±1.24
7	2.58±1.19	2.8±1.24
10	2.12±1.19	2.15±1.24
13	1.91±1.19	2.15±1.24
16	1.74±1.19	1.77±1.24
19	1.74±1.19	1.61±1.24
22	1.74±1.19	1.66±1.24
25	1.91±1.19	1.83±1.24
28	2.24±1.19	2.05±1.24

the feed, the feeding system, ambient temperature, and individual metabolic efficiency (Acres-Aviagen, A, 2014). When evaluating the productive efficiency factor (PEF), a better result was obtained from the group exposed to conventional feed, recording a value of 196.6, while the experimental diet group reached 184.2. The PEF is another important indicator that combines performance in terms of weight gain and feed efficiency, providing a comprehensive view of the productivity of the feeding system. Both groups achieved favorable PEF results, exceeding the threshold of 180 considered "excellent" in most national poultry farms (Ingalls Herrera & Ortiz Muñiz, 2006). This result is a positive indicator that the incorporation of Mexican pinion seeds in the diet does not negatively affect the productive efficiency of the chickens.

CONCLUSIONS

The results indicate that the non-toxic residual paste of Mexican *J. curcas* seeds or pinion seeds is a promising ingredient for broiler diets, without negatively affecting their growth, feed conversion, productive efficiency, or health. The performance was comparable to that of the commercial diet, with no significant differences in the weight of the chickens. This supports the viability of integrating Mexican pinion seeds or *J. curcas* seeds into poultry feeding, contributing to the diversification and sustainability of this industry.

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Colegio de Postgraduados

Effect of meta-Topolin on the *in vitro* Propagation of Strawberry (*Fragaria×ananassa* Duch)

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ABSTRACT

Objective: Strawberry cultivation (*Fragaria* sp.) is globally relevant due to the appeal of its fruit and its organoleptic characteristics, which increase its demand. However, it is necessary to implement new propagation methods that allow the establishment of commercial plantations, such as plant micropropagation, in order to obtain thousands of plants in a reduced period of time. Therefore, the objective of this study was to determine the effect of meta-Topolin (mT) during the *in vitro* multiplication of strawberries.

Design/Methodology/Approach: *In vitro* strawberry plants were used in MS medium with 30 g L^{-1} of sucrose, evaluating concentrations of meta-Topolin (mT: 0, 0.5, 1.0, 1.5 mg L^{-1}) and 6-benzylaminopurine (BAP: 0, 0.5, 1.0, 1.5 mg L^{-1}), under conditions of 24±2 °C and a photoperiod of 16:8 h light/darkness.

Results: mT increased the number of shoots compared to BAP, with 1.0 mg/L yielding 4.72 shoots per explant and 1.5 mg/L yielding 3.50. At 1.5 mg/L, the shoots reached 3.41 cm. The roots formed during multiplication facilitated acclimatization.

Limitations/Implications: Although the use of meta-Topolin showed promising results in the *in vitro* multiplication of strawberries, its performance needs to be evaluated in later developmental stages, field growth, and response to different environmental conditions.

Findings/Conclusions: mT increased the number of shoots compared to BAP, with 1.0 mg L^{-1} yielding 4.72 shoots per explant and 1.5 mg L^{-1} yielding 3.50. At 1.5 mg L^{-1}, the shoots reached 3.41 cm. The roots formed during multiplication facilitated acclimatization.

Keywords: micropropagation, strawberry, commercial propagules, cytokinins.

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INTRODUCTION

The strawberry fruit (*Fragaria* sp.) is globally valued for its organoleptic properties (Carole *et al.*, 2024). Additionally, this fruit contains various nutrients such as proteins, calcium, potassium, iron, copper, and vitamins (Kannaujia & Asrey, 2021). The cultivation of this species contributes to the economy of different countries around the world, including

Mexico, where approximately 443,552.81 tons of strawberries are produced annually, with Michoacán being the leading producer, accounting for 326,191.10 tons (SIAP, 2024).

The propagation of this crop is mainly carried out asexually through cuttings or by seed germination (Li *et al.*, 2020). However, propagation through cuttings does not meet the demand for propagules required for the establishment of commercial orchards (Dhukate *et al.*, 2021). On the other hand, seed germination has low efficiency and promotes genetic segregation, limiting its use. For this reason, it is essential to implement strategies that optimize and increase the propagation capacity of this crop (Valliath & Mondal, 2023). Consequently, plant tissue culture, known as micropropagation, has been successfully implemented in this plant species (Neri *et al.*, 2022). This technique allows the production of a large number of plants in relatively short periods and in limited spaces. Additionally, it generates plants with greater genetic uniformity, vigor, and high phytosanitary quality. However, to reach its commercial potential, it is necessary to develop protocols that maximize plant production (Abdalla *et al.*, 2022). In this context, the proper selection of the type and concentration of plant growth regulators (PGRs) is crucial to ensure the success of micropropagation (Papafotiou *et al.*, 2023). The 6-Benzylaminopurine (BAP) is one of the most commonly used PGRs to stimulate shoot formation in various plant species (Krishna *et al.*, 2021; Monthony *et al.*, 2021; Nugrahani *et al.*, 2024). However, other cytokinins can be evaluated to determine their efficacy in *in vitro* shoot formation. One such alternative is meta-Topolin (mT, 6-(3-hydroxybenzylamino) purine), a synthetic cytokinin that activates different morphogenetic regeneration pathways in *in vitro* cultures (Gantait & Mitra, 2021). mT has been successfully used in the micropropagation of species such as vanilla (*Vanilla planifolia* Andrews) (Manokari *et al.*, 2021) and gerbera (*Gerbera jamesonii* Bolus ex Hook.f.) (Mahanta *et al.*, 2023), proving to be more efficient than BAP in shoot generation. Therefore, the objective of this study was to evaluate the effect of meta-Topolin on the *in vitro* multiplication of strawberry (*Fragaria*×*ananassa* Duch).

MATERIALS AND METHODS

Plant Material

In vitro strawberry plants (*Fragaria*×*ananassa* Duch), previously established from apical meristems, were used. These plants, cultivated at the National Center for Genetic Resources, served as the source of explants for the study.

Shoot Proliferation

Individual 1 cm long shoots of strawberry (*Fragaria*×*ananassa* Duch) were selected and transferred to MS (Murashige & Skoog, 1962) culture medium supplemented with 30 g L^{-1} of sucrose. Different concentrations of meta-Topolin (mT: 0, 0.5, 1.0, and 1.5 mg L^{-1}) and 6-benzylaminopurine (BAP: 0, 0.5, 1.0, and 1.5 mg L^{-1}) were evaluated. The medium was adjusted to a pH of 5.8±0.1, and 7 g L^{-1} of agar was added as a gelling agent. A total of 35 mL of the medium was dispensed into 250 mL glass jars, which were sterilized in an autoclave at 1.5 kg cm^2 and 121 °C for 15 min. The plants were maintained under controlled conditions of 24±2 °C, with a photoperiod of 16 h light and 8 h darkness, under an irradiation of 50 μmol/m^2/s. After 45 days of culture, the following parameters were evaluated: number of shoots per explant, shoot length, number of leaves, as well as number and length of roots.

Acclimatization

Shoots measuring 3 cm in length, with a well-developed root system, were transferred to plastic domes measuring 24×16×7 cm, containing a sterile substrate composed of peat moss and perlite in a 1:1 (v/v) ratio. The plants were maintained under controlled greenhouse conditions with 50% shade, a temperature of 30±5 °C, and relative humidity gradually adjusted: 90±5% during the first week, 80±5% in the second week, and 65±5% in the third week. After 30 days, the plant survival rate was recorded.

Statistical Analysis

All experiments were conducted in duplicate, following a completely randomized design. The data obtained were statistically analyzed using IBM SPSS Statistics software (version 21). An analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparison test ($p \leq 0.05$) to identify statistically significant differences between treatments.

RESULTS AND DISCUSSION

Sprouting Proliferation

After 45 days of cultivation, statistically significant differences were observed among the treatments evaluated (Table 1). In general, mT produced a higher number of shoots compared to BAP (Figure 1). A total of 4.72 shoots per explant were obtained in the culture medium containing 1.0 mg L^{-1} of mT, followed by 3.50 shoots in 1.5 mg L^{-1} of mT and 3.17 shoots in 1.0 mg L^{-1} of BAP. The lowest number of shoots per explant (1.20) was observed in the control treatment (0 mg L^{-1} of BAP or mT). Regarding shoot length, 1.5 mg L^{-1} of mT produced shoots of 3.41 cm, followed by 2.59 cm obtained in the control treatment and 1.68 cm in 1.0 mg L^{-1} of mT. The shortest shoots were observed with 1.0 and 1.5 mg L^{-1} of BAP, measuring 0.83 and 0.85 cm, respectively. The highest number of leaves (5.10) was recorded in the control treatment, while the lowest (1.87) was observed in 1.5 mg L^{-1} of mT. Strawberry shoots generated roots during the multiplication stage.

Table 1. Effect of 6-Benzylaminopurine and meta-Topolin on the proliferation of strawberry shoots (*Fragaria*×*ananassa* Duch) after 45 days of cultivation.

Treatment (mg L^{-1})	Number of shoots	Length of shoots (cm)	Number of sheets	Number of roots	Length of roots (cm)
0 BAP/mT	1.20±0.37d	2.59±0.75ab	5.10±0.09a	5.40±0.63a	1.08±0.29a
0.5 BAP	2.75±0.33bc	1.27±0.15c	2.02±0.11c	0.60±0.19bc	0.23±0.08bc
1.0 BAP	3.17±0.32bc	0.83±0.09c	2.05±0.10c	0.89±0.26bc	0.33±0.08bc
1.5 BAP	1.83±0.30cd	0.85±0.09c	2.16±0.11c	0.00±0.00c	0.00±0.00c
0.5 mT	2.41±0.39bc	1.23±0.22c	3.67±0.08b	1.50±0.45b	0.36±0.11b
1.0 mT	4.72±0.22a	1.68±0.30bc	2.79±0.13bc	0.54±0.31bc	0.13±0.08bc
1.5 mT	3.50±0.26ab	3.41±0.27a	1.87±0.09c	0.00±0.00c	0.00±0.00c

The data are presented as mean±standard error. Different letters indicate significant differences (Tukey, $p \leq 0.05$).

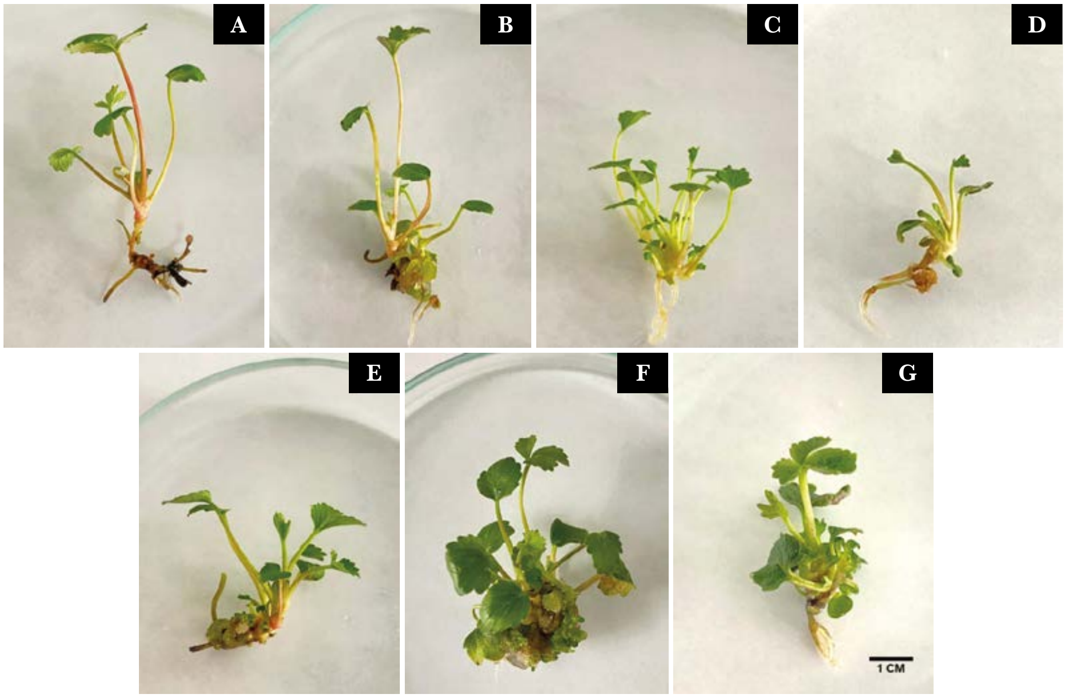

Figure 1. Effect of 6-Benzylaminopurine (BAP) and meta-Topolin (mT) on the proliferation of strawberry shoots (*Fragaria×ananassa* Duch). A) Control treatment, B-D) 0.5, 1.0, and 1.5 mg L^{-1} of BAP, left to right, E-G) 0.5, 1.0, and 1.5 mg L^{-1} of mT, left to right.

However, the highest number of roots (5.40) was observed in the control treatment. The treatment with 1.5 mg L^{-1} of mT did not produce roots. Roots with a length of 1.08 cm were obtained in the control treatment, whereas the root length in the other treatments was shorter.

Acclimatization

Strawberry plants (*Fragaria×ananassa* Duch) subjected to the acclimatization process showed an 85% survival rate after 30 days of cultivation (Figure 2). The plants were transferred to plastic bags.

The micropropagation of various varieties derived from *Fragaria* sp. crosses has proven to be an effective method for obtaining a large number of disease-free plants with high

Figure 2. Acclimatization process of strawberry (*Fragaria×ananassa* Duch). A) *In vitro* plants used and B and C) Plants after 30 days of acclimatization.

genetic uniformity (Quiroz *et al.*, 2017; Neri *et al.*, 2022). In this study, an increase in the *in vitro* proliferation of strawberry (*Fragaria*×*ananassa* Duch) shoots was confirmed through the use of meta-Topolin (mT), an aromatic cytokinin characterized by a benzyl ring with a hydroxyl group in the meta position (Strnad, 2021). This plant growth regulator (PGR) is widely used in the *in vitro* propagation of plants, standing out for its high efficiency based on criteria such as a high multiplication rate, vigorous shoots, optimal rooting, and greater acclimatization capacity (Zaytseva *et al.*, 2021). In this study, the addition of meta-Topolin to the culture medium resulted in a higher number of shoots per explant compared to the addition of 6-benzylaminopurine (BAP). The concentration of 1.0 mg L^{-1} of mT significantly promoted the proliferation of strawberry (*Fragaria*×*ananassa* Duch) shoots, while 1.5 mg L^{-1} of mT produced longer shoots. Although BAP is the most commonly used cytokinin in plant tissue culture due to its positive response in most species (Krishna *et al.*, 2021), in this study, mT demonstrated greater efficiency in generating shoots per explant. These results align with those reported by Kumar *et al.* (2024), who observed a similar response in winter cherry (*Withania somnifera* (L.) Dunal), where the use of 2.5 mg L^{-1} of mT outperformed various concentrations of BAP in the production of shoots per explant. The advantages offered by mT compared to other cytokinins include counteracting the effects of hyperhydricity caused by the use of BAP, preventing shoot necrosis by containing a hydroxyl group that allows the reversal of O-glycosides generated by BAP use, avoiding the formation of chimeras, and not inhibiting root formation during acclimatization (Cardoso, 2021; Werbrouck, 2021). Although it is mentioned that the use of topolins is common in laboratories dedicated to commercial micropropagation, these protocols are industrial and therefore confidential (Ahmad & Strnad, 2021; Werbrouck, 2021).

Meta-Topolin has proven to be highly effective in the micropropagation of various plant species, to the point of being considered, a few years ago, a panacea in plant tissue culture (Aremu *et al.*, 2012). In vanilla (*Vanilla planifolia* Andrews), the use of mT produced a record 62 shoots per explant, the highest reported for this species (Manokari *et al.*, 2021). In sweet potato (*Ipomoea batatas* (L.) Lam.), mT achieved the highest number of shoots compared to other plant growth regulators (PGRs) (Bansal *et al.*, 2023). Similarly, in gerbera (*Gerbera jamesonii* Bolus ex Hook.f.), a concentration of 1.5 mg L^{-1} of mT generated approximately 14 shoots per explant, surpassing results obtained with BAP, kinetin, thidiazuron, and zeatin (Mahanta *et al.*, 2023). In this study, using strawberry (*Fragaria*×*ananassa* Duch), 4.72 shoots per explant were obtained with 1.00 mg L^{-1} of meta-Topolin. Comparatively, Dhukate *et al.* (2021) reported in strawberry (*Fragaria*×*ananassa*) cvs. 'Sweet Charlie' and 'Winter Dawn' the production of 4-5 shoots per explant using 0.5 mg L^{-1} of BAP and 1.0 mg L^{-1} of kinetin. Similarly, Neri *et al.* (2022) reported in strawberry (*Fragaria*×*ananassa* Duch.) cv. 'Aroma' the production of 4.20 shoots per explant with the addition of 1.0 mg L^{-1} of zeatin. These results highlight the efficacy of mT compared to other plant growth regulators.

Acclimatization is one of the most challenging steps in the plant micropropagation process (De Stefano *et al.*, 2022). This process involves the gradual adaptation of *in vitro* plants to *ex vitro* conditions, which is essential for achieving high survival rates (Mahendra *et al.*, 2020). In this study, an 85% survival rate was achieved in strawberry (*Fragaria*×

ananassa Duch) plants obtained through plant tissue culture. In comparison, Dhukate *et al.* (2021) reported survival rates of 95% and 90% in the strawberry varieties 'Sweet Charlie' and 'Winter Dawn,' respectively. Similarly, Neri *et al.* (2022) achieved a 100% survival rate in the strawberry variety 'Aroma.' These results reflect the variability in plant response during acclimatization, depending on the variety and the specific conditions of the process.

CONCLUSIONS

The use of meta-Topolin resulted in a higher number of shoots per explant compared to the use of BAP. The concentration of 1.0 mg L^{-1} of mT produced the highest number of shoots per explant, while 1.5 mg L^{-1} generated the longest shoots. Additionally, an 85% survival rate was achieved during the gradual acclimatization process. These results represent a significant advancement in the propagation of strawberry (*Fragaria*×*ananassa* Duch) contributing to the production of propagules for the establishment of commercial plantations.

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